

The effect of *Phytolacca decandra* in the prophylaxis of bovine mastitis.

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I hereby declare that this research represents my own work both in concept and execution.

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This research is dedicated to the blessed few who are true teachers of
the art of healing, and who do so selflessly

AND/EN

Die navorsing dra ek ook op aan Laurens en Susan, wie se sorg en
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Abstract

The purpose of this study was to determine the effect of the homoeopathic remedies *Phytolacca decandra* 12CH and *Phytolacca decandra* 200CH on the incidence of acute clinical mastitis, on the somatic cell count and on the butterfat, protein and lactose levels of composite milk samples obtained from dairy cows. It was hypothesised that the remedy would have an effect on the incidence of acute clinical mastitis, on the somatic cell count and on the butterfat, protein and lactose levels of composite milk samples obtained from the two respective treatment groups in the dairy herd used.

A placebo-controlled double blind study design was used. The trial group consisted of 252 Jersey cows from a farm in the Underberg district of KwaZulu-Natal, South Africa. These cows were ranked according to age before being randomly divided into three groups consisting of 84 lactating cows each. The random allocation of treatments in this manner ensured an even spread of treatments across all age classes and lactation number. The three groups were painted with different colours of enamel paint to ensure easy and accurate dispensing of medicine and placebo. All identifiable variables were the same for the three groups for the duration of the 100-day study.

The homoeopathic remedies *Phytolacca decandra* 12CH, *Phytolacca decandra* 200CH, and the placebo were supplied in granules in plastic sachets that were randomised and colour coded by an independent Homoeopath. Dispensing took place after milking while the cows were being fed and was done by the person responsible for the feeding. Five millilitre medicated or unmedicated granule doses were dispensed into the dry feed by matching the colour code on the container of the granules of placebo or verum with that of the cow and then sprinkling the granules over the dry food with a 5ml-measuring spoon.

Composite milk samples were obtained the day before treatment commenced to obtain baseline readings for the whole test population. Sampling occurred on day 1, 35, 70 and 100. The milk samples were analysed at Taurus Central Laboratory to determine values for somatic cell count, butterfat, protein and lactose on the day of the milk recording.

The data from 191 cows from the test population was used after selection criteria were applied. There were 65 cows in the *Phytolacca decandra* 200CH, 67 cows in the *Phytolacca decandra* 12CH, and 59 cows in the placebo group. All cases of acute clinical mastitis were recorded for the test population, and at

the culmination of the trial each cow had four results for the somatic cell count, butterfat, protein and lactose content of their milk.

The number of acute cases of clinical mastitis was expressed as a percentage, and as the incidence of cases per hundred cows per annum. Data concerning the milk constituents inside and between the respective groups was analysed using parametric ANOVA tests to isolate any significant difference at the 5% level of confidence. Where differences were found, Bonferroni tests were done to identify any significant difference within and between the groups at the 5% level of confidence.

The incidence of acute clinical bovine mastitis was less in the treatment groups than in the placebo. For the duration of the trial, the *Phytolacca decandra* 200CH treatment group had an incidence of 16.3 cases per 100 cows per annum, the *Phytolacca decandra* 12CH treatment group had an incidence of 38.7 cases per 100 cows per annum and the Placebo group had an incidence of 68.1 cases per 100 cows per annum.

There was no statistically significant difference at the 5% level of confidence between the measurements from the *Phytolacca decandra* 12CH treatment group, the *Phytolacca decandra* 200CH treatment group and the Placebo group.

Statistically significant differences occurred between butterfat levels measured in milk samples collected on Day 35 and Day 100 and between Day 70 and Day 100 from the *Phytolacca decandra* 12CH treatment group. Statistically significant differences occurred between butterfat levels measured in milk samples collected on Day -1 and Day 35 and between Day 70 and Day 100 from the Placebo group.

Statistically significant differences occurred between total protein levels measured in milk samples collected on Day -1 and Day 70, between Day -1 and Day 100 and between Day 35 and Day 100 from the *Phytolacca decandra* 12CH treatment group. Statistically significant differences occurred between total protein levels measured in milk samples collected on Day -1 and Day 70, between Day -1 and Day 100 and between Day 35 and 100 from the *Phytolacca decandra* 200CH treatment group. Statistically significant differences occurred between total protein levels measured in milk samples collected on Day -1 and Day 100 and between Day 35 and 100 from the Placebo group.

No statistically significant differences were observed for measurements concerning lactose levels and somatic cell counts within and between the groups.

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Definition of terms

- Clusters** - A teat cluster consists of four teat cups and a claw, which is attached to the teat cups by milk tubes. (Randles, 2000)
- Teat cups** - Teat cups consist of an outer shell and an inner teat cup liner that is in contact with the teat skin during milking.
(South Africa. Department of agriculture, 1995: 261 and Rebhun, 1995: 300)
- Composite milk** - A sample representing the total combined volume of milk from each functional quarter of the udder. (Randles, 2000)
- Parenteral** - Administered by any way other than through the mouth
(Martin, 1994: 487).
- Placebo** - Placebo refers to a non-medicated substance, which is relatively inert pharmacodynamically. It is sometimes administered to contrast the effects of a non-medication in controlled experiments with those of medication in comparable groups of patients. (Gaier, 1991: 426)
- Prophylaxis** - Therapeutic intervention to prevent disease (Gaier, 1991: 473), such as immunization (Martin, 1994: 539).
- Sebum** - The oily substance secreted by the sebaceous glands. Sebum provides a thin film of fat over the skin, which slows the evaporation of water and also has an antibacterial effect
(Martin, 1994: 593).
- Somatic cell count** - Somatic cells are white blood cells, and are part of the body's defence against bacteria or other invading microorganisms (South Africa. Department of agriculture, 1995: 259). Large mononuclear cells, formerly epithelial cells, account for 65-70% of cells in normal milk. Neutrophils comprise 0-8%, and lymphocytes about 5%. About 18-25% of the cells have degenerated. With infection or inflammation, neutrophils increase dramatically and may comprise up to 95% of the cells in mastitic milk. (Amstutz, 1980: 1050)
- Virulence** - The disease producing ability of a microorganism
(Martin, 1994: 705).

Chapter 1 Introduction

1.1 The effect of bovine mastitis on milk production and quality

With the exception of some limited regions, South Africa is a country where, despite the potential for markedly improved yields, milk is generally produced under conditions less than favourable to efficient dairy farming. The dairy industry is characterised, therefore, by relatively low production and relatively high expenditure. The situation is aggravated by the increasing demands of a rapidly expanding population, making milk products luxuries instead of the common daily source of highly assimilable animal protein for the majority of the population. (Giesecke, 1983: 1) The conclusion that should be drawn is that efficient modern control of mastitis would allow most South African dairy farmers to maintain, or even improve present milk production with fewer cows. They would also realise greater profits and would be producing milk of higher quality. (Giesecke, du Preez and Petzer, 1994:167)

1.2 Milk as food

Milk is a complete food in itself. Not only does it contain more solids than any beverage, but has even more solids than tomatoes, peaches, lettuce and melons. In its unprocessed state, milk contains a wide range of highly digestible and nutritional constituents. (Giesecke, 1983: 2)

In addition, milk as a source of animal protein is more readily assimilable than any other, with 0,5kg of Cheddar cheese providing as much protein as 0,785kg sirloin steak or 0,945kg of chicken (Giesecke, 1983: 2).

Man requires such proteins to obtain the necessary amino acids for tissue growth. Since the body cannot store proteins for tissue growth, they are essential ingredients in food for daily building and maintenance of body tissue, especially in children. (Giesecke, 1983: 2)

Milk protein is therefore the most obvious choice for providing the amino acids required by man, more so because it supplies what is lacking in other proteins (e.g. cereals lack the essential amino acid lysine found in milk) (Giesecke, 1983: 2).

1.3 Treatment

1.3.1 Allopathic treatment

Despite the widespread use of various antibiotics and other chemotherapeutic agents like steroids, antibacterial treatment of mastitis has generally been less effective than desirable (du Preez, 1993; and Searcy, Ryes and Guajardo, 1995).

Disadvantages of these drugs are that they are expensive and have low efficacy even with sensitivity testing for individual cases. Their use reduces the leukocyte response to the mammary gland and predisposes to antibiotic resistance and other problems such as antibiotic hypersensitivity. (Searcy, Ryes and Guajardo, 1995)

A serious consequence of the use of antibiotics in treating mastitis is their effect on the manufacture of dairy products and the development of sensitivity syndromes in humans (Radostits, Blood and Gay, 1994: 573).

A program for mastitis control involves overall prevention in the herd as well as drug treatment of individual cows. Research has shown that drug treatment alone is ineffective. (Michigan State University, 1962: 19)

1.3.2 Homoeopathic treatment

Homoeopathy offers a safe and effective alternative to the use of antibiotic and other chemotherapeutic drugs. It can be applied prophylactically and in the treatment of individual cases. (Macleod, 1981: 50-51) It is generally thought that a system of preventative medicine is the ideal state of affairs, an approach for which homoeopathy is well suited (Macleod, 1981: preface).

Homoeopathic treatment aims to improve overall health, thereby reducing the cows' susceptibility to bacteria that cannot be eradicated (Hansford and Pinkus, 1998: 26).

1.4 The aim of the study

The aim of this study was to determine the efficacy of the homoeopathic remedy *Phytolacca decandra* in two different potencies in preventing bovine mastitis in terms of the somatic cell counts in composite milk samples and the incidence of acute cases of clinical mastitis during the 100-day study period.

1.5 Statement of the objectives

1.5.1 The first objective

Objective one was to evaluate the effect of *Phytolacca decandra* 200CH on somatic cell counts of composite milk samples obtained from dairy cattle.

1.5.2 The second objective

Objective two was to evaluate the effect of *Phytolacca decandra* 200CH on the incidence of cases of acute clinical mastitis in dairy cattle.

1.5.3 The third objective

Objective three was to evaluate the effect of *Phytolacca decandra* 12CH on somatic cell counts of composite milk samples obtained from dairy cattle.

1.5.4 The fourth objective

Objective four was to evaluate the effect of *Phytolacca decandra* 12CH on the incidence of cases of acute clinical mastitis in dairy cattle.

1.5.5 The fifth objective

Objective five was to evaluate the effect of *Phytolacca decandra* 12CH and 200CH on the butterfat content of composite milk samples collected from dairy cattle.

1.5.6 The sixth objective

Objective six was to evaluate the effect of *Phytolacca decandra* 12CH and 200CH on the lactose content of composite milk samples collected from dairy cattle.

1.5.7 The seventh objective

Objective seven was to evaluate the effect of *Phytolacca decandra* 12CH and 200CH on the protein content of composite milk samples collected from dairy cattle.

1.6 The Hypotheses

1.6.1 Hypothesis one

It is hypothesised that the homoeopathic remedy *Phytolacca decandra* will influence the somatic cell count of composite milk samples obtained from dairy cattle.

1.6.2 Hypothesis two

It is hypothesised that the homoeopathic remedy *Phytolacca decandra* will influence the incidence of acute cases of clinical mastitis in dairy cattle.

1.6.3 Hypothesis three

It is hypothesised that the homoeopathic remedy *Phytolacca decandra* will influence the butterfat content of composite milk samples obtained from dairy cattle.

1.6.4 Hypothesis four

It is hypothesised that the homoeopathic remedy *Phytolacca decandra* will influence the lactose content of composite milk samples obtained from dairy cattle.

1.6.5 Hypothesis five

It is hypothesised that the homoeopathic remedy *Phytolacca decandra* will influence the protein content of composite milk samples obtained from dairy cattle.

1.7 Delimitation

1.7.1 Delimitation one

The emphasis of this study is to observe any significant differences in the composition of composite milk samples collected from dairy cattle, and not on the mechanism of action of the medicine, nor of the process of lactogenesis.

1.7.2 Delimitation two

The study is limited to the observation and recording of the acute cases of clinical mastitis in dairy cattle, and not the identification of mastitogenic microorganisms responsible for its development or the effect of the medicine on certain strains of pathogens.

1.7.3 Delimitation three

This study is limited to the treatment of animals; only lactating Jersey cows received the remedy and placebo treatments.

1.7.4 Delimitation four

This study is limited to prophylaxis only, and will not attempt the homoeopathic treatment of acute cases of clinical mastitis or any other condition to be found or to develop for the duration of the study.

1.7.5 Delimitation five

This study will not investigate any other medicines or potencies other than those stipulated.

1.8 Assumptions

1.8.1 The first assumption

It is assumed that the three groups of Jersey cows were homogeneous with respect to age and lactation number.

1.8.2 The second assumption

It is assumed that exposure to and the influence of environmental conditions on the cows and the measurements was the same for the 3 groups of cows for the duration of the study.

1.8.3 The third assumption

It is assumed that the homoeopathic medicines used in the trial were prepared according to the monographs in the French Homoeopathic Pharmacopoeia as stipulated.

1.8.4 The fourth assumption

It is assumed that the homoeopathic remedies were functionally active at the time of dispensing.

1.8.5 The fifth assumption

It is assumed that the dry feed in which the homoeopathic medicine was dispensed was homoeopathically inert.

1.8.6 The sixth assumption

It is assumed that any demonstrable difference in the groups that received homoeopathic medicines was due to the action of the medicine and not attributable to "suggestion" or the "placebo" effect.

Chapter 2

Relate of the relevant literature

2.1 Introduction

The term mastitis means, literally, inflammation of the breast or udder, and it is derived from the Greek words *mastos* meaning breast, and *itis* meaning inflammation. The term mastitis includes all inflammatory conditions in the udder. (Davis, 1963: 659)

By selective breeding, and forcing for high milk yields, the dairy cow is now yielding quantities of milk far in excess of what was intended by nature. Already in 1938, before the farmers club in London, Dr. W.M. Cumber said that the cause for this great increase in mastitis is that farmers endeavour to get the cow to do too much work. This puts a great strain on the animal. The cow should produce milk to rear her calf, and 250 gallons (1 136,5 l) of milk per lactation keeps it in good condition. The endeavours of dairymen led to the breeding of first 1000-gallon (4 546 l per lactation) cows and then 2000-gallon (9 092 l per lactation) cows. Trying to force so much out of the cow is not natural. (Little and Plastridge, 1946: 257-258)

2.2 Historical overview

Danish veterinarian and researcher Bernhard Bang recognised by 1889 that "in general mastitis must not be considered as a proper infectious disease caused by one definite factor, but that due to external factors, bacteria from the environment, manure and urine accidentally had found their way through the teat canal" (Van Horn and Wilcox, 1992: 440).

The first cases of clinical mastitis in South African dairy cattle were reported at the end of the nineteenth century; by this time 70 years of research had already been conducted in Switzerland, France and Germany (Giesecke, du Preez and Petzer, 1994: 163).

Between 1910 and 1937 no records of any noteworthy progress on mastitis control in South Africa were kept, suggesting that the control of clinical mastitis remained limited to the 'single cow' approach, a tradition still overemphasised by many milk producers (Giesecke, du Preez and Petzer, 1994: 163).

In 1938 the attention of South African research switched to aspects of subclinical mastitis. This changed between 1945 and 1965, to a non-standardised type of specific single herd approach, adopted if necessary to supplement the single cow approach in herds with serious problems of clinical mastitis. (Giesecke, du Preez and Petzer, 1994: 163)

Complications occurred between 1968 and 1970, when dairy farmers began to administer antibiotics indiscriminately instead of using them, as directed, to supplement good parlour management (Giesecke, du Preez and Petzer, 1994: 163).

From 1976 onwards, mastitis control progressed to a standardised extensive regional approach supported in a limited but growing number of herds. There was regular monitoring of somatic cell count values in herd milk as well as veterinary advice on various aspects of the prevention and control of subclinical and clinical mastitis. (Giesecke, du Preez and Petzer, 1994:163)

2.3 Economic perspective

“Mastitis is the disease syndrome that causes the most wastage in Dairy Farming” (Day, 1995). The cost of this disease to the farmer is staggering. The latest estimation by Petzer (1996) determined that financial losses in South African dairy herds due to mastitis totals R525 million *per annum*. The average loss per herd is estimated at R39 537 per annum and the loss per cow at R414 per annum.

Mastitis affects the farmer economically in two ways: Through direct costs and indirect costs.

1. Direct costs:

- discarded milk (Blowey and Edmondson, 1995: 3), both during treatment and in the compulsory 72-hour period of withholding after last antibiotic treatment (Van den Heever, Giesecke and March, 1971: 5).
- drug and veterinary costs. (Blowey and Edmondson, 1995: 3)

2. Indirect costs:

- decreased milk yield during remainder of lactation due to udder damage and/or subclinical infection.
- penalties because of increased somatic cell count.
- extra labour requirements for treating and nursing.
- higher culling and replacement rates leading to loss of genetic potential.
- deaths. (Blowey and Edmondson, 1995: 3)

2.4 Aetiology

Mastitis is a multifactorial disease (Giesecke, du Preez and Petzer, 1994: 219). In the broad context, the dynamics of udder health and mastitis depend on the interrelationships between three main classes of factors, namely (i) those associated with the cow, i.e. genetic factors, (ii) those associated with the cow's environment, and (iii) those associated with the microbes challenging the cow's udder. These factors may contribute towards the development of mastitis, provided they provoke pathological injury of mammary epithelium. (Giesecke, *et al.* 1988: 19)

Although mastitis is caused by both non-infectious (traumatic or toxic) and infectious agents, microorganisms are the most important aetiological agents. The udder is constantly exposed to a variety of microorganisms, but whether mastitis develops or not depends on the nature of the organism, its virulence and numbers, and the susceptibility of the udder to infection. The risk of mastitis is higher in early lactation and in high producing cows, and increases with the age of the cow. Poor hygiene is also an important predisposing factor in the development of infectious mastitis. (Coetzer, Thomson and Tustin, 1994: 1565) Milking machines may damage the teat, allowing pathogens access to the gland through the teat canal, and may transfer pathogens from one cow to another through contaminated clusters (Coetzer, Thomson and Tustin, 1994: 1565).

2.4.1 Contagious and environmental mastitogenic microorganisms

Streptococcus agalactiae was the most common cause of mastitis prior to 1940, but with the widespread use of antibiotics in the control of mastitis, the prevalence of *S. agalactiae* mastitis has decreased, while mastitis caused by *Staphylococcus aureus*, coliform bacteria, *Mycoplasma* spp.,

Streptococcus uberis and *Staphylococcus epidermis* has increased globally (Coetzer, Thomson and Tustin, 1994: 1564). In most countries *Staphylococcus aureus* is the predominant cause of subclinical mastitis (Andrews, Blowey, Boyd and Eddy, 1992: 296).

2.4.1.1 Contagious organisms

The most common classification divides mastitis pathogens into contagious and environmental organisms. Contagious organisms include *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Staphylococcus aureus*. Mycoplasma is also mastitogenic, but can be involved in other disease processes in the cow. All those organisms have only limited survival spans in the environment of the cow. (Van Horn and Wilcox, 1992: 442)

2.4.1.2 Epidemiology of contagious organisms

The mammary gland and/or teat cistern are reservoirs of infection. Organisms are transmitted from the carrier cow or quarter to the teats of non-infected cows/quarters during the milking process. Colonies become established at the teat end and slowly grow through the canal over 1-3 days. Dry cow therapy and post-milking teat disinfection are important means of control. Herds with a high incidence of infections often have high cell counts but normal total bacterial counts. (Blowey and Edmondson, 1995: 30)

2.4.1.3 Environmental organisms

Environmental organisms include *Streptococcus uberis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* (Van Horn and Wilcox, 1992:442), *Citrobacter* spp., *Bacillus cereus*, *Bacillus licheniformis*, *Pasteurella* spp., *Streptococcus faecalis*, fungi and yeasts (Blowey and Edmondson, 1995: 29). They survive and proliferate in the environment of cows. *Pseudomonas aeruginosa* in general is recovered from water lines (Van Horn and Wilcox, 1992:442), and thrives in wet conditions (Randles, 2000). Contamination of the teat canal and cistern with environmental organisms during milking is frequent if proper pre-milking hygiene procedures are not applied (Van Horn and Wilcox, 1992:442).

2.4.1.4 Epidemiology of Environmental Organisms

The environment is the reservoir of infection. Organisms are transferred from the environment to the teats between milkings. Dry cow therapy is of no value as environmental infections do not persist subclinically and are not carried from one lactation to the next. Pre-milking teat disinfection is thought to assist with the control of environmental infections. Herds with a high incidence of environmental infections may have low cell counts but high total bacterial counts. (Blowey and Edmondson, 1995: 29)

Holding areas (pre- and post-milking) where cows congregate are also important, especially as the teat canal is not fully re-sealed until about half an hour after machine milking. Overmilking predisposes to teat canal damage and inadequate sealing, leading to a greater chance of infection. (Randles, 2000)

A cow's metabolic adjustments and immunological responses are its most important natural mechanisms for protecting the integrity of its tissues and its life (Giesecke, du Preez and Petzer, 1994: 70). Giesecke *et al.* (1988) (as cited by Farrow, 1997:12-13) states that the lactating cows high metabolic requirements make them particularly susceptible to the stressful conditions associated with metabolic environmental factors. These factors include a hot climate, exertion, transportation, starvation and lactation, and may affect the metabolism and integrity of mammary tissue.

The teat canal has several anatomical features that serve as barrier to penetration by bacteria, but their efficiency decreases progressively with the number of lactations. The teat canal's stratified squamous epithelial lining, spiral longitudinal mucosal folding and sphincter, as well as the sebum-like material containing long chain fatty acids within its lumen, and Furstenberg's rosette (a series of folds in which the teat canal terminates proximally), play important roles in its defence mechanism. (Coetzer, Thomson and Tustin, 1994: 1568)

There is a variable delay between infection and detection of mastitis by means of diagnostic techniques because visual detection of mastitis by the appearance of floccules in the milk, swelling of the udder and/or erythema usually only occurs once the infection has progressed to an advanced stage. Since increased time delays result in increased tissue damage, minimizing time delays is a factor of vital importance for the successful therapy of mastitis. (Giesecke, du Preez and Petzer, 1994: 6)

2.5 Classification of mastitis

A classification based on clinical criteria is probably the most practical approach, and is desirable for various reasons, such as prognosis, therapy, prevention and control of mastitis (Giesecke, *et al.* 1988: 21).

2.5.1 Subclinical mastitis

In the case of subclinical mastitis the milk looks normal (Giesecke, *et al.* 1988: 21), but a range of tests can be applied to detect either the infecting organism (directly or indirectly) or the cytological and biochemical changes to the secretion as a consequence to inflammation (Andrews, *et al.* 1992: 292).

2.5.1.1 Latent infection

In the case of latent infection pathogenic microorganisms capable of causing mastitis are present in the mammary gland without any evidence of mastitis and the milk has a normal somatic cell count (Coetzer, Thomson and Tustin, 1994: 1571).

2.5.1.2 Aseptic or non-specific subclinical mastitis.

Trauma or toxins usually cause aseptic or non-specific subclinical mastitis, and pathogenic microorganisms cannot be detected in the milk or quarter. Evidence of inflammation may or may not be detectable in the udder tissue. Inflammatory products are present in the secretion, and the milk has an increased somatic cell count (>500 000/ml). (Coetzer, Thomson and Tustin, 1994: 1571)

2.5.1.3 Infectious subclinical mastitis

In this case pathogens and the products of the inflammatory reaction can be detected in the milk by laboratory methods, but there are no macroscopic changes either in the milk or the udder tissue (Coetzer, Thomson and Tustin, 1994: 1571).

2.5.1.4 Teat canal infections

Teat canal colonisation by pathogenic microorganisms can be detected by samples collected through the teat canal. The milk may have a normal (<500 000cells/ml) or sometimes increased somatic cell count. (Coetzer, Thomson and Tustin, 1994: 1571)

2.5.1.5 Diagnosis of subclinical mastitis

Milk from a healthy, uninfected bovine mammary gland contains somatic cells comprising macrophages, neutrophils and lymphocytes. The number of these is usually <250 000cells/ml milk. A somatic cell count of between 250 000-500 000cells/ml of a composite milk sample could indicate an infected quarter, and a somatic cell count of over 500 000 cells/ml of a composite milk sample indicates infection in at least one quarter. (Andrews, *et al.* 1992: 309)

The somatic cell count in milk is a very sensitive indicator of udder health. Increased somatic cell count values signal udder disease, decreased milk production, changed composition and usefulness of milk, reduced hygienic quality (safety for human consumption) of milk, deficient management of udder health, and most importantly increased production costs and decreased profits for the farmer. (Giesecke, du Preez and Petzer, 1994: 111)

For optimum efficiency in modern herd management of udder health, obtaining somatic cell counts of herd milk is the most practical way presently available to farmers to monitor and control mastitis (Giesecke, du Preez and Petzer, 1994: 111).

Diagnosis of subclinical mastitis is mostly done by demonstrating certain changes in normal looking milk (Giesecke, *et al.* 1988: 21), through a range of tests that can be applied to the milk directly or indirectly to detect either the infecting organism or the cytological and biochemical changes to the secretion as a consequence to inflammation (Andrews, *et al.* 1992: 292).

2.5.1.5.1 Bacteriological examination

The secretion from a normal mammary gland is sterile, but may acquire bacteria from a colonised teat duct during collection of the sample.

Detection of pathogens in aseptically collected milk samples therefore indicates infection. After collection, samples should be tested with minimum delay. A volume of milk is plated out as a two- or three-way streak over the surface of an agar plate and is incubated for 24-48 hours at 37°C. Mastitogenic bacteria can be isolated and identified and subjected to antimicrobial sensitivity testing. (Andrews, *et al.* 1992: 292)

Bacteriologic examination of milk samples is the standard for establishing if an udder is infected. Drawbacks of bacteriologic examination for routine mastitis control procedures is that numbers of mastitogenic bacteria shed from infected cows are sometimes too low to culture with standard methods, the need for experienced milk samplers to take aseptic samples and the time and expense involved in sampling and laboratory procedures. (Van Horn and Wilcox, 1992: 443)

2.5.1.5.2 Cytological examination

This should be done in conjunction with bacteriological examination.

Indirect estimation:

The California milk cell test is based on a gelling reaction between the nucleic acid of the white blood and alveolar epithelial cells and a reagent containing a purple pH indicator dye. Reactions are categorized from 0 to 4. Reactions 3 and 4 have a high probability of infection being present. (Andrews, *et al.* 1992: 293) Gel formation (degree of reaction) is directly proportional to the somatic cell count (Searcy, Ryes and Guajardo, 1995).

Microscopic somatic cell count

The microscopic method is a direct visual method for determining the number of cells in milk, but is primarily a reference method (Van den Heever, Katz, Prinsloo, Giesecke, Rawlins and Jones, 1983: 1). The primary application of microscopic counting is the checking or calibration of other counting techniques (Andrews, *et al.* 1992: 306).

Electronic Particle counting

Two different principles can be used to count somatic cells in milk. The Coulter counter is an electronic particle counter while a Fossomatic detects somatic cells specifically by detecting light emitted from the cell nucleus once the DNA has been stained with a fluorescent dye.

With the aid of a Coulter counter it is possible to determine rapidly and accurately the number of particles over a certain size in a suspension.

Prior to the determination of the number of cells, the milk is treated as follows:

- 1 Cells are stabilized (fixed) to make them resistant to further treatments,
- 2 Milk samples to be examined are diluted with an electrolyte, and
- 3 The fat globules are dispersed to a size below the chosen Coulter counter threshold.

In the Coulter counter, the treated milk is passed through a 100 μ m aperture located between two electrodes. When a particle passes through the aperture, a small quantity of highly conductive liquid in the circuit is displaced by a particle of lower conductivity. The increased resistance raises the voltage, producing a voltage pulse proportional to the volume of the particle. The pulses are fed into a threshold circuit so that only those including and exceeding a particular threshold value are counted. (Andrews, *et al.* 1992: 307; and Van den Heever, *et al.* 1983: 3)

The Fossomatic instrument is an automatic microscope for counting cells in liquids. The cell nuclear DNA is stained with ethidium bromide and the sample is then exposed to blue light from a high-energy lamp, causing fluorescent light to be emitted at a characteristic wavelength. (Andrews, *et al.* 1992: 307; and Van den Heever, *et al.* 1983: 3) These fluorescent pulses are detected and recorded as the number of somatic cells per millilitre milk. (Van Niekerk, 1999).

2.5.1.5.3 Antibody detection in milk

A diagnostic test that detects antibodies in milk samples does not necessarily indicate the presence of bacteria in the udder. Recommendations for anti-mastitis actions cannot therefore be based on this principle. (Van Horn and Wilcox, 1992: 443)

2.5.1.5.4 Biochemical and other tests

These tests detect compositional changes in milk due to inflammation. Significant changes in ionic composition occur, with both sodium and chloride levels increasing and potassium decreasing. These changes affect the electrical conductivity of milk, which is usually increased in mastitic milk. The lactose concentration in mastitic milk is decreased from 48mg/ml to 44mg/ml. (Andrews, *et al.* 1992: 293)

Enzymatic changes also occur. Associated with an increased somatic cell count is an up to 20-fold increase in catalase. With damage to the secretory epithelium there is a 6-fold increase in *N*-acetyl glucose aminidase. Serum components such as antitrypsin and bovine serum albumin also leak into the mastitic gland. These can be detected and are indicators of inflammation. (Andrews, *et al.* 1992: 293)

2.5.2 Clinical mastitis

Clinical mastitis is most frequently infectious, and is classified according to its severity and rapidity of onset and duration into peracute, acute, subacute and chronic forms (Coetzer, Thomson and Tustin, 1994: 1571).

2.5.2.1 Peracute mastitis

Peracute mastitis is characterised by sudden onset and a severe, acute inflammatory reaction involving one or more quarters of the udder, and signs of systemic involvement such as fever, depression, shivering, anorexia, rapid weak pulse, dehydration, weakness and rapid loss of weight (Coetzer, Thomson and Tustin, 1994: 1571).

Milk secretion is severely affected, and may cease altogether with little milk present at milking. The affected quarter is distinctly swollen, hardened, painful and warm. (Giesecke, *et al.* 1988: 21) The milk is visibly abnormal and may be bloody, watery, purulent or fibrinous, and may contain clots. Peracute mastitis may lead to loss of a quarter or even death of the cow. (Coetzer, Thomson and Tustin, 1994: 1571)

2.5.2.2 Acute mastitis

Acute mastitis is characterised by an inflammatory reaction in the udder with swelling (hardening), warmth, reddening, pain (cow kicks when udder is touched) and reduced function (less milk, altered secretion) (Van Horn and Wilcox, 1992: 442). There is an accompanying mild fever, and the animal is slightly depressed. The milk is visibly abnormal, with a reduced milk yield. (Coetzer, Thomson and Tustin, 1994: 1572) The milk is seen to contain purulent floccules on the strip-cup, and the cow may kick during strip cup testing because of pain. Symptoms may rapidly develop within several hours to 1 to 2 days. (Giesecke *et al.* 1988: 21) In severe cases of acute clinical mastitis the somatic cell count values

frequently exceed 15-20-million cells/ml milk (Giesecke, du Preez and Petzer, 1994: 112).

2.5.2.3 Subacute mastitis

The signs of inflammation in the udder and the changes in the milk are milder than those present in acute mastitis (Coetzer, Thomson and Tustin, 1994: 1572). The cow shows no general symptoms of disease, and manifests normal behaviour and body temperature. Development of symptoms is gradual and takes several days. (Giesecke, *et al.* 1988: 21)

2.5.2.4 Chronic mastitis

The inflammatory process persists for months, or from one lactation to the next, with periodic relapses into subacute or acute forms. Affected quarters fail to respond to treatment and there is progressive fibrosis that leads to enlargement and asymmetry of the gland. (Coetzer, Thomson and Tustin, 1994: 1572) The milk may or may not be altered to become serum-like. No marked swelling and pain is evident on palpation, and the cow is generally healthy. (Giesecke, *et al.* 1988: 21)

2.6 Mastitis control

General strategies for mastitis control should include four key elements:

- 1- Regular monitoring of herd management of udder health by means of standard somatic cell count determinations in herd milk.
- 2- Preservation of normal udder health.
- 3- Elimination of existing cases of mastitis
- 4- Prevention of new cases of mastitis. (Giesecke, *et al.* 1988: 37)

2.6.1 Preventing new infections

“Mastitis is a managerial disease”

Good dairy management, parlour hygiene and milking procedure, and good maintenance of operation of the milking machine, are the main ingredients of successful mastitis control (South Africa. Department of Agriculture, 1995: 260).

Milking hygiene is the most important part of mastitis control because the teat skin is the critical starting point for all infections of the mammary

gland. In contagious mastitis hygiene of the milking procedure and the milking machine is critical. In environmental mastitis the disinfection of the teat skin and the cleanliness of the environment are additional high priorities. (Radostits, Blood and Gay, 1994: 606)

2.6.1.1 Udder washing and teat sanitization

At present the commonest procedure to prepare the cow for milking and to improve the milk quality is to wash the teats by hand and with running water, preferably warmed. Although not a highly rated procedure in mastitis control, the dilution of contamination on the skin appears to reduce environmental intramammary infection. Drying of the teats with individual paper towels before the cups are put on, and avoidance of wetting the hairs of the lower udder are vital parts of the pre-milking procedure. (Radostits, Blood and Gay, 1994: 606)

More recently introduced practices are cluster backflush between cows and pre-dipping of teats in sanitizing solutions with thorough drying off before attachment of milk unit clusters. Pre-dipping teats as well as the use of a wash sanitizer followed by manual drying with single-use paper towel, can reduce bacterial load on teat skin by 80%. (Van Horn and Wilcox, 1992: 445)

2.6.1.2 Teat stripping

Significant reduction in the new infection rate has been demonstrated if the teats are stripped (a little milk removed) before pre-milking washing, provided it is done in such a way that there is no chance of refluxing milk from the strip cup into the udder (Radostits, Blood and Gay, 1994: 606).

2.6.1.3 Teat dipping or spraying

Pre-milking

The disinfectant is applied just before milking, and the teats must be wiped clean of disinfectant before the cluster is attached (Blowey and Edmondson, 1995: 93) to avoid contamination of the milk. This technique reduces total bacterial count of the milk and the incidence of clinical mastitis, but has no effect on herd cell count or milk yield. (Radostits, Blood and Gay, 1994: 606)

Post-milking

There are three major reasons for carrying out post-milking teat disinfection:

- 1- removal of mastitis bacteria from the teat skin
- 2- removal of bacteria from teat sores
- 3- improving teat skin quality. (Blowey and Edmondson, 1995: 93)

The disinfectant is applied as soon as the milking unit is removed. The open teat canal sphincter has to be covered to protect the teat canal from infection after milking, before it closes properly. Teats do not need to be wiped dry after the post-dip has been applied. Post-milking teat disinfection is of a major importance in the control of contagious mastitis (Blowey and Edmondson 1995: 93), including infection with *Staphylococcus aureus* (Radostits, Blood and Gay, 1994: 606).

Teat cup disinfection/back-flushing

Both these procedures significantly reduce bacterial contamination of the teat cup liners, but this seems to have little effect on the infection rate. It is safe to eliminate it if mastitis prevalence is low, especially if teat dipping and dry-period treatments are carried out meticulously. (Radostits, Blood and Gay, 1994: 606)

2.6.1.4 Milking machine operation

The milking machine is an essential part of the dairy industry but it is assumed that its use, proper or improper, has been the principle factor in the increase of subclinical mastitis in recent years. Identifiable ways in which it can influence the new infection rate in the udder are:

- Carrying pathogens from one cow to the next. This may continue for up to six subsequent cows after the infected one
- Carrying infection from one quarter to another in the same cow by permitting reflux of milk from one infected quarter to another
- Traumatizing the teat end and thus predisposing the quarter to invasion by bacteria and causing mastitis
- Allowing the passage of pathogens past the teat canal by an impact mechanism, created by an abrupt loss off milking vacuum pressure. (Radostits, Blood and Gay, 1994: 606)

Other factors pertaining to the proper functioning of the milking machine and the effect it has on mastitis:

- Vacuum level
- Vacuum reserve
- Vacuum regulator function
- The pulsation system (rate and ratio)
- Liners and rubberware
- The wash-up routine. (Blowey and Edmondson, 1995: 66-67)

2.6.1.5 Milking order

Milking should take place in the following order: young cows should be milked first, and then the older cows followed by the known infected cows. Cows with clinical mastitis should be milked last. In very large herds separate strings of animals can be maintained for each of these categories. (Radostits, Blood and Gay, 1994: 606)

2.6.1.6 Stress

One of the most significant and prevalent predisposing factors to mastitis is stress. South African dairy cattle, particularly Friesian and Holstein breeds, frequently are subjected to poor nutrition and heat stress. These animals require carefully monitored feeding, sufficient drinking water and shade. (Giesecke, du Preez and Petzer, 1994: 76-77) Unless these requirements are met, cows would be less resilient toward acute stresses related to parturition, lactation, fluctuating temperatures and elevated humidity (Giesecke, *et al.* 1988: 13).

2.6.1.7 Additional factors affecting udder health and milk quality.

2.6.1.7.1 Stage of lactation and age of the cow

Clinical mastitis is most common at calving, in the first 5 weeks of lactation, and in the early dry period. The incidence of clinical mastitis increases with the age of the cow (Andrews, *et al.* 1992: 290). There is less effective healing of mammary lesions in older cows (Giesecke, *et al.* 1988: 16). Teat canal deficiency leads to easier penetration of the teat duct by pathogens (Andrews, *et al.* 1992: 290; and Giesecke, *et al.* 1988: 16).

2.6.1.7.2 Climate

Temperatures of above 30 °C or below freezing point may slightly increase the butterfat content of milk. Milk protein may be decreased by 10 – 20% at temperatures above 27 °C, and increased at temperatures below 0 °C. (South Africa. Department of Agriculture, 1995: 237-239)

Elevated humidity adds to the stress that predisposes to the development of mastitis (Giesecke, *et al.* 1988:13). Warm, wet conditions are favourable for environmental mastitogenic bacteria, like *Pseudomonas aeruginosa* and for *Streptococcus uberis* (Randles, 2000), as described in 2.4.1.3. Research done by Farrow (1997: Appendix 4) on the effect of homoeopathic nosode therapy as a prophylaxis for bovine mastitis shows an increase in the somatic cell counts recorded during the months for which the rainfall recorded was more than 100mm.

2.7 Treatment of mastitis

The main objective in the treatment of mastitis is to eliminate infection from the udder. Treatment can be administered at two different stages in the lactation cycle:

During lactation

Lactating cow therapy is administered while cows are in milk.

At the end of lactation

Dry cow therapy is aimed at removing any infection present in the udder at the end of lactation to prevent carry-over to the next lactation. It is also effective in reducing the number of new infections contracted during the dry period. (Blowey and Edmondson, 1995: 146)

Treatment administration routes:

- intramammary treatment is infused into the udder through the teat canal
- parenteral treatment is given by subcutaneous, intra-muscular or intravenous injection
- oral therapy (drenching) is given in liquid, powder, pill or bolus form by mouth. (Blowey and Edmondson, 1995: 146)

Irrespective of the cause of mastitis, there are several reasons why some form of therapy (not necessarily antibiotic) should be instigated:

- To restore productivity of the cow, thereby allowing her milk to be sold again as soon as possible.
- to prevent the mastitis from getting any worse.
- to prevent long-term and possibly irreversible udder damage, which would have a deleterious effect on yield and may affect milk quality.
- to prevent the spread of infection to other animals.
- to improve the overall health and hence welfare of the cow.

(Blowey and Edmondson, 1995: 146)

2.7.1 Treatment of mastitis in the lactating cow

2.7.1.1 Clinical mastitis

Clinical mastitis should be treated as soon as it is diagnosed (Bramley, 1981: 119). Intramammary treatment is the technique of choice for subclinical, or acute clinical mastitis, and is by far the most common method. Failure following intramammary antimicrobial therapy of peracute or acute clinical mastitis is due to poor or uneven drug distribution through the intensely swollen udder parenchyma, with the milk duct system being either compressed or blocked (Coetzer, Thomson and Tustin, 1994: 1576) by the process and products of inflammation, including pus and desquamated epithelial cells (Randles, 2000). Under these circumstances parenteral antimicrobial therapy is indicated (Coetzer, Thomson and Tustin, 1994: 1576).

In the presence of systemic symptoms, antibiotic therapy of acute clinical mastitis should consist of both parenteral and intramammary administration of the antibiotic agent used (Coetzer, Thomson and Tustin, 1994: 1579; and Smith, 1996:1191). Comparatively satisfactory results can be expected only if correct emergency treatment is given within the first six to eight hours after the onset of the udder infection directly responsible for the clinical mastitis (Giesecke, du Preez and Petzer, 1994: 62).

Although antibiotic sensitivity testing collects information of historical interest only to the clinical case in question, it provides a picture of the resistance patterns of the pathogens causing clinical mastitis in the herd. This could be of use in future cases and in the selection of a therapeutic agent at drying off. (Bramley, *et al.* 1980: 124)

The single most important factor for the successful treatment of clinical mastitis is the complete and almost continuous removal of secretion from the affected quarter/s. The more frequently such removal is repeated, the better the chances of rapid and successful therapy. (Smith, 1996: 1179) This observation has led to the formulation of enthusiastic statements such as the following: The quickest way to cure a cow of all udder ailments is to let a vigorous calf suckle her, making sure the calf feeds from the affected quarters. (de Bairacli Levy, 1988: 254)

Parenteral treatment (antibiotics and antimicrobials) is advisable in all cases of mastitis in which there is a marked systemic reaction (Radostits, Blood and Gay, 1994: 571). For clinical mastitis cases in which the cow is showing systemic signs as well, further treatments can include analgesics to combat pain, multivitamins to support liver function, cortisone for relief of swelling and inflammation, antihistamines to combat shock and inflammation, liquids administered orally to further combat shock, and heart stimulants and calcium replacements if necessary (Giesecke, du Preez and Petzer, 1994: 297).

2.7.1.2 Subclinical mastitis

As a rule, treatment of subclinical mastitis during lactation is indicated only when the dairy farmer is in danger of losing his milk market due to a high percentage of cows being affected (Coetzer, Thomson and Tustin, 1994: 1579). In general terms, subclinically infected quarters are best left untreated until the dry period to avoid discarding milk withheld from sale because of antibiotic content. If the infection in the herd is predominantly *Staphylococcus aureus*, the recovery rate for treatments of subclinical infections during lactation is so low that it is not recommended. (Radostits, Blood and Gay, 1994: 604)

2.7.2 Dry period treatment

Dry cow treatment is carried out at the end of the last milking when the cow is dried off. The objective is to treat any infection present in the udder at the end of lactation to prevent carry-over to the next lactation, with *Staphylococcus aureus* as the main target. Dry cow treatment also serves to protect against new infections that may occur during the dry period. (Radostits, Blood and Gay, 1994: 604)

The general recommendation is that all quarters of all cows be treated. This is undoubtedly the correct procedure when the infection rate is 40-50% of quarters, but in herds with a low prevalence of infection and low average cell count, no significant gain in production or profitability is achieved by blanket dry period treatment. (Radostits, Blood and Gay, 1994: 605)

Selective dry period therapy saves cost, especially when only 8-10% of quarters are actually infected, but untreated cows will be susceptible to new infections, especially at the beginning and end of this period. The available evidence strongly supports the treatment of all quarters. (Radostits, Blood and Gay, 1994: 605)

2.7.3 Supportive therapy

In addition to antibiotics, a wide range of supportive treatments has been suggested for different types of mastitis (Blowey and Edmondson, 1995: 154).

2.7.3.1 Fluid therapy

Toxins can induce a state of shock by vasodilatation, followed by a drop in blood pressure, with a consequent decrease in tissue perfusion. The animal appears dehydrated because the fluids are extravasated. Dehydration may frequently reach 7-10% of body weight. This means that for an average 600kg cow 40-60 litres of fluid need to be replaced to restore the circulation to normality. Large volumes of isotonic solutions to restore the circulating volume, or about 2 litres of a hypertonic solution can be infused intravenously to stimulate the thirst centres. Fluids can also be pumped into the rumen. Warm water and electrolyte solutions should always be available to the sick cow. (Blowey and Edmondson, 1995: 156)

2.7.3.2 Anti-inflammatory drugs

Shock can also be counteracted by the use of anti-inflammatory drugs such as cortisone, aspirin and phenylbutazone. Cortisone reduces the inflammatory response, but allows greater bacterial multiplication. (Blowey and Edmondson, 1995: 156)

2.7.3.3 Administration of Calcium

Some sick cows are naturally hypocalcaemic. Calcium is also said to aid detoxification of the liver. (Blowey and Edmondson, 1995: 156)

2.7.3.4 Administration of Glucose

Cows with acute *E. coli* mastitis can be hypoglycaemic, and may benefit from intravenous dextrose infusions (Blowey and Edmondson, 1995: 156).

2.7.3.5 Non-antibiotic Intramammary Infusions

Infusing natural live yoghurt into the udder can decrease the raised pH of mastitic milk and eliminate residual mastitic organisms by the probiotic effect of the natural live *Lactobacilli* (Blowey and Edmondson, 1995: 157).

2.7.3.6 Topical Preparations

Cai-Pan Japanese peppermint oil ('Uddermint') has been recommended for topical application. Stimulating warmth to the skin and leading to vasodilation, it produces a feeling of well being in the affected cow. (Blowey and Edmondson, 1995: 157)

2.7.3.7 Homoeopathy

At present homoeopathic treatment has the attraction of offering treatment without a milk withdrawal period. Homoeopathic preparations have also been used to prevent mastitis. Although there are plenty of anecdotal reports showing a benefit, specific trial work is lacking. Firm data will be needed before homoeopathic treatment for mastitis can be recommended. (Blowey and Edmondson, 1995: 157) Section 2.8 (Homoeopathy) is devoted to the discussion of the relevant homoeopathic principles and discusses the homoeopathic approach regarding the prophylaxis of bovine mastitis.

2.7.4 Vaccination

Attempts to reduce the incidence and severity of bovine mastitis through immunization with various antigens have been made throughout the last several decades, with little documented benefit for either the cow or the producer. For a mastitis immunization program to be of value to the dairy producer, the vaccine must do at least one of the following:

- eliminate chronic mammary gland infections
- prevent new intramammary infections
- reduce the incidence and severity of new intramammary infections.

(Smith, 1996: 1193)

All genera and species of gram-negative bacteria contain common gram-negative core antigens that are present within the deeper layers of the bacterial cell wall. One of these gram negative core antigens, endotoxin is thought to play an important role in the production of clinical signs, biochemical and haematologic alterations and pathologic lesions, which are associated with coliform mastitis. *E. coli*, *Klebsiella* spp., or *Enterobacter aerogenes* variously cause coliform mastitis. (Van Horn and Wilcox, 1992: 552)

In three different clinical trials, the incidence of clinical coliform mastitis in dairy cows was reduced 69, 72 and 80%, by vaccination with an *E. coli* vaccine (Van Horn and Wilcox 1992: 552). The use of a three-shot regimen for this immunogen has been consistently documented to be safe, efficacious, and cost effective (Smith, 1996: 1193).

Although inconsistent outcomes are reported, a variety of experimental and commercial vaccines have been brought to bear against *Staphylococcus aureus* mastitis (Smith, 1996: 1193). In a field trial with a staphylococcal bacterin-toxoid preparation, encouraging results were obtained. Fewer new infections, less culling and lower somatic cell counts were evident in vaccinated cows than in controls. (Andrews, *et al.* 1992: 345)

Macrophages from the non-lactating bovine gland phagocytose *Staphylococcus aureus* readily, but kill them very slowly. In normal glands there is a 24-hour lapse between entry of bacteria into the gland and accumulation of 500 000 polymorphonuclear leucocytes (PMN)/ml. This delay allows infection to become established. Vaccination can decrease this lag period to six hours. (Andrews, *et al.* 1992: 345)

2.7.5 Culling chronic cases

The maintenance of a high culling rate of senior cows with lumpy udders or a history of repeated attacks of mastitis is probably the most important tactic in herds where the prevalence of mastitis is high (Radostits, Blood and Gay, 1994: 606). A cow that has had mastitis on more than three occasions in the course of a lactation, or that has not responded to dry cow treatment, should be put on the cull list. If only one quarter is chronically affected with *Staphylococcus aureus*, that quarter can be culled. (South Africa. Department of Agriculture, 1995: 260)

2.7.6 Antibiotic contamination of milk

Food safety has become one of the most visible and emotional issues confronting affluent societies (Van Horn and Wilcox, 1992: 556). Antibiotic contamination of dairy and meat products poses a potential health risk to a small percentage of the population (Smith, 1996: 1192). A serious consequence of the use of antibiotics in treating mastitis is their effect on the manufacture of dairy products and the development of sensitivity syndromes in humans (Radostits, Blood and Gay, 1994: 573). Adverse reactions in people consuming residue-contaminated foods pertain mostly to penicillin-contaminated dairy products. Most victims have a prior history of penicillin allergy. (Van Horn and Wilcox, 1992: 559-560) Antibiotic contamination of the milk supply is of additional concern to creameries because antibiotic residues are capable of inhibiting the growth and activity of bacterial cultures used in the processing of many dairy products (Smith, 1996: 1192).

Unfortunately, data suggests that in South Africa antibiotic residues in milk supplies are still a considerable problem requiring increased attention as well as appropriate counter measures from health authorities, milk processors and in particular from dairy farmers (Giesecke, du Preez and Petzer, 1994: 56-59).

Specific on-farm residue avoidance programs aid in reducing health risks to the consumer (Smith, 1996: 1192). A total-quality management program (i.e. animal identification, records, understanding of drug usage, employee training and monitoring) is important in preventing violative drug residues (Musser and Anderson, 1999).

Veterinarians have the responsibility of warning farmers of the need to withhold milk and they should be aware of the withholding times of each

product, detail of which is usually required to be included on its label (Radostits, Blood and Gay, 1994: 573).

In South Africa it is an offence to sell milk polluted with the residues of remedies, disinfectants, cleaning agents and other chemicals (Giesecke, du Preez and Petzer, 1994: 56-59; and South Africa, 1977: 2).

2.7.7 Limitations of antibiotic therapy

The major shortcoming of conventional antibiotic therapy is bacterial resistance. In some instances mastitogenic bacteria inherit and acquire characteristics that make them resistant to antibiotic or antimicrobial drugs. For example, certain strains of the most common mastitogenic microorganism, *Staphylococcus aureus*, produce the enzyme beta-lactamase (penicillinase), which destroys penicillin and renders the bacteria resistant to this antibiotic. (Giesecke, du Preez and Petzer, 1994: 295)

Further limitation to this form of treatment includes poor penetration of certain antibiotics from the blood stream to the udder tissues. Others, like *streptomycin*, dissolve poorly in fats and are thus unable to move efficiently or in sufficient concentration through the tissues to reach the bacteria and destroy them. (Giesecke, du Preez and Petzer, 1994: 295) Lipid solubility and dissociation constants at different levels of pH affect the ability of a molecule to cross cell membranes, including capillary cell walls (Randles, 2000).

Certain antibiotics, furthermore, chelate with the magnesium and calcium in milk, and are thus rendered inactive. Others still have a very short half-life; if not administered repeatedly as prescribed, the duration of the therapeutic concentration required to kill the bacteria in the udder is too short and the treatment fails. (Giesecke, du Preez and Petzer, 1994: 295)

2.8 Homoeopathy

2.8.1 The Origins of Homoeopathy

Dr Samuel Hahnemann, a German physician and chemist, first discovered homoeopathy in 1790. He started by experimenting on himself by taking cinchona bark (quinine), half an ounce of the bark twice a day for several days and then noted the symptoms or effects produced in a healthy body.

By building up a picture called a 'proving', Hahnemann and his fellow workers continued this process of using progressively smaller doses with numerous different substances and twenty-five years later, the first *Materia Medica Pura* was published. He discovered that what a remedy can cause it could also cure by stimulating the self-healing process inherent in living organisms. This homoeopathic principle is expressed in Latin as *similia similibus curenter* - 'let like be treated by like'. (Hansford and Pinkus, 1998: 2) In practice, this means that a medicine capable of producing certain effects when taken by a healthy human being is capable of curing any illness that displays similar effects (Sankaran, 1992: 1).

2.8.2 Preparation of the Homoeopathic dose

Hahnemann observed that a medicine given in large doses caused side effects and aggravation. By extreme dilution the curative effect was increased without the aggravation. He also discovered that almost all poisonous materials were made safe after the 3CH potency. (Hansford and Pinkus, 1998: 3)

Homoeopathic remedies are all obtained from natural sources (Blowey and Edmondson, 1995: 157).

2.8.3 Potentisation

In order to make a remedy, dilution and succussion (vigorous shaking) is necessary, which results in potentisation. Centesimal potencies are produced by taking one drop of the mother tincture and mixing it with ninety nine drops of alcohol/distilled water and succussing forty to fifty times to produce a 1 in 100 dilution called a 1CH. A 2CH is a 1 in 10 000 dilution, a 3CH is a 1 in 1 000 000 dilution, a 30CH is a 1 in 10^{-60} dilution and so on. After a 12CH dilution, there is no trace of the original substance left. (Hansford and Pinkus, 1998: 3)

2.8.4 Nosodes

Nosodes are homoeopathic remedies prepared from bacteria, viruses or diseased tissue (Hansford and Pinkus, 1998: 3), having their own full, distinct drug picture (Gaier, 1991). There is no infective potential in these medicines, nor are they strictly speaking vaccines (with demonstrable antigenic material) (Day, 1992), although they may act in a similar way.

Homoeopathic vets often use nosodes to try and prevent the spread of some of the more common diseases (Hansford and Pinkus, 1998: 4), and also to prevent the development of specific disease syndromes (Day, 1995: 30).

With nosode therapy, more attention is paid to the causative agent than to the symptoms themselves. This enables treatment to be instituted on a wide scale that requires less time and energy from the veterinary practitioner. (Day, 1992) Nosode therapy seems to be reasonably effective, and provides an easy approach to farmers who experience difficulty in selecting the appropriate remedy for specific individual cases of mastitis (Hansford and Pinkus, 1998: 28). Section 2.8.7 considers previous trials in which homoeopathic remedies were used in the prophylaxis of bovine mastitis. Three of the four studies related administered nosodes as the prophylactic remedy.

2.8.5 The Single Remedy Approach

A single homoeopathic remedy is the best option (Day, 1995: 32). Day (1995: 27) suggested a treatment and prevention strategy that consists of treating the group holistically as if it were a single animal. Every symptom observed in the group is seen as symptom of the hypothetical individual. If this is done correctly, it should be possible to find a single remedy to perform the required task.

“It follows undeniably that the sum of all the symptoms and conditions in each individual case of disease must be the sole indication, the sole guide to direct us in the choice of the remedy” (Hahnemann, 1995: 106). “In no case under treatment is it necessary and therefore not permissible to administer to a patient more than one single, simple medicinal substance at a time” (Hahnemann, 1995: 296).

According to Macleod (1991), *Phytolacca decandra* is probably the best remedy for the treatment of mastitis. Most of the symptoms of *Phytolacca decandra* are in the glands (Morrison, 1993: 297), with special affinity for the breasts and glands of woman. It is indicated in mastitis and other breast disorders. (Murphy, 2000: 1360) The root is used to regulate any abnormality in the milk of cows, including scanty; thick; watery or curdy milk; and milk containing blood or pus. In breast indurations and abscesses of nursing woman and even in cancers, its action has been well confirmed. (Clarke, 1991: 804)

2.8.6 Method, route and frequency of remedy administration

The route of remedy administration needs to be suited to the facilities available and the class of animal being treated. Available routes are in-water, in-feed, aerosol and individual dosing. (Day, 1995: 31) Hansford and Pinkus (1998: 8 and 28) suggest using a spray applicator at milking time. To administer a dose, lift the tail and wipe away the faecal matter and spray two jets onto the mucous membrane of the vulva. Alternatively the remedy can be sprayed onto the nose. Care should be taken by the dispenser not to inhale the spray.

Remedy administration may need to be continuous, or at predetermined intervals in preventative medicine (Day, 1995:33).

2.8.7 Previous trials in homoeopathic prophylaxis of bovine mastitis.

Day (1986) did two pilot studies investigating the homoeopathic prevention of mastitis by means of nosode therapy. Study 1 was done on a herd of pedigree Friesian dairy cows that was randomly split into two groups of 41 cows each. Day was of the opinion that the milk yield, age or calving date could materially affect results obtained from this trial. Combined mastitis nosode 30CH was used for the treatment group. Both placebo and verum treatments were administered via the water troughs of the respective groups. Bulk milk samples were analysed and recorded, with favourable results. The incidence of mastitis cases in the placebo group was ten times greater than those recorded in the treatment group.

Study 2 was done on a problem herd of Friesian dairy cows. High-risk cows were separated from the low-risk ones according to their individual somatic cell counts, mastitis history, and age. Only the high-risk group (n = 50) received the combined mastitis nosode in their drinking water, with the low-risk group (n = 80) acting as a control. Before treatment, there were three times as many cases of mastitis in the high-risk group than in the low-risk group. After treatment the incidence of mastitis in the high-risk group decreased to twice as many as in the low-risk group, and then further reduced to 75% of that recorded in the low-risk group. For the first four months of this six month trial, the somatic cell counts gradually decreased from 1 259 000 to 788 000 until an upsurge in the last two months, which occurred in both groups.

Searcy, Ryes and Guajardo (1995) conducted a placebo-controlled trial in which 26 cows were divided into 2 groups of 13 animals each, both groups homogeneous according to age, monthly milk production and mastitic status. A homoeopathic combination of *Phytolacca decandra* 200CH (50%), *Phosphorus* 200CH (30%), and *Conium maculatum* 200CH (20%) was administered to the treatment group over a period of 30 days. The animals received liquid oral doses of 50ml each, starting with a dose every 48 hours for the first 2 weeks, after which they received twice weekly doses for 1 week and finally a single dosage in the last week. The California Milk Cell Test was used to evaluate the mastitic status of each udder. The results of the California Milk Cell Test were converted to a numerical equivalence scale for each animal in both groups. Of the functional quarters in the control group, 37 had a positive reaction to the test, against 17 quarters in the treated animals. The proportion of affected quarters according to the California Milk Cell Test was 32% in the treated group, and 68% in the control group. This study confirms the benefits that can be achieved by homoeopathic treatment in disease control in animals.

Farrow (1997) studied the prophylactic treatment of bovine mastitis using nosode therapy. A placebo controlled double-blind study was done. Of the 57 cows that completed the trial, 28 animals were in the treatment group and 29 in the placebo group. The nosode was produced from a sample of infected milk containing the pathogens *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae* and *Corynebacterium* species. A 30CH of this combination was produced and administered in 50ml liquid oral doses, once a month over the five-month study period. Two Sample T-tests and the Mann-Whitney test were used to compare each group, while the Wilcoxon's two-sample tests were used to determine whether animals within the same group improved as treatment progressed. No significant differences were noted between the 2 groups, based on the mean somatic cell counts and the incidence of clinical and subclinical mastitis.

Hansford and Pinkus (1998: 26-29) report positive observations concerning the effectiveness of nosode therapy as a homoeopathic prophylactic method against mastitis. These reports show records of three dairies where nosodes were regularly administered to all cows in the dairy over varying periods of time. The three dairies mentioned show lasting decreases in measured somatic cell counts over time, but the evidence is not beyond question, as there were no placebo or control groups.

Chapter 3 Materials and methods

3.1 Study design and protocol

This was a placebo controlled double-blind study. Two different medicines and a placebo were obtained from Natura Homoeopathic Laboratory and were randomised and colour coded by an independent Homoeopath. Three groups of cows were each marked with enamel paint corresponding with the colour codes of the randomised medicine and placebo. This ensured accurate dispensing throughout the duration of the trial.

The three groups of cows were on the same farm for the duration of the trial. This ensured that they were exposed to the same daily routine, diet, milking procedures, environmental conditions and stressors. Therefore any significant differences in the measurements between the respective groups will be attributable to the action of the medicine only.

3.1.1 Sample selection

A well-managed herd of lactating Jersey cows in the Underberg district of KwaZulu-Natal was used for this study with the written consent of the owner (Appendix 1).

The initial sample size consisted of 252 lactating cows, 84 in each of the three respective groups. The cows were ranked according to age (date of birth). The treatments were numbered 1, 2 and 3. A random number between 1 and 3 was generated using the Microsoft Excel function "Randbetween (bottom, top)" where bottom = 1 and top = 3. This function returns a random integer between the upper and lower limit each time the worksheet is recalculated. The first cow on the list was allocated this treatment without replacement. The procedure was repeated for the second cow. The third cow was allocated the remaining treatment. This sequence of randomly allocated treatments was applied repetitively to the remainder of the cows to ensure an even spread of treatments across all age classes and lactation number. This approach is advantageous in short-term trials in that significant differences in trial results between the groups can be identified quickly (Searcy, Ryes and Guajardo, 1995).

According to the criteria governing the admissibility of data, only 191 cows completed the trial. Nine cows lost production from one quarter of the udder, 5 died, and 26 cows were dried off before the last milk recording. Twenty cows missed one of the 4 milk recordings, rendering data gathered from them inadmissible. There was a loss of 17, 19 and 25 cows from the *Phytolacca decandra* 200CH, *Phytolacca decandra* 12CH and placebo treatments respectively, a total of 61 animals.

3.1.2 Exclusion and inclusion criteria

Selection criteria for cows to be included in this trial were as follows:

Inclusion criteria:

- All lactating cows, with all four quarters functional,
- Cows contracting any form of mastitis during the course of the trial and treated according to traditional veterinary methods (antibiotic treatment),
- Cows receiving antibiotic therapy at the commencement of the trial.

Exclusion criteria:

- Dry cows or any other cows not present in the milking herd for the duration of the study, or for any other reason,
- Cows not productive in all four quarters of the udder.

3.2 Intervention: The medicine and the placebo

Phytolacca decandra 12CH and *Phytolacca decandra* 200CH and the placebo were prepared, in granule form, by Natura Homoeopathic Laboratory. These centesimal potencies were prepared according to the French Homoeopathic Pharmacopoeia, and by Hahnemann's bottle-by-bottle method. (Stoffberg, 2000) The mother tincture was prepared from the whole plant in a 45% V/V ethanolic solution (French Homoeopathic Pharmacopoeia, 1989). One part mother tincture was diluted in 99 parts per volume of a 43% ethanolic solution and was succussed 100 times with a Dynamat 5 C potentising machine. This is the 1CH potency. Of the 1CH solution, one part was diluted in 99 parts per volume of a 20%

ethanolic solution and succussed 100 times. This is the 2CH potency, and this process was repeated up to the 10CH potency. The 11CH and 12CH potencies were prepared using 96% alcohol as diluent, which facilitates the impregnation of the granules in the 12CH potency. One part of the 12CH potency was diluted per volume in 99 parts of 20% alcohol and succussed 100 times by machine to give the 13CH potency. The process is repeated, bottle-by-bottle in a 1:100 ratio of dilution and succussed 100 times by machine at every step until the 198CH potency. For 198CH, 199CH and 200CH, 96% alcohol is used as the diluent to facilitate the impregnation of the granules with the 200CH potency. (Stoffberg, 2000)

The potencies and the placebo were packaged and sealed in durable plastic bags, colour coded according to the randomisation and dispensed to the researcher in quantities of 1 litre of granules per bag.

3.2.1 Dosage and route of administration

Individual in-feed dosing was used as it provided the most practical means of dispensing for the research setting, without incurring undue duress upon the cattle (cf. 2.8.6 Method, route and frequency of remedy administration).

Dispensing took place after milking while the cows were being fed, and was done by the person responsible for the feeding. Five millilitres of medicated or placebo granules were dispensed into the dry feed by matching the colour code on the container of placebo or verum to that of the cow and then sprinkling the granules over the dry food with a 5 ml measuring spoon. Each container had its own spoon to prevent cross-contamination of the potencies. This method ensured easy identification and accurate dispensing.

3.2.2 Treatment regimen

The duration of this trial was 100 days. Treatment commenced the day after the first milk recording. *Phytolacca decandra* 12CH, *Phytolacca decandra* 200CH and the placebo were administered twice weekly (on days 1 and 4) to the respective predetermined groups for the first 35 days. For the last 65 days of the trial the remedies were administered on the first day of the seven-day week.

3.3 Primary data

3.3.1 Milk sampling

Composite milk samples from each cow were collected on the farm every five weeks during the afternoon milking by the dairy manager and the researcher. The samples were collected from the recording jar and thus contained the total volume of milk from all four quarters of the udder. The samples were analysed at the Taurus Central Laboratory in Irene where the somatic cell counts were determined using a Fossomatic somatic cell counter. Fossomatic instruments are designed to detect somatic cells in milk. The DNA in each cell is dyed with ethidium bromide. Blue light causes the dye to fluoresce. A thin film of milk and ethidium bromide on the flat rim of a rotating disk passes a detector. Fluorescence of each cell nucleus is detected and recorded as an electronic pulse and thus the number of somatic cells per millilitre milk is measured. (Van Niekerk, 1999)

Milk samples were collected on four occasions, with the first batch being collected the day before the commencement of the trial (Day -1), the second after 35 days (Day 35), the third after 70 days (Day 70), and the fourth on the last day of the 100-day trial (Day 100).

3.3.2 Recording of acute cases of mastitis

All lactating cows are handled and inspected by the dairy manager at each milking, morning and evening. Acute clinical mastitis was diagnosed when purulent floccules were visible on the strip-cup. Other signs and symptoms indicating an acute inflammatory reaction in the udder, like swelling (hardening), warmth, reddening, and pain (cow kicks when udder is touched) were also used as criteria for the diagnosis of acute clinical mastitis.

The ear tag numbers and affected quarters of any cow with clinical mastitis are noted, as are the intra-mammary and/or parenteral treatment of these cows. One case is one quarter affected at one time. Other disease conditions and their treatments were also accurately recorded.

3.4 Statistical Analysis

The somatic cell counts, protein, butterfat, and lactose content of the milk of all cows tested on each of the four occasions were tabulated together with the observed cases of clinical mastitis over this period.

The Repeated Measures Multi-factorial Analysis of Variance method was used for analysis of the somatic cell count, protein, butterfat, and lactose values for all three groups. Where significant differences were found, the Bonferroni test was used to identify between which groups or recordings they occurred.

The data gathered from the recording of acute cases of clinical mastitis was processed and expressed as a percentage of the relevant group, and as the incidence per 100 cows per year within the group.

The statistical package SPSS was used for data entry and analysis.

Chapter 4 Results

4.1 Criteria governing admissibility of data

- Only data obtained from milk samples collected on the farm by the dairy manager and the researcher was used.
- Only data obtained from milk samples collected from the cows in the selected groups, and on the 4 specified milk recording days was used.
- Only data obtained from the results of milk analysis by Taurus Central Milk Laboratory was used.
- Only data obtained from the observations and records of the dairy manager about the incidence of acute clinical mastitis was used.

4.2 Analysis of the incidence of acute clinical mastitis

This is best expressed as a percentage and as the number of cases recorded per 100 cows per annum.

Phytolacca decandra 12CH-group

This group consisted of 66 cows after selection criteria were applied. Seven cases of acute clinical mastitis were recorded in the group receiving *Phytolacca decandra* 12CH as prophylaxis. The incidence of acute cases of clinical mastitis for the 100-day period was 10.606% or 38 cases per 100 cows per annum.

Phytolacca decandra 200CH-group

This group consisted of 67 cows after selection criteria were applied. Three cases were recorded in the group receiving *Phytolacca decandra* 200CH as prophylaxis. The incidence of acute cases of clinical mastitis for the 100-day period was 4.478% or 16.344 cases per 100 cows per annum.

Placebo-group

This group consisted of 59 cows after the selection criteria were applied. Eleven cases were recorded in the group receiving the Placebo. The incidence of acute cases of clinical mastitis for the 100-day period was 18.644% or 68.051 cases per 100 cows per annum.

Graph 4.1 presents the incidence of acute cases of clinical mastitis graphically.

Table 4.1 Comparison of the incidence of acute cases of clinical mastitis in Groups 1, 2 and 3

<i>Phytolacca decandra</i> 12CH group	38.712 per 100 cows per annum
<i>Phytolacca decandra</i> 200CH group	16.344 per 100 cows per annum
Placebo group	68.051 per 100 cows per annum

4.3 Statistical analysis of the butterfat, total protein and lactose levels, and somatic cell counts between the *Phytolacca decandra* 12CH treatment group, the *Phytolacca decandra* 200CH treatment group and Placebo group

The sample size of the study is large enough to warrant the use of the Multifactorial Analysis of Variance (ANOVA) method of data analysis. The data gathered from the trial was analysed using the Parametric ANOVA method. This method tested for significant differences in the same group between the four different milk recordings, and between the three groups for the same milk recording. Where significant differences were found, the Bonferroni test was used to identify between which groups or recordings they occurred. The null hypothesis (assuming no statistically significant change) was rejected at the 5% level of significance if the observed p-value was less than or equal to 0.05 (i.e. $p \leq 0.05$).

4.3.1 Day -1: Oneway ANOVA analysis of butterfat, total protein, and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Table 4.2 Day -1 (baseline levels): Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Measurement	Level of significance
Butterfat	0.987
Protein (total)	0.366
Lactose	0.752
Somatic cell count	0.275

The null hypothesis cannot be rejected, because none of the above values are significant at the $\alpha = 0.05$ level. Table 4.2 indicates that there was no statistically significant difference between the baseline

measurements obtained from the three groups of cows at the beginning of the trial. Graphs 4.2 and 4.3 illustrate the measurements for Day -1 graphically.

4.3.2 Day 35: Oneway ANOVA analysis of butterfat, total protein, and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Table 4.3 *Day 35: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts between Groups 1, 2 and 3*

Measurement	Level of significance
Butterfat	0.887
Protein (total)	0.059
Lactose	0.326
Somatic cell count	0.179

Table 4.3 indicates that there was no statistically significant difference between the data gathered from the three groups of cows at the $\alpha = 0.05$ level of significance on day 35 of the trial.

At the $\alpha = 0.06$ level of significance, the null hypothesis can be rejected for protein. A Bonferroni test was done which indicated that a significant difference between protein levels occurred between the *Phytolacca decandra* 200CH treatment group and the Placebo group, with the level of significance of the outcome as 0.055. Graphs 4.4 and 4.5 illustrate the measurements for Day 35 graphically.

4.3.3 Day 70: Oneway ANOVA analysis of butterfat, total protein, and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Table 4.4 *Day 70: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts between Groups 1, 2 and 3*

Measurement	Level of significance
Butterfat	0.997
Protein (total)	0.367
Lactose	0.354
Somatic cell count	0.186

Table 4.4 indicates that there was no statistically significant difference between the data gathered from the three groups of cows at the $\alpha = 0.05$ level of significance on day 70 of the trial. Graphs 4.6 and 4.7 illustrate the measurements for Day 70 graphically.

4.3.4 Day 100: Oneway ANOVA analysis of butterfat, total protein, and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Table 4.5 Day 100: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts between Groups 1, 2 and 3

Measurements	Level of significance
Butterfat	0.423
Protein (total)	0.531
Lactose	0.791
Somatic cell count	0.114

The results in Table 4.5 are insignificant at the $\alpha = 0.05$ level of significance, and the null hypothesis cannot be discarded for the alternative hypothesis. There is therefore no statistically significant difference between the treatment groups and the placebo in this instance. Graphs 4.8 and 4.9 illustrate the measurements for Day 100 graphically.

4.4 Statistical analysis of the butterfat, total protein and lactose levels, and somatic cell counts within the *Phytolacca decandra* 12CH treatment group, the *Phytolacca decandra* 200CH treatment group and the Placebo group

4.4.1 *Phytolacca decandra* 12CH treatment group: Oneway ANOVA and Bonferroni analysis of the butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100

Table 4.6 *Phytolacca decandra* 12CH treatment group: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100

Measurement	Level of significance
Butterfat	0.015
Protein	0.000
Lactose	0.200
Somatic cell count	0.665

The results for the lactose levels and the somatic cell counts are not below the $\alpha = 0.05$ level of significance, and the null hypothesis cannot be rejected for these measurements.

The result of the analysis of the butterfat for the *Phytolacca decandra* 12CH treatment group is below the $\alpha = 0.05$ level of significance, indicating a statistically significant difference between one or more of the measurements. The Bonferroni test established that there is a significant difference between the measurements taken on Day 35 and Day 100, and between Day 70 and Day 100 for butterfat.

The result of the analysis of the protein for the *Phytolacca decandra* 12CH treatment group is below the level of significance, indicating a statistically significant difference between one or more of the measurements. The Bonferroni test established that significant differences in the protein levels occurred between the measurements taken on Day -1 and Day 70, between Day -1 and Day 100 and also between Day 35 and Day 100. Graphs 4.10 and 4.11 illustrate the measurements for the *Phytolacca decandra* 12 CH treatment group graphically.

4.4.2 *Phytolacca decandra* 200CH treatment group: Oneway ANOVA and Bonferroni analysis of butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100

Table 4.7 *Phytolacca decandra* 200CH treatment group: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100

Measurement	Level of significance
Butterfat	0.225
Protein (total)	0.000
Lactose	0.139
Somatic cell count	0.590

There is no statistically significant difference between the measurements taken on Days -1, 35, 70 or 100 for the butterfat and lactose levels and the somatic cell counts for the *Phytolacca decandra* 200CH treatment group.

The result of the analysis of the protein levels for the *Phytolacca decandra* 200CH treatment group is below the level of significance, indicating a statistically significant difference between one or more of the measurements. The Bonferroni test done determined that the significant differences in protein levels occurred between the values determined for Day -1 and Day 70, between Day -1 and Day 100 and also between Day 35 and Day 100. Graphs 4.12 and 4.13 illustrate the measurements for the *Phytolacca decandra* 200CH treatment group graphically.

4.4.3 Placebo group: Oneway ANOVA and Bonferroni analysis of butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100

Table 4.8 *Placebo group: Results of Oneway ANOVA analysis of butterfat, total protein and lactose levels, and somatic cell counts on Days -1, 35, 70 and 100*

Measurements	Level of significance
Butterfat	0.042
Protein (total)	0.000
Lactose	0.854
Somatic cell count	0.622

The null hypothesis is valid for the lactose levels and the somatic cell count at the $\alpha = 0.05$ level of significance.

The results of the ANOVA analysis for the butterfat levels was less than the $\alpha = 0.05$ level of significance, making it statistically significant. The null hypothesis is rejected in favour of the alternative hypothesis. The Bonferroni test done to identify between which milk recordings the difference was observed, indicated that there were significant differences between measurements taken on Day -1 and Day 35, and on Day 70 and Day 100. These differences can be observed in Graphs 4.14 and 4.16.

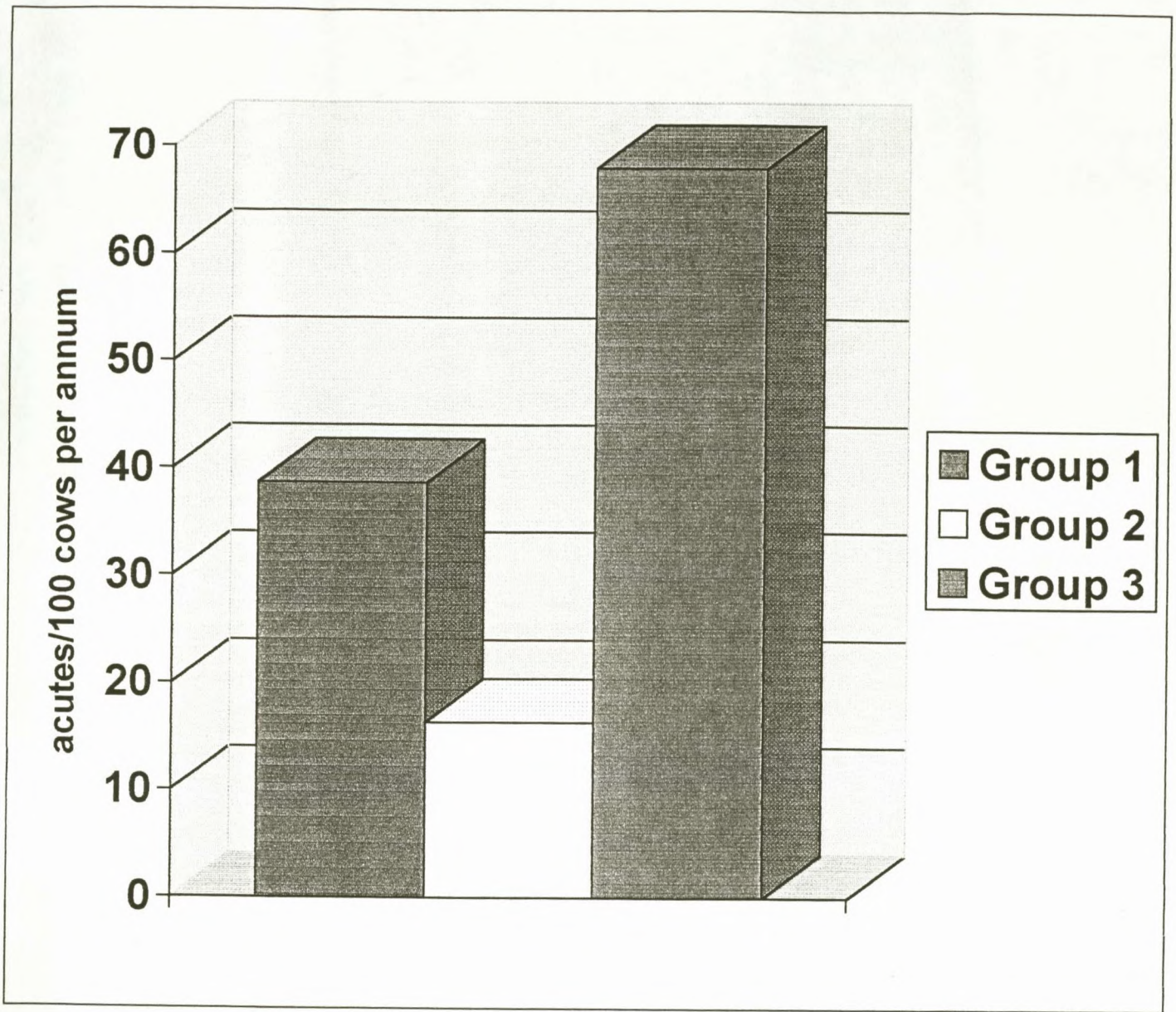
The result of the analysis of the protein for the Placebo group is below the $\alpha = 0.05$ level of significance, indicating a statistically significant difference between one or more of the measurements. The Bonferroni test done determined that the significant differences occurred between measurements taken on Day -1 and Day 100.

Table 4.9 *Explanation of the legend for Graphs 4.1 - 4.9*

Group 1	<i>Phytolacca decandra</i> 12CH treatment group
Group 2	<i>Phytolacca decandra</i> 200CH treatment group
Group 3	Placebo group

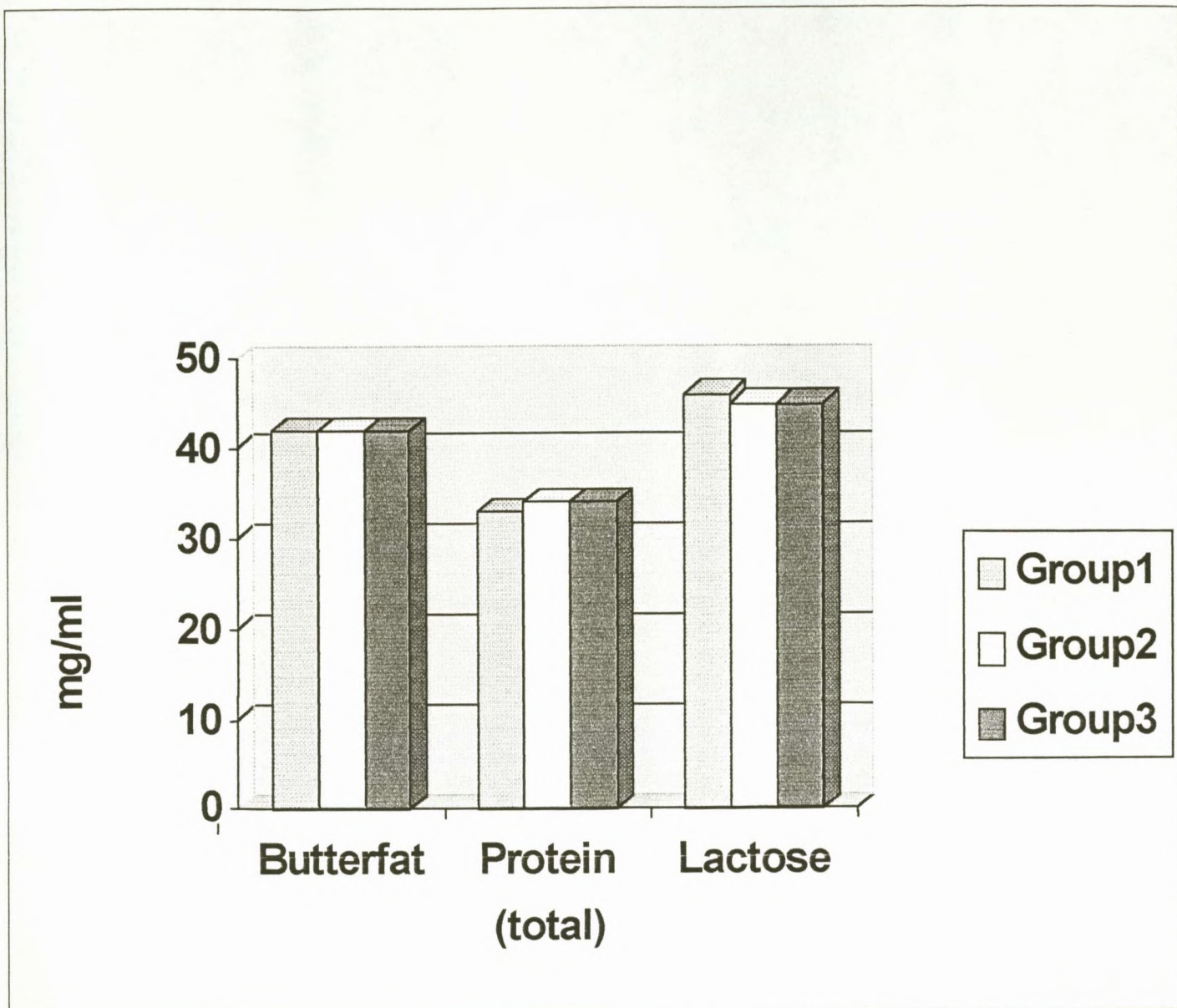
Table 4.10 *Explanation of the legend for Graphs 4.10 - 4.19*

Day -1	Milk recording 1
Day 35	Milk recording 2
Day 70	Milk recording 3
Day 100	Milk recording 4



Graph 4.1 Incidence of acute cases of clinical mastitis per 100 cows per annum in Groups 1, 2 and 3 from Day -1 to 100

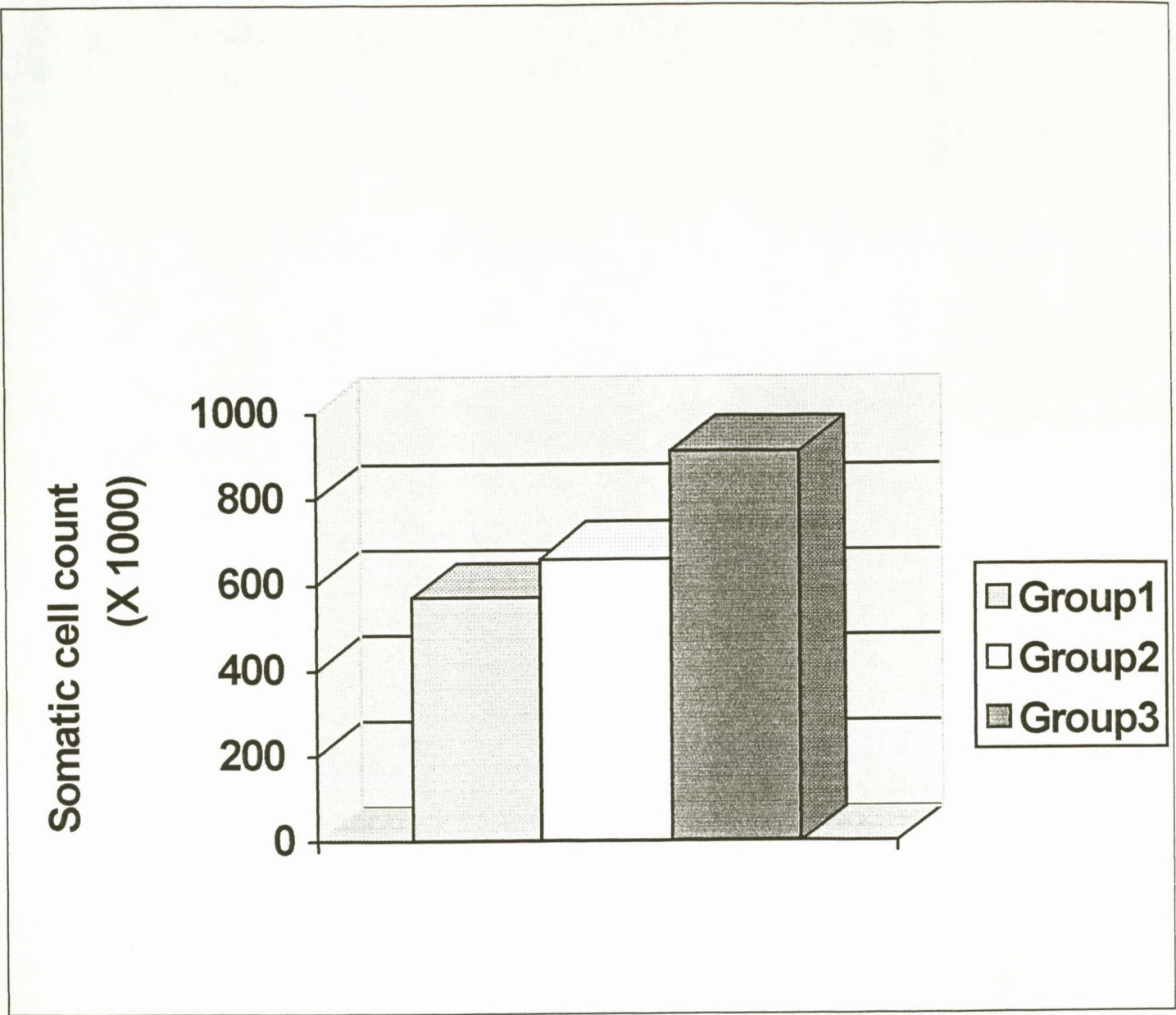
Consult Table 4.1 for the values of the incidence of acute cases of clinical mastitis.



Graph 4.2 Day -1: Average butterfat, total protein and lactose levels in Groups 1, 2 and 3

Table 4.11 Day -1: Average butterfat, total protein and lactose level values in Groups 1, 2 and 3 in mg/ml

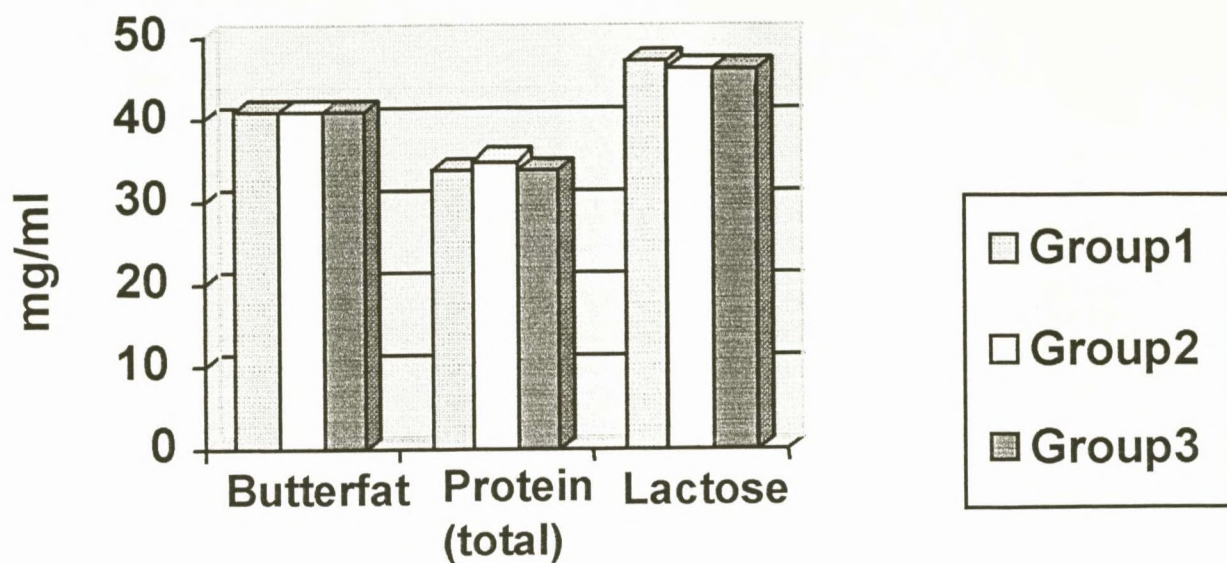
	Butterfat	Protein (total)	Lactose
Group 1	42.2	33.3	45.9
Group 2	42.1	34.1	45.3
Group 3	42.3	33.6	45.4



Graph 4.3 – 1: Average somatic cell count in Groups 1, 2 and 3

Table 4.12 Day -1: Average somatic cell count values in Groups 1, 2 and 3 in thousands/ml

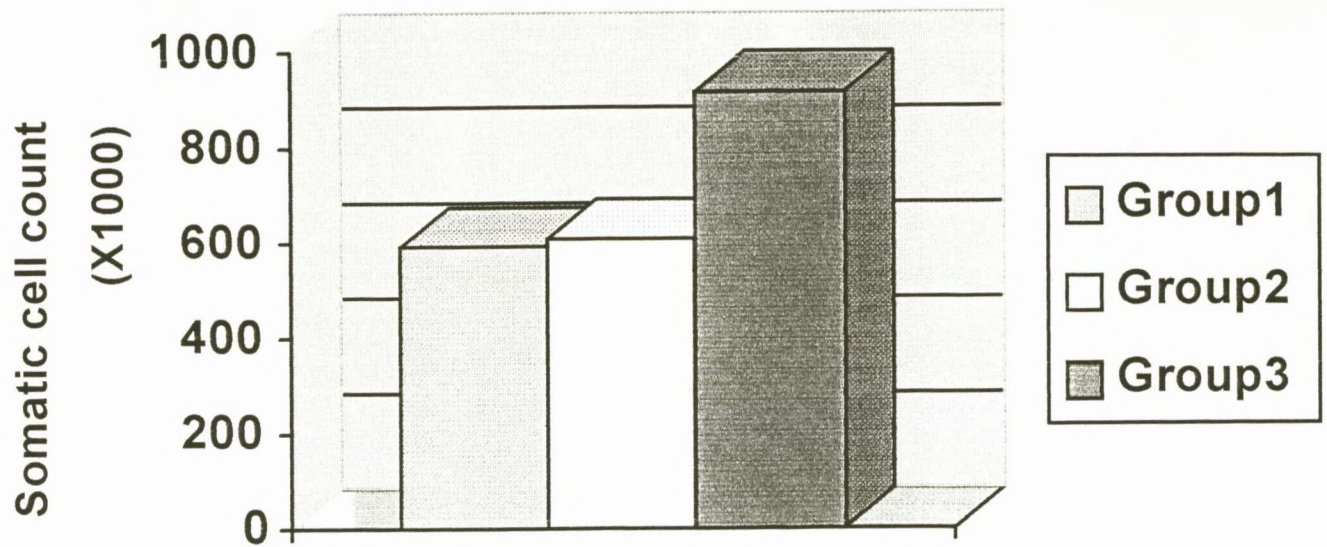
	Somatic cell count
Group 1	568.49
Group 2	663.57
Group 3	908.49



Graph 4.4 Day 35: Average butterfat, total protein and lactose levels in Groups 1, 2 and 3

Table 4.13 Day 35: Average butterfat, total protein and lactose level values in Groups 1, 2 and 3

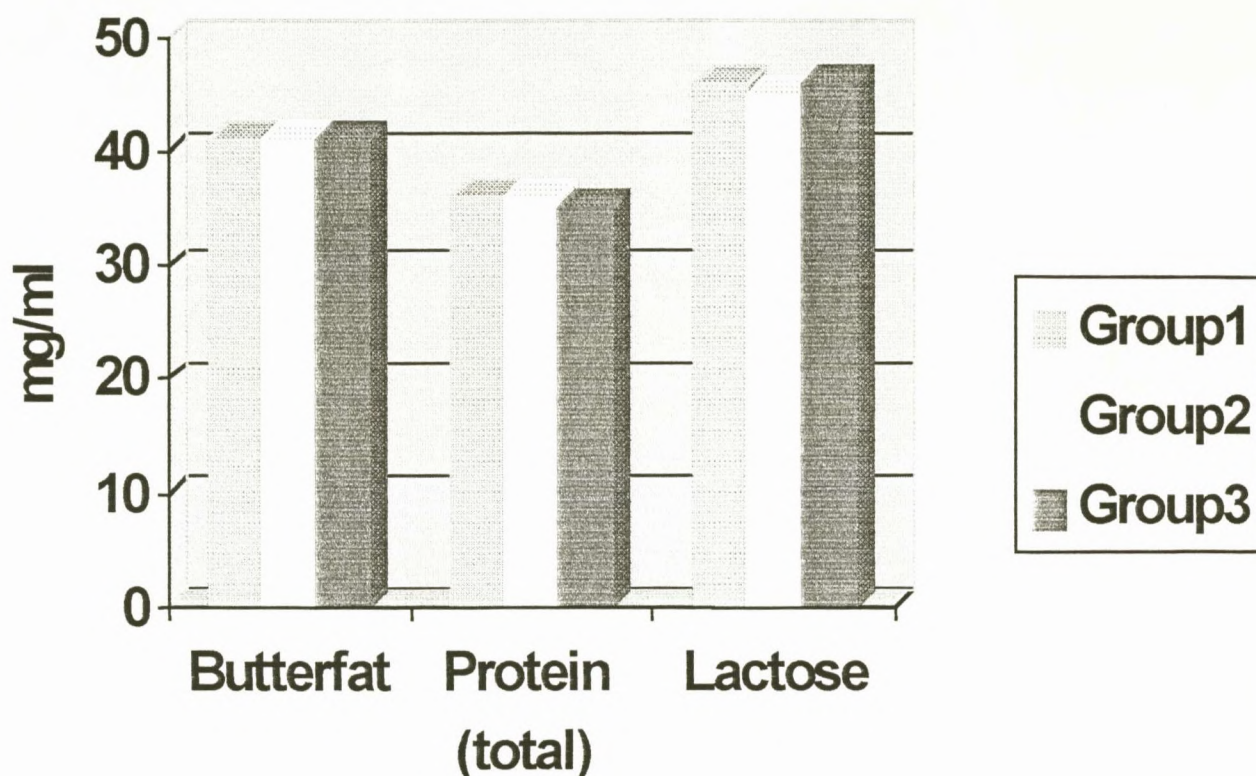
	Butterfat	Protein (total)	Lactose
Group 1	41.8	34.3	46.7
Group 2	41.2	35.4	46.4
Group 3	42.1	34.1	46.3



Graph 4.5 Day 35: Average somatic cell counts in Groups 1, 2 and 3

Table 4.14 Day 35: Average somatic cell count level values in Groups 1, 2 and 3 in thousands/ml

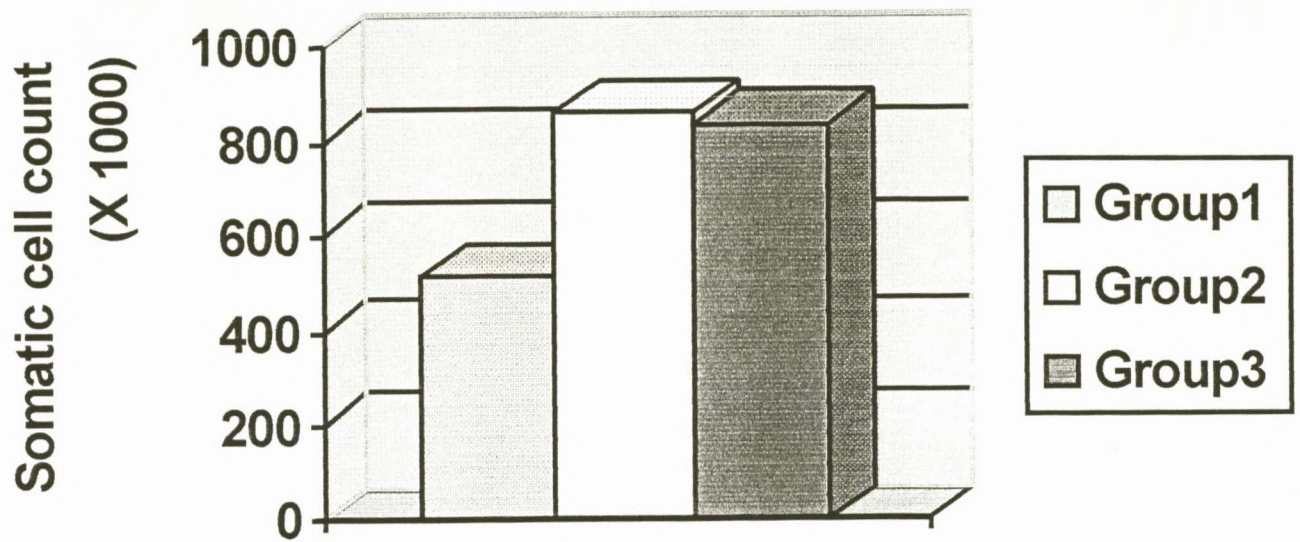
	Somatic cell counts
Group 1	594.71
Group 2	605.16
Group 3	917.70



Graph 4.6 Day 70: Average butterfat, total protein and lactose levels in Groups 1, 2 and 3

Table 4.15 Day 70: Average butterfat, total protein and lactose level values in Groups 1, 2 and 3 in mg/ml

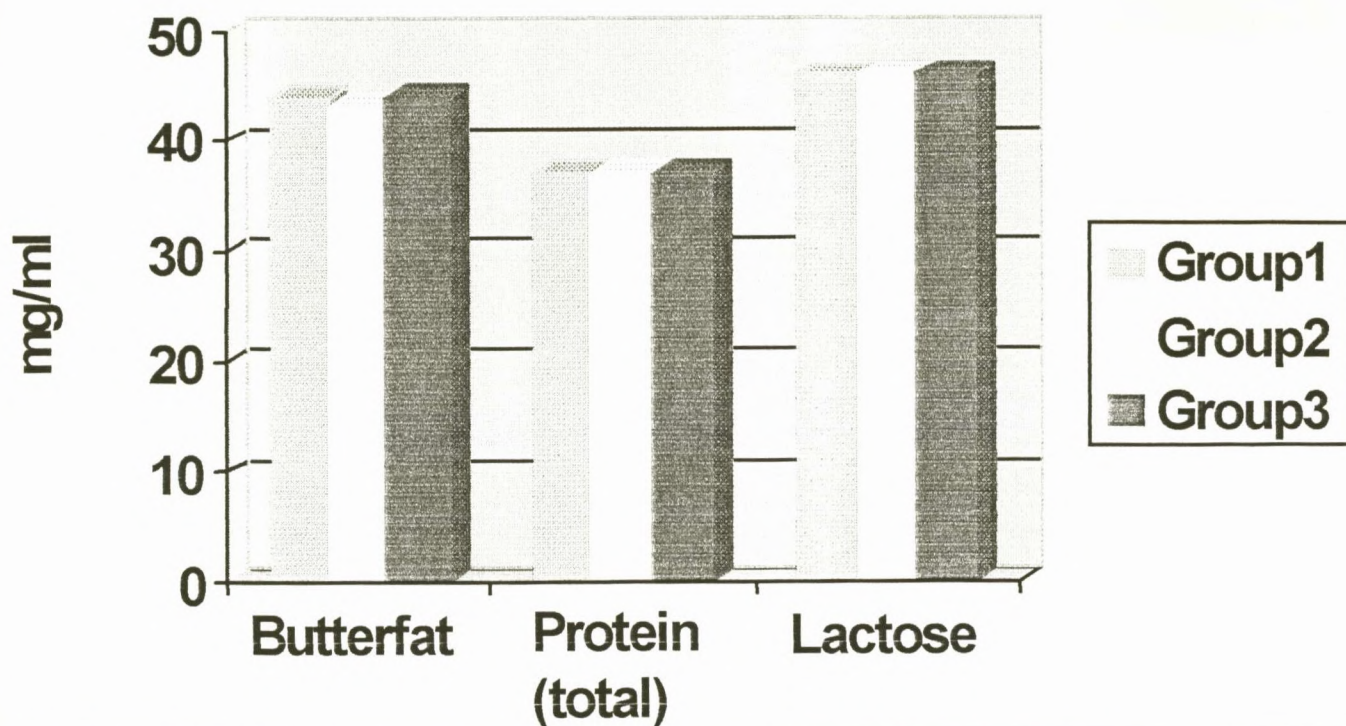
	Butterfat	Protein (total)	Lactose
Group 1	40.8	35.9	45.8
Group 2	40.8	36.0	45.2
Group 3	40.8	35.3	46.1



Graph 4.7 Day 70: Average somatic cell counts in Groups 1, 2 and 3

Table 4.16 Day 70: Average somatic cell count values in Groups 1, 2 and 3 in thousands/ml

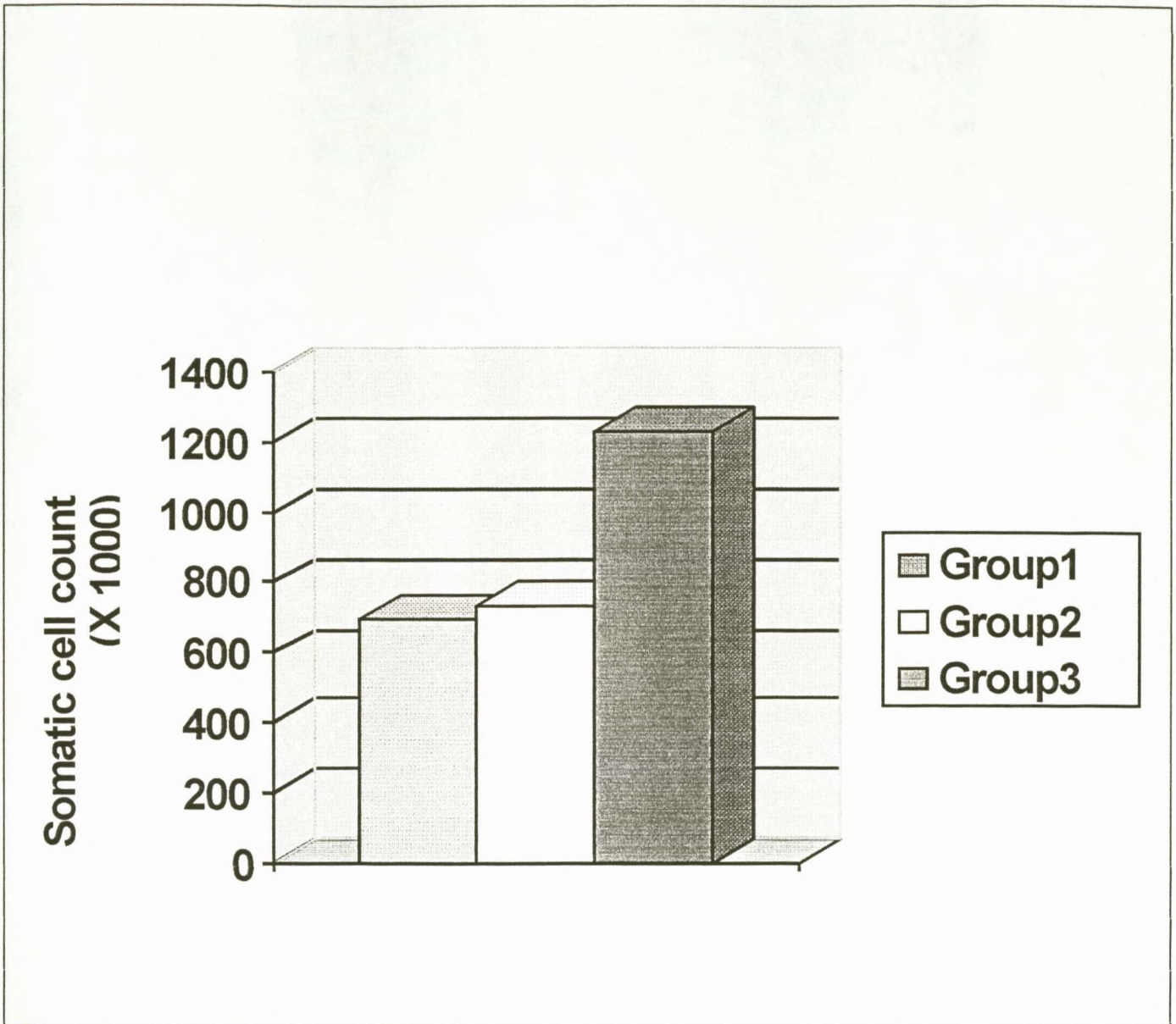
	Somatic cell count
Group 1	515.48
Group 2	856.71
Group 3	835.91



Graph 4.8 Day 100: Average butterfat, total protein and lactose levels in Groups 1, 2 and 3

Table 4.17 Day 100: Average butterfat, total protein and lactose level values in Groups 1, 2 and 3

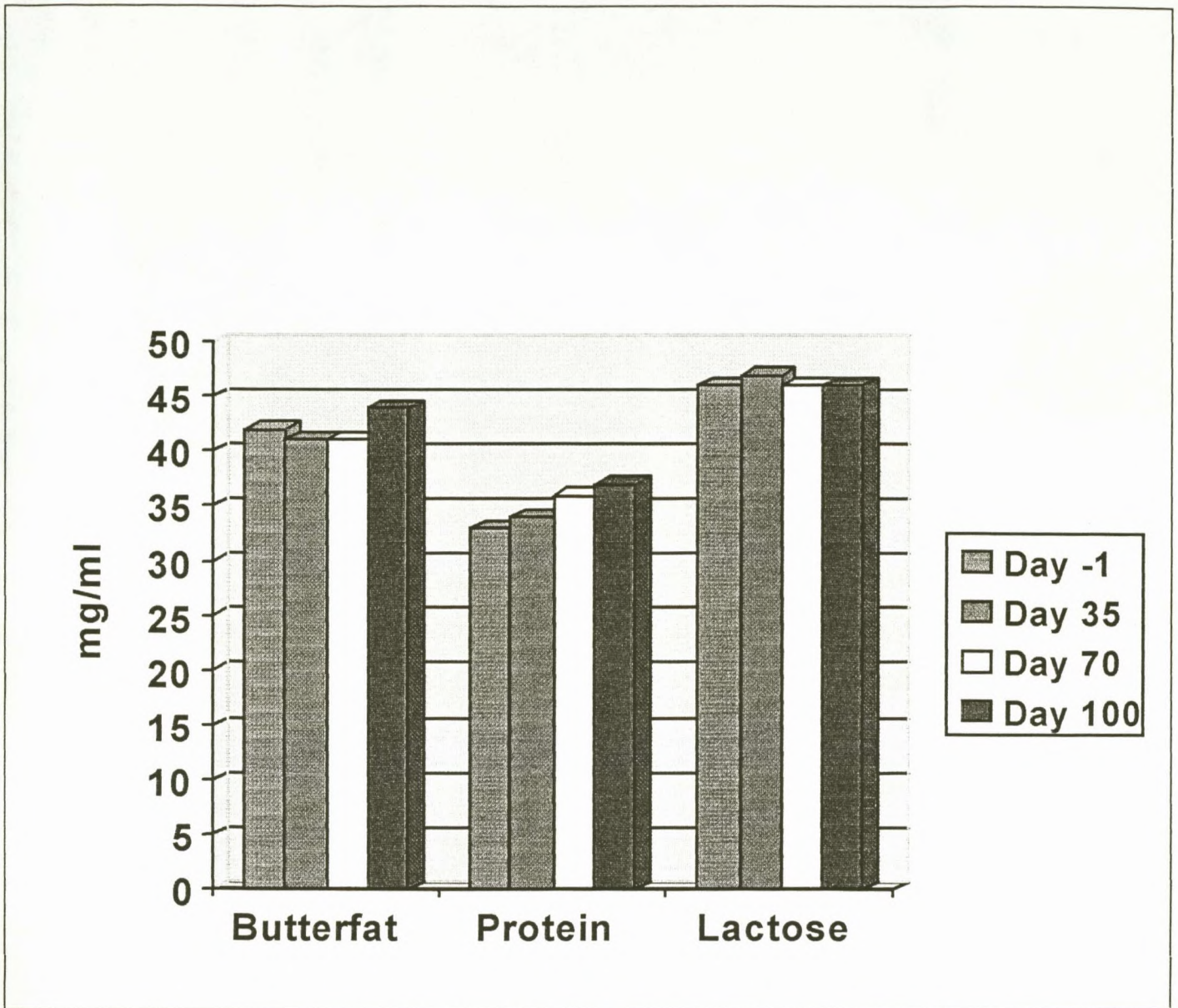
	Butterfat	Protein (total)	Lactose
Group 1	43.8	37.0	46.2
Group 2	42.8	37.3	45.8
Group 3	44.0	36.7	46.0



Graph 4.9 Day 100: Average somatic cell counts in Group 1, 2 and 3

Table 4.18 Day 100: Average somatic cell count values in Group 1, 2 and 3 in thousands/ml

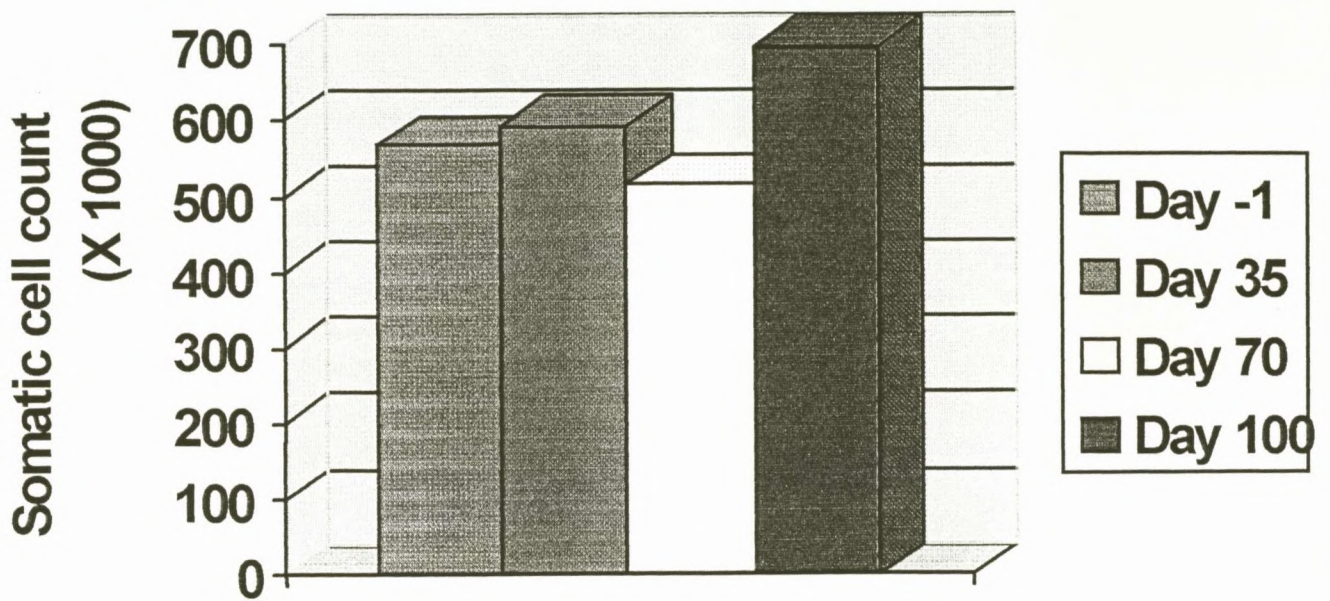
	Somatic cell count
Group 1	698.78
Group 2	733.51
Group 3	1228.85



Graph 4.10 Group 1: Average butterfat, total protein and lactose levels for Days -1, 35, 70 and 100

Table 4.19 Group 1: Average butterfat, total protein and lactose level values for Days -1, 35, 70 and 100 in mg/ml

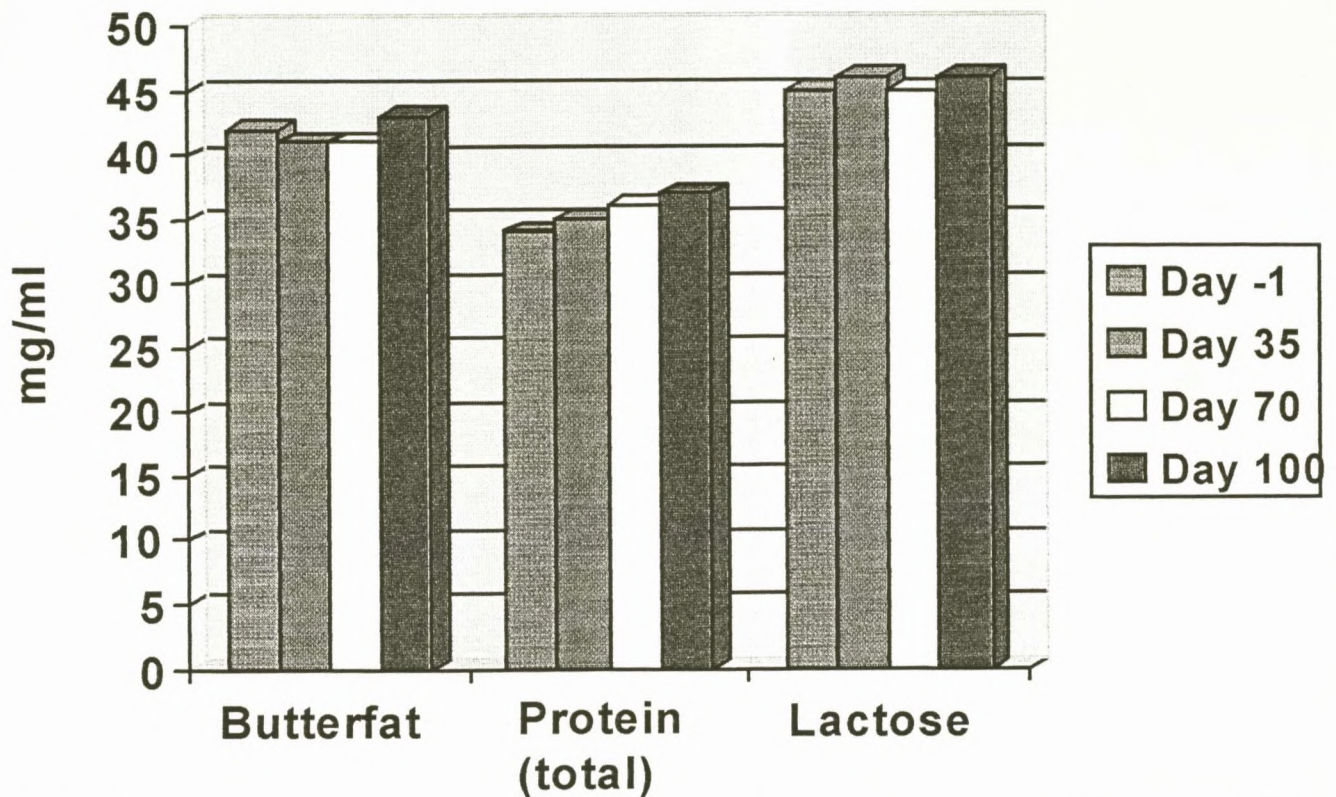
	Butterfat	Protein (total)	Lactose
Day -1	42.2	33.3	45.9
Day 35	40.8	34.3	46.7
Day 70	40.8	35.9	45.8
Day 100	43.8	37.0	46.2



Graph 4.11 Group 1: Average somatic cell counts for Days -1, 35, 70 and 100

Table 4.20 Group 1: Average somatic cell count levels for Days -1, 35, 70 and 100 in thousands/ml

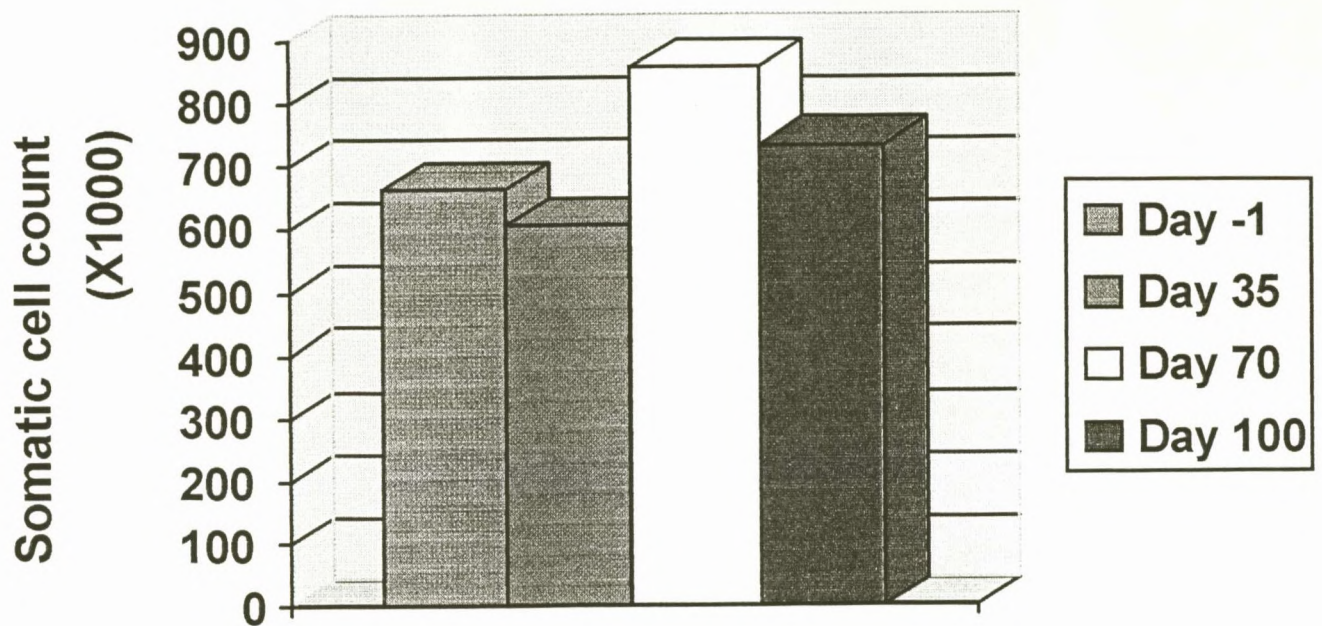
	Somatic cell count
Day -1	568.55
Day 35	594.71
Day 70	515.48
Day 100	698.78



Graph 4.12 Group 2: Average butterfat, total protein, and lactose levels for Days -1, 35, 70 and 100

Table 4.21 Group 2: Average butterfat, total protein, and lactose level values for Days -1, 35, 70 and 100 in mg/ml

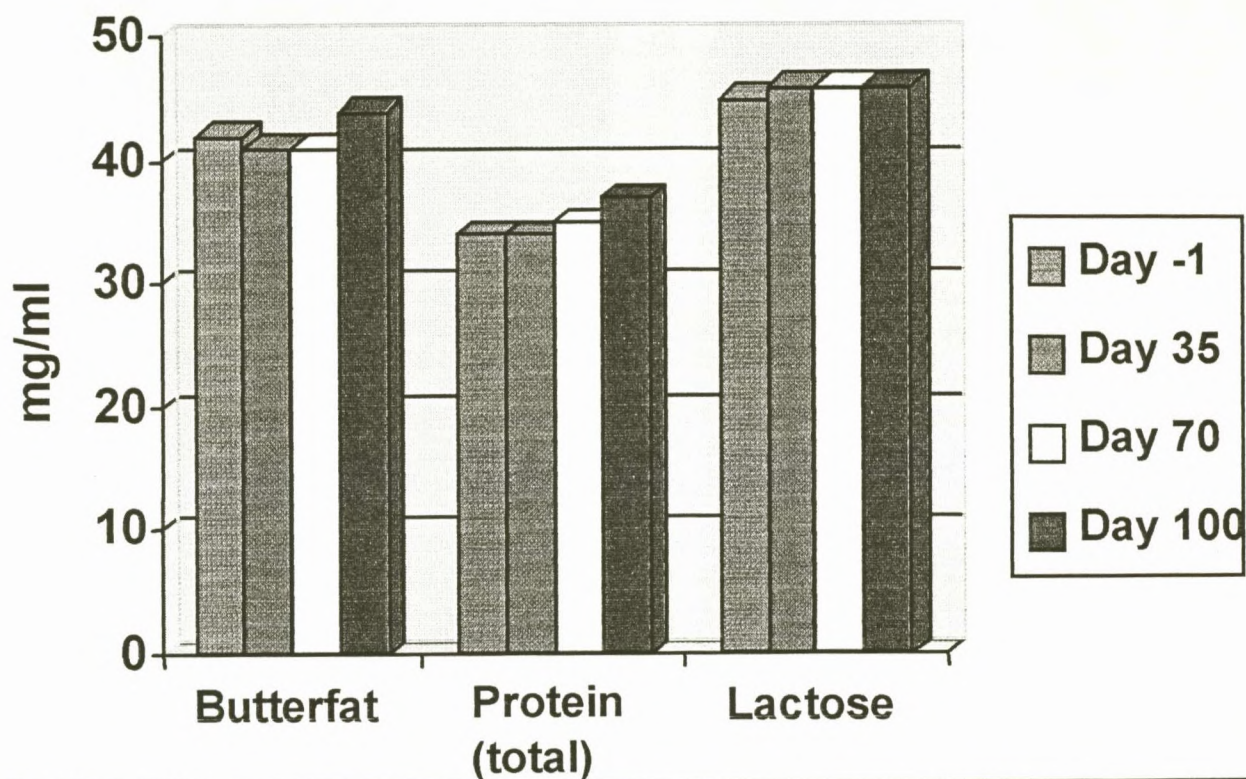
	Butterfat	Protein (total)	Lactose
Day -1	42.1	34.1	45.3
Day 35	41.2	35.4	46.4
Day 70	40.8	36.0	45.2
Day 100	42.8	37.3	45.8



Graph 4.13 Group 2: Average somatic cell counts for Days -1, 35, 70 and 100

Table 4.22 Group 2: Average somatic cell count levels for Days -1, 35, 70 and 100 in thousands/ml

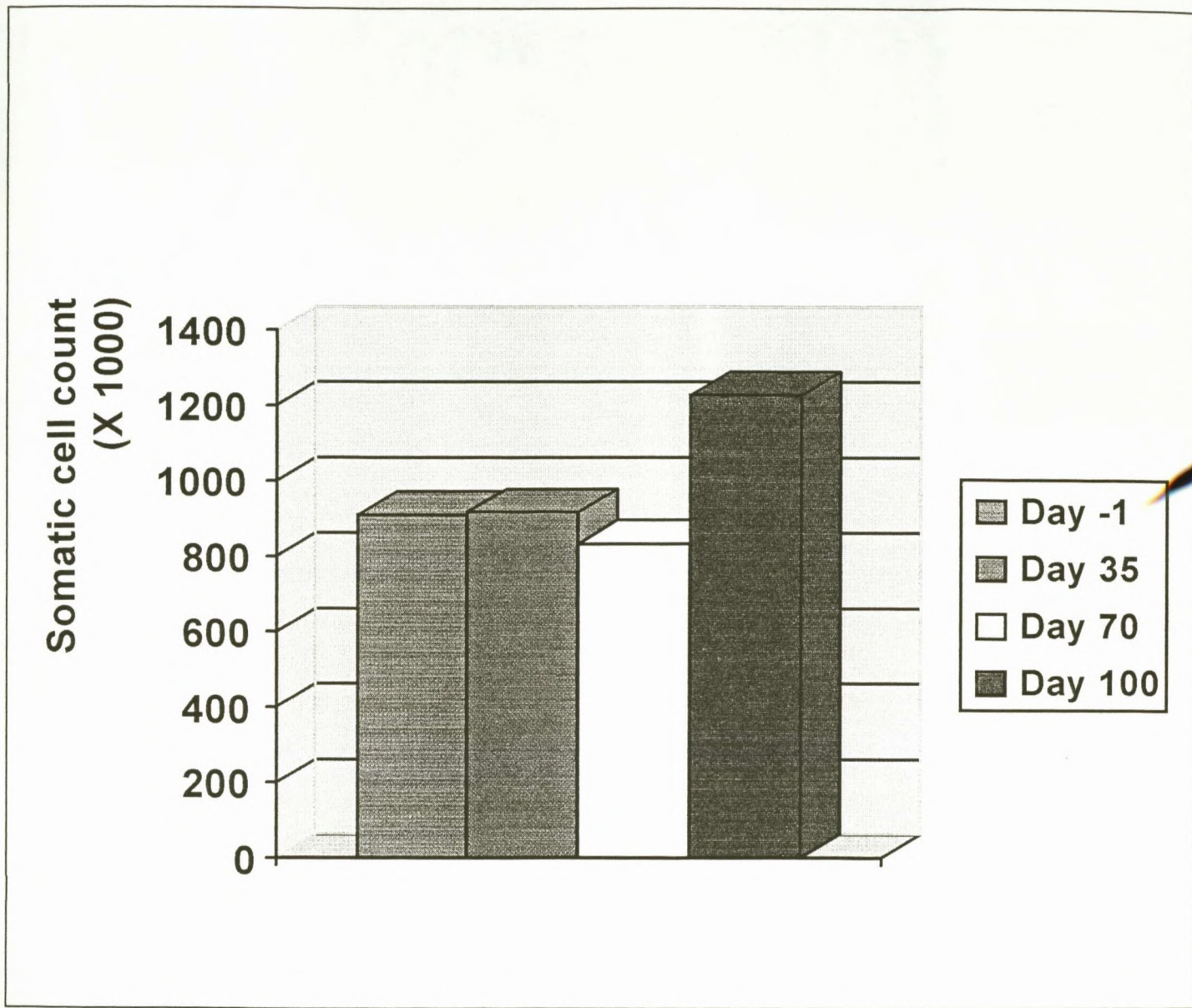
	Somatic cell count
Day -1	662.57
Day 35	605.16
Day 70	856.71
Day 100	733.51



Graph 4.14 Group 3: Average butterfat, total protein and lactose levels for Days -1, 35, 70 and 100

Table 4.23 Group 3: Average butterfat, total protein and lactose level values for Days -1, 35, 70 and 100 in mg/ml

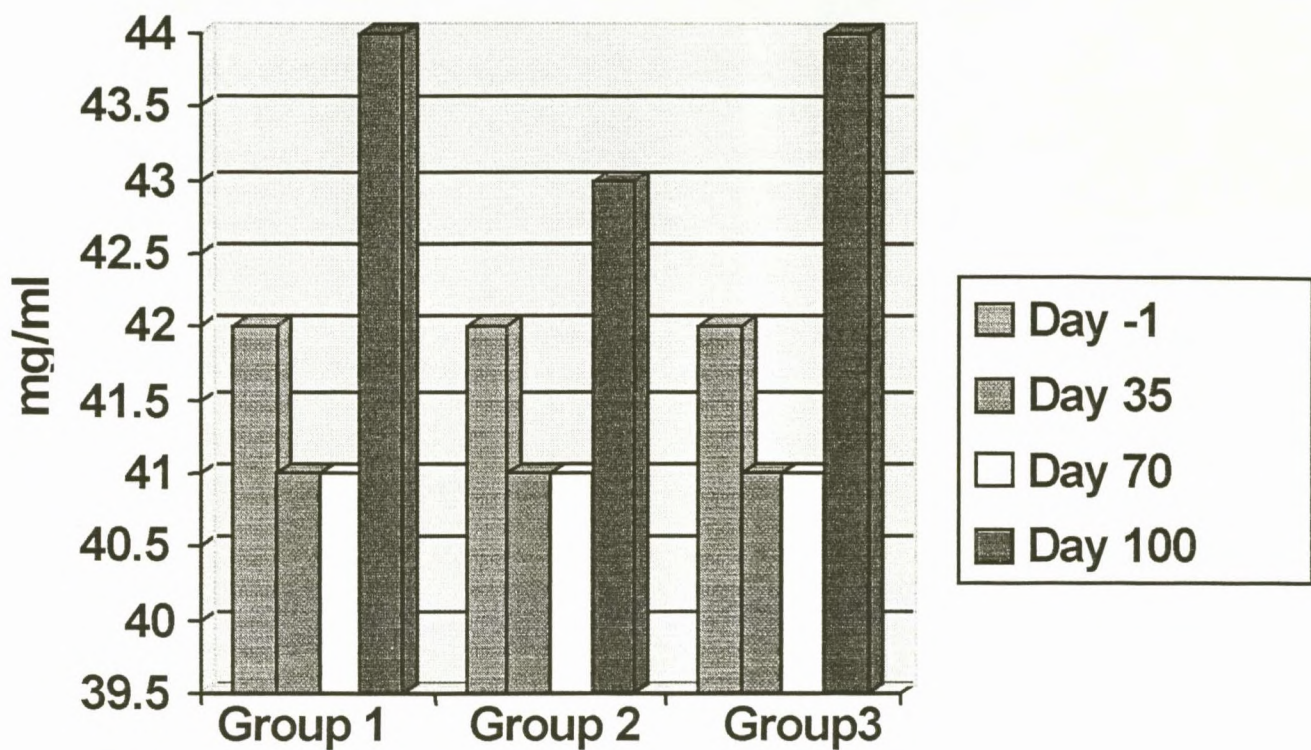
	Butterfat	Protein (total)	Lactose
Day -1	42.3	33.6	45.4
Day 35	42.1	34.1	46.3
Day 70	40.8	35.3	46.1
Day 100	44.0	36.7	46.0



Graph 4.15 Group 3: Average somatic cell counts for Days -1, 35, 70 and 100

Table 4.24 Group 3: Average somatic cell count values for Days -1, 35, 70 and 100 in thousands/ml

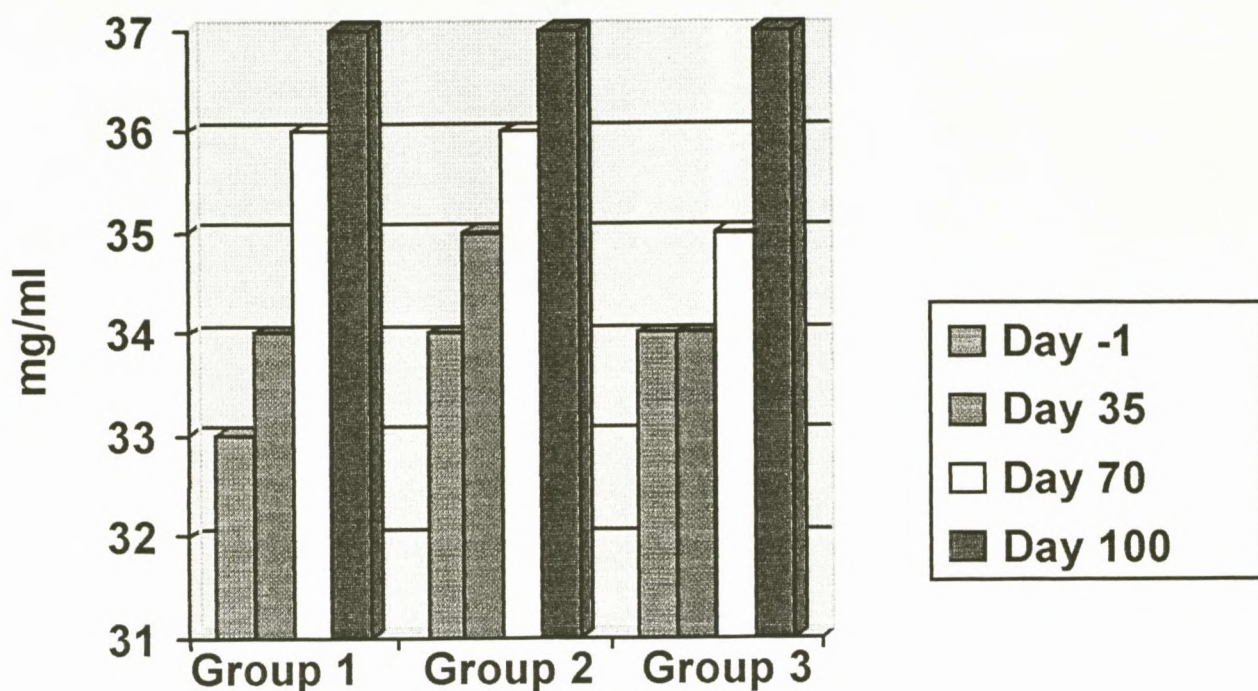
	Somatic cell count
Day -1	908.49
Day 35	917.70
Day 70	835.91
Day 100	1228.85



Graph 4.16 Butterfat: Average butterfat levels in Groups 1, 2 and 3 for Days -1, 35, 70 and 100

Table 4.25 Butterfat: Average butterfat level values in Groups 1, 2 and 3 for Days -1, 35, 70 and 100 in mg/ml

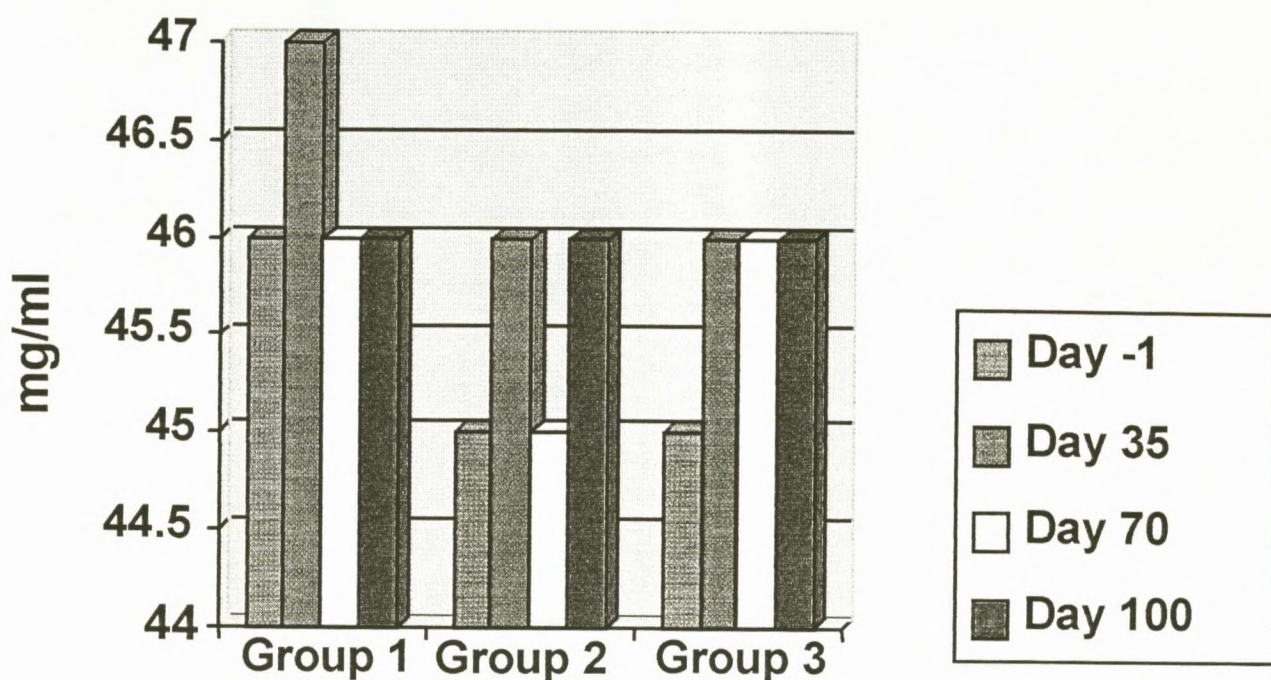
	Group 1	Group 2	Group 3
Day -1	42.2	42.1	42.3
Day 35	40.8	41.2	42.1
Day 70	40.8	40.8	40.8
Day 100	43.8	42.8	44.0



Graph 4.17 Total protein: Average total protein levels in Groups 1, 2 and 3 for Days -1, 35, 70 and 100

Table 4.26 Total protein: Average total protein level values in Groups 1, 2 and 3 for Days -1, 35, 70 and 100 in mg/ml

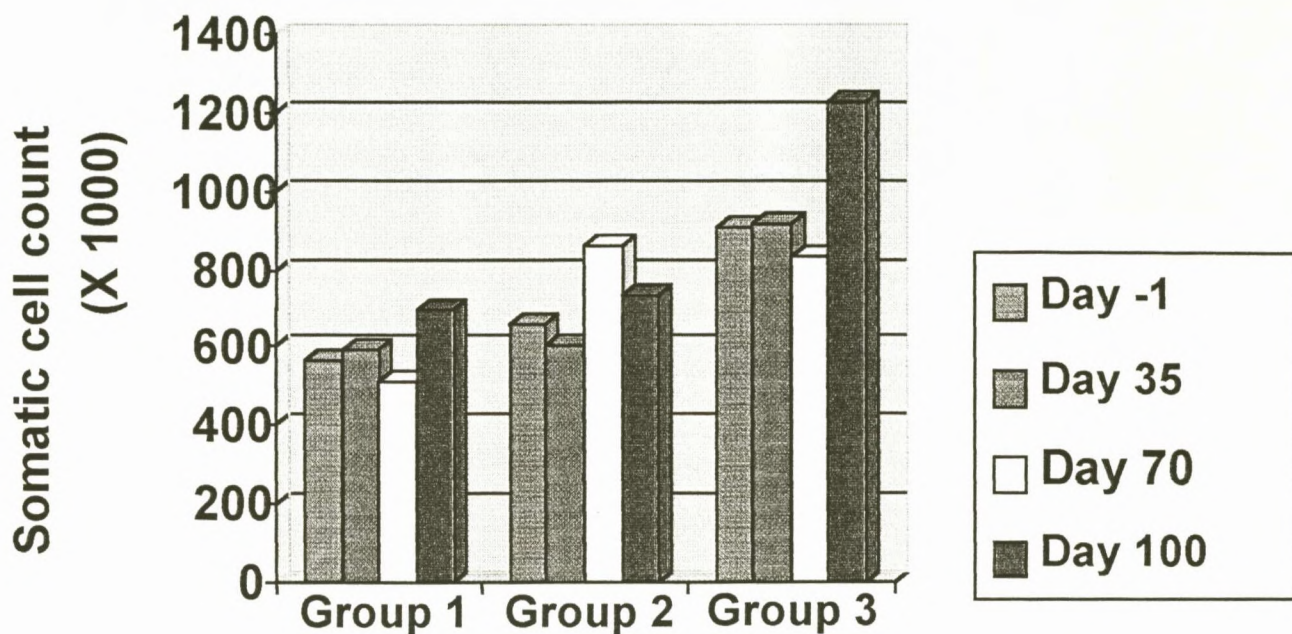
	Group 1	Group 2	Group 3
Day -1	33.3	34.1	33.6
Day 35	34.3	35.4	34.1
Day 70	35.9	36.0	35.3
Day 100	37.0	37.3	36.7



Graph 4.18 Lactose: Average lactose levels in Groups 1, 2 and 3 for Days -1, 35, 70 and 100

Table 4.27 Lactose: Average lactose level values in Groups 1, 2 and 3 for Days -1, 35, 70 and 100 in mg/ml

	Group 1	Group 2	Group 3
Day -1	45.9	45.3	45.4
Day 35	46.7	46.4	46.3
Day 70	45.8	45.2	46.1
Day 100	46.2	45.8	46.0



Graph 4.19 Somatic cell count: Average somatic cell counts in Groups 1, 2 and 3 for Days -1, 35, 70 and 100

Table 4.28 Somatic cell count: Average somatic cell count values in Groups 1, 2 and 3 for Days -1, 35, 70 and 100 in thousands/ml

	Group 1	Group 2	Group 3
Day -1	568.55	662.57	908.47
Day 35	594.71	605.16	917.70
Day 70	515.48	856.71	835.91
Day 100	698.78	733.51	1228.85

Chapter 5 Discussion

This study endeavoured to observe the effect of *Phytolacca decandra* 12CH and *Phytolacca decandra* 200CH on the incidence of acute clinical bovine mastitis and on the butterfat, total protein and lactose levels, and the somatic cell counts obtained from composite milk samples in dairy cattle.

5.1 Discussion of statistical evaluations

The incidence of acute clinical mastitis and the butterfat, total protein and lactose measurements and somatic cell counts of composite milk samples were used to test the hypotheses. The diagnosis of acute clinical mastitis depends on the observation of clinical signs and symptoms, as discussed in Chapter 2. The measurements concerning the butterfat, total protein and lactose measurements and somatic cell counts of composite milk samples were all done in a recognised and specialised laboratory with modern equipment. All are objective and accurate measurements, giving a representative and comprehensive reflection of the status of mastitis in the herd. This also enabled easy and accurate comparisons between the measurements of the different groups.

The methods used for the analysis of the data obtained from this study are explained in Chapter 4.

5.1.1 The incidence of acute clinical mastitis

The results from the trial show that *Phytolacca decandra* does decrease the incidence of acute clinical mastitis, as illustrated in Table 4.1. With the placebo group as reference, the *Phytolacca decandra* 12CH treatment group shows an incidence of 53.2% less, and the *Phytolacca decandra* 200CH group shows an incidence of 76.1% less. The *Phytolacca decandra* 200CH treatment group had an incidence of 57.89% less than that of the *Phytolacca decandra* 12CH treatment group.

Of the 21 cases of acute clinical mastitis recorded for this trial, nine cases (three cases in the *Phytolacca decandra* 12CH treatment group, one case in the *Phytolacca decandra* 200CH treatment group, and five cases in the Placebo group) occurred in cows that are 10 – 12 years of age. Eight cases (three cases in the *Phytolacca decandra* 12CH treatment group, one case in the *Phytolacca decandra* 200CH treatment group and four cases

in the Placebo group) of acute clinical mastitis were recorded for the 6 – 9 year old cows during the 100-day trial period. Five cases of acute clinical mastitis (one case in the *Phytolacca decandra* 12CH treatment group, two cases in the *Phytolacca decandra* 200CH treatment group and two cases in the Placebo group) were reported for the 2 – 5 year old cows during the 100-day trial period. This is in accordance with observations in the literature regarding the effects of age on the incidence of mastitis, as discussed in Chapter 2 (2.6.1.7.1 Stage of lactation and age of the cow).

The above analysis shows that for the cows older than 6 years, the *Phytolacca decandra* 200CH was effective in decreasing the incidence of acute clinical mastitis. This is an important observation, as it indicates a possible sensitivity to this remedy in the high-risk group for the development of acute clinical mastitis.

5.1.2 Butterfat

Statistically significant differences were noted within the *Phytolacca decandra* 12CH treatment group and within the Placebo group between milk recordings, as discussed in Chapter 4. Graph 4.16 shows that the statistically significant differences between the values of the four consecutive milk recordings of the above mentioned groups of cows are reflected to a lesser degree by the *Phytolacca decandra* 200CH treatment group.

Graph 4.16 represents all butterfat values measured in the trial. In light of section 2.6.1.7 (Additional factors affecting udder health and milk quality (2.6.1.7.2 Climate)), the effect of temperature (cf. Appendix 3 and Table 4.30) on the butterfat content of composite milk samples can be observed. During the 20 days preceding the trial, the average maximum temperature was 24.6 °C, with five maximum temperature recordings equalling or exceeding 27 °C. The highest daytime temperature recorded is 29 °C on 3 March 2000 (Day –17). These temperatures approach 30 °C and this could be responsible (according to 2.6.1.7) for the higher butterfat levels (as compared to the next two measurements) for all three groups on Day -1. The absence of these relatively high (or low) temperatures in the intervals between Day –1 and Day 35 and between Day 35 and Day 70 could be responsible for the statistically insignificant decrease in the *Phytolacca decandra* 12CH and *Phytolacca decandra* 200CH treatment groups and the statistically significant decrease between recorded butterfat levels in the Placebo group. The *Phytolacca decandra* 12CH treatment group had statistically significant increases in butterfat levels

between Day 35 and Day 100, and between Day 70 and Day 100. The Placebo group had a statistically significant increase in butterfat levels between Day 70 and Day 100. This increase in butterfat levels could be due to a decrease in average minimum temperature from Day 70 to Day 100 to -2.5°C (cf. 2.6.1.7.2 and Table 4.30). The coldest minimum temperature for the entire trial was recorded during the night before the last milk recording, and was recorded as -6.6°C . Minimum temperatures recorded for Days 98 and 97 of the trial is -6.1°C , and for Day 96 is -5.4°C .

Despite the temperature fluctuations during the trial, there was no statistically significant difference between butterfat values between the four milk recordings in the *Phytolacca decandra* 200CH treatment group. This resistance to temperature fluctuations could be due to the action of this remedy.

5.1.3 Total protein

Graph 4.17 compares the average total protein, values of all four milk recordings of the three groups. Although there are statistically significant differences between the different measurements within the groups, the same pattern is observed for both treatment groups and the placebo. These in-group differences cannot be ascribed to the action of the homoeopathic remedies.

Section 2.6.1.7.2 provides a possible reason for the gradual increase in the total protein levels in milk for the consecutive measurements. The discussion of average minimum and maximum temperature measured during the trial period in 5.1.2 (Butterfat) applies to the discussion for protein. A possible explanation for the increase in total protein levels at each successive milk recording for all three groups is the progressive decrease in the average and highest maximum temperatures as well as the progressive decrease in the average and lowest minimum temperatures recorded during the trial (cf. Appendix 3).

5.1.4 Lactose

No statistically significant differences were shown for lactose levels between and within the groups on the four milk recordings, for the duration of the trial.

5.1.5 Somatic cell counts

Graph 4.19 illustrates the comparison of the somatic cell count measurements taken for the three groups on the four consecutive milk recordings. Despite the randomisation to obtain homogeneous groups, there was an inequality in baseline somatic cell counts between the three groups of cows that is continued proportionally for the three following measurements, as illustrated in Graph 4.3.

Graph 4.19 shows promising results when comparing the different measurements for the three groups. There are however no statistically significant differences between the measurements within and between the groups regarding the somatic cell counts, and the configuration observed for both treatment groups is reflected in the placebo group. Neither *Phytolacca decandra* 12CH nor *Phytolacca decandra* 200CH had any effect on this measurement for the duration of this trial.

The observation made by Farrow (1997: Appendix 4) (cf. 2.6.1.7.1) regarding the effect of rainfall on the somatic cell counts is not reflected in this trial. Farrow conducted his trial from July to December, i.e. starting in winter and ending in summer. This trial was conducted from March to June, i.e. starting in late summer and ending in winter. This discrepancy could be due to different climatic conditions other than rainfall.

5.2 Discussion of previous trials in homoeopathic prophylaxis of bovine mastitis

With respect to the studies mentioned in Chapter 2 (2.8.5 Relate of previous trials in homoeopathic prophylaxis of bovine mastitis) certain aspects of study design should be mentioned. There is no standard reference or method regarding the division of study populations into treatment and placebo groups. Age, stage of lactation, mastitic status and milk production of the dairy cows were taken into account in short term trials done by Searcy, Ryes and Guajardo, (1995) and in problem herds by Day (1986), but not in a six month trial done by Day (1986) since he states that it would affect the results materially.

In the above studies and in the current study discussed, there is enough evidence to suggest the potential and merit of nosode therapy as used by Day (1986), Hansford and Pinkus (1998) and Farrow (1986), using combinations of different homoeopathic remedies at different potency

levels in a complex as done by Searcy, Ryes and Guajardo, (1995), and in the use of a single remedy selected on the clinical presentation of mastitis as was done in this trial. Further studies are called for to establish clinical criteria for the indication of usage of these methods in specific circumstances.

The treatment regimen has also varied greatly in the past. Day (1986) administered nosode therapy via the drinking water. Farrow (1997) administered 50ml liquid oral doses of a 30CH nosode once a month for five months, and Searcy, Ryes and Guajardo, administered liquid oral doses of the chosen complex every 48 hours for the first two weeks, two doses in the second week, and one dose in the last week of their 30 day trial. Frequency of dosage administration should be adapted according to the nature of the medicinal substance used, the form and method of dispensing, the duration of the trial and the reactivity of the animals treated.

6.1 Conclusion

This trial was effective in achieving the second objective and in proving the second hypothesis. *Phytolacca decandra* 200CH did affect the incidence of acute clinical mastitis in the dairy cows treated. A favourable response was observed when using the placebo treatment as reference.

This trial was also effective in achieving the fourth objective and in proving the second hypothesis. *Phytolacca decandra* 12CH did affect the incidence of acute clinical cases of mastitis in the dairy cows treated, as illustrated in Table 4.1. A favourable response was observed when comparing the incidence of acute clinical mastitis in this group to that of the Placebo group and the *Phytolacca decandra* 12CH treatment group.

This trial was ineffective in proving the other hypotheses and in achieving the other objectives set. Despite sporadic increases and decreases in any given variable during the trial, one cannot reasonably conclude that the remedies given as prophylaxis did positively affect the somatic cell counts or butterfat, total protein and lactose levels in the milk of the three groups of cattle studied. Most differences observed within a group were not consistent, and were mirrored in the other groups. The observation made about the effect of *Phytolacca decandra* 200CH on the butterfat level changes related to temperature is not one of increasing the measurements, but of decreasing the effect of this environmental factor on the cows milk production.

The researcher is of the opinion that the criteria selected to measure possible differences within and between the three groups gave a good indication and reflection of the udder health and mastitic status of the groups, and that all of these measurements should be included in future trials.

6.2 Recommendations for future trials

The duration of the acute cases of clinical mastitis diagnosed should be recorded because it gives an indication of the severity of the

inflammation and of the ability of the cows' immune system to resolve the infection.

A minimum trial period of 12 months is recommended for future studies. This will enable a more accurate and reliable observation of seasonal trends, which was not possible in this case due to the short duration of this study.

Marking the cows with enamel paint proved to be very time consuming, costly and ineffective because all the groups had to be remarked every five weeks. Tagging the cows with different coloured ear tags as was done by Farrow (1997) is a much more efficient way of marking the cows.

Alternative methods of dispensing should be investigated. This is discussed in Chapter 2 (2.8.6 Method, route and frequency of remedy administration). Spray application of the remedy at milking time looks like a good alternative method of remedy administration if done correctly. When taking into account that spray application involves handling of the cow, and will also involve a change of the dairy routine, this method should only be considered if it can be done without causing any undue duress to the cows. Vithoukias (1981: 103) cautions that by mere succussion of the fluid without further dilution (as when handling the spray applicator), a remedy can be raised to the next potency level. A change in remedy dynamics is not desirable.

Different treatment regimes or combinations of nosode therapy, symptomatic or clinical prescribing (as done in this trial), and constitutional treatment should be investigated. Hansford and Pinkus (1998: 44) hold constitutional treatment to be beneficial in chronic conditions such as chronic cases of mastitis where symptomatic prescribing has failed to provide lasting cure. In a discussion with Mr. Pinkus, co-author of *The Herdsman's introduction to Homoeopathy* and director of Ainsworths Homoeopathic Pharmacy, he suggested that a combination of constitutional remedies and nosode therapy is an effective way to approach the problem of bovine mastitis homoeopathically.

Constitutional prescribing requires some very precise information that is often not readily available or discernible, since the loose-housed cow loses her identity to some extent in a large herd. This is true at least as far as the outside observer or the unenlightened herdsman is concerned (Day, 1986).

This statement is applicable to the South African context because herds tend to be large and are generally loose-housed, which make astute observation of individual animals more difficult. Accurate constitutional prescribing in a 350-cow herd is a rather daunting challenge if all the animals have to be treated, even if the animals are handled daily and handlers have the necessary skills and knowledge. Research on the efficacy of constitutional prescribing would be executed more accurately in smaller herds, where each cow is known and can be better observed. For homoeopathic veterinary medicine to be accepted as safe, effective and economical on commercial dairy farms, more evidence and further investigation of different treatment regimes is needed.

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Appendix 1

Consent form for Homoeopathic research

I,, of the farm.....
agree to allow three hundred of my dairy herd to be used in a research program to observe the effect of Homoeopathic treatment on the somatic cell count of composite milk samples, and on the incidence of acute cases of clinical mastitis.

The following terms and conditions are agreed to:

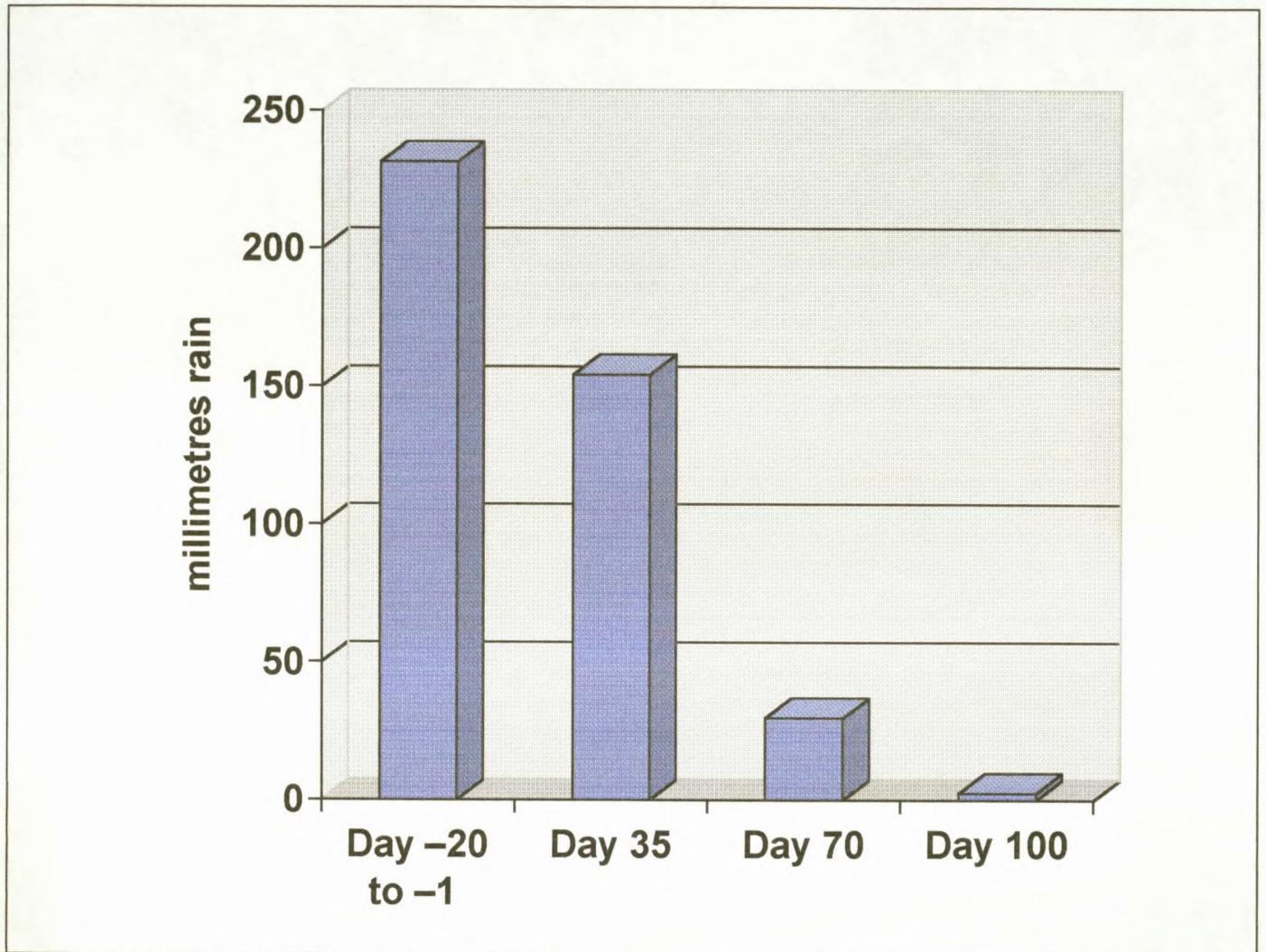
1. I fully understand the nature and methodology of the research, and also the role my herd is to play.
2. I understand that 300 cows (in three herds of 100 cows each) will be divided into treatment and placebo groups, implying that not all animals will be receiving medication.
3. I understand that I am under no obligation to continue with this research until its completion, and that I may withdraw if the trial is (with reasonable certainty) compromising the health and production of my dairy herds.

Signed at On

Farmer

Researcher

Appendix 2



Graph 4.20 Rainfall recorded at the Underberg Meteorological Station (Shaleburn) from Day -20 to Day 100

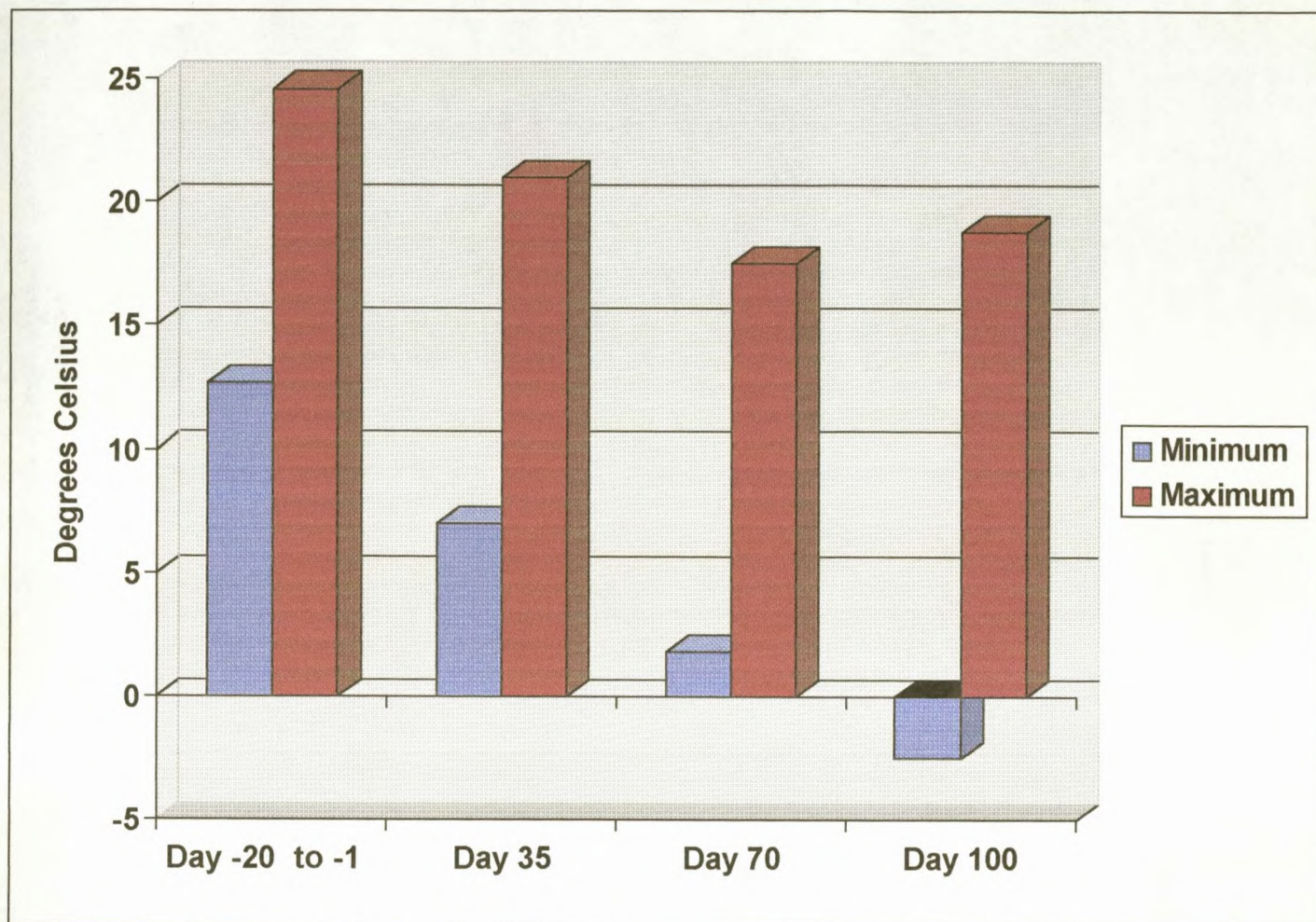
Table 4.29 Rainfall figures recorded at the Underberg Meteorological Station (Shaleburn) from Day -20 to Day 100 in millimetres

Time period:	Day -20 to -1	Day 35	Day 70	Day 100
Millimetres rain	231.4	154.3	29.7	2.5

Reference:

South African Weather Bureau, 2000.

Appendix 3



Graph 4.21 Average minimum and maximum temperatures recorded at the Underburg Meteorological Station (Shaleburn) from Day -20 to Day 100

Table 4.30 Average minimum and maximum temperature values recorded at the Underburg Meteorological Station (Shaleburn) from Day -20 to Day 100 in degrees Celsius

	Day -20 to -1	Day 35	Day 70	Day 100
Minimum	12.7	7	1.8	-2.5
Maximum	24.6	21	17.5	18.8

Reference:

South African Weather Bureau, 2000.