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INTRAMAMMARY ANTIBIOTICS IN DAIRY GOATS: WITHDRAWAL  
PERIODS AND TISSUE TOLERANCE

By

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## ABBREVIATIONS

A: Atrophy  
 AB: Antibiotics  
 ACD: Acid Degree Values  
 ADI: Acceptable Daily Intake  
 A.M.D.E.: Absorption, Metabolism, tissue Distribution, Elimination or Excretion  
 AOAC: Association of Official Analytical Chemists  
 ARC: Agricultural Research Council  
 AUC: Area Under Curve  
 B Lymphocytes: B cells  
 BsDA: *Bacillus sterothermophilus* disc assay  
 BTA: Bovine blood Triptose Agar  
 C: Control group for Trial 3  
 C1: Control group for Trial 1  
 C2: Control group for Trial 2  
 CAE: Caprine Arthritis Encephalitis  
 CAEV: Caprine Arthritis Encephalitis Virus  
 CFM: Cubic Feet per Minute  
 C<sub>max</sub>: Maximal Concentration  
 T<sub>max</sub>: Time of Maximal Concentration  
 CMCT: California Milk Cell Test  
 cm Hg: Centimeters of Mercury  
 CNS: Coagulase Negative Staphylococci  
 COR: *Corynebacterium pseudotuberculosis*  
 d.f.: Degrees of Freedom  
 DNA: Deoxyribonucleic Acid  
 EC: European Community  
 ELISA: Enzyme Linked Immuno Absorbent Assay  
 ENT: *Enterobacterium*  
 EU: European Union  
 F: Fibrosis  
 FDA: Food and Drug Administration  
 Fig.: Figure  
 FPT: Failure of Passive Transfer  
 GIT: Gastrointestinal Tract  
 GLA: Goat Lymphocyte Antigen  
 GLP: Good Laboratory Practice  
 h: hours  
 Hg: Mercury  
 H<sub>2</sub>O<sub>2</sub>: Hydrogen Peroxide  
 HPLC: High Performance Liquid Chromatography  
 Ig: Immunoglobulin  
 IgA: Immunoglobulin A  
 IgG: Immunoglobulin G  
 IgG<sub>1</sub>: Immunoglobulin G<sub>1</sub>  
 IgG<sub>2</sub>: Immunoglobulin G<sub>2</sub>  
 IgM: Immunoglobulin M  
 IM: Intramuscular  
 IMI: Intramammary Infection  
 IU: International Units  
 IV: Intravenous  
 KLE: *Klebsiella*  
 KOH: Potassium Hydroxide  
 kPa: Kilo Pascal  
 L: Left udder half of goat



LD: Lymphocyte Defined  
LP: Lactoperoxidase  
MHC: Major Histocompatibility Complex  
MIC: Minimal Inhibitory Concentration  
MICR: *Micrococcus*  
MRL: Maximum Residue Level  
MRLs: Maximum Residue Levels  
MYC: *Mycoplasma*  
NAGase: N-Acetyl-B-D-glucosaminidase  
NCIMS: National Conference of Interstate Milk Shippers  
N: Nodules  
NS: Non-Significant  
OTAU: Onderstepoort Teaching Animal Unit  
PABA: p-Aminobenzoic Acid  
PAE: Post Antibiotic Effect  
PAS: *Pasteurella haemolytica*  
PD: Pharmacodynamic  
PK: Pharmacokinetic  
PK/PD: Pharmacokinetic-Pharmacodynamic Modelling  
PMN: Polymorphonucleates  
PMO: Pasteurised Milk Ordinance  
PNA: Peanut Agglutinin  
PSE: *Pseudomonas*  
R: Right udder half of goat  
RNA: Ribonucleic Acid  
Rx1: First Antibiotic Treatment  
Rx2: Second Antibiotic Treatment  
Rx3: Third Antibiotic Treatment  
SABS: South African Bureau of Standards  
SAG: *Streptococcus agalactiae*  
SC: Subcutaneous  
SCC: Somatic Cell Count  
SCN: Thiocyanate  
SD: Serologically Defined  
SD<sub>1</sub>: Serologically Defined, type 1  
SD<sub>2</sub>: Serologically Defined, type 2  
SDY: *Streptococcus dysgalactiae*  
se: Standard Error  
STA: *Staphylococcus aureus*  
STE: *Staphylococcus epidermidis*  
SUB: *Streptococcus uberis*  
T1: Treated with Curaclox LC (Trials 1 & 3)  
T2: Treated with Spectrazol Milking Cow (Trial 2)  
T3: Treated with Rilexine 200 LC (Trial 3)  
T Lymphocytes: T cells  
TMR: Total Mixed Rations  
TRIS: Thermo Resistant Inhibitory Substances  
US: United States  
USA: United States of America  
USFDA: United States Food and Drug Administration  
WMT: Wisconsin Mastitis Test  
WP: Withdrawal Period  
\*\*\*: Significant at the 0.1% level of significance  
\*\*\*: Significant at the 1% level of Significance  
\*: Significant at the 5% level of Significance

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## SUMMARY

### INTRAMAMMARY ANTIBIOTICS IN DAIRY GOATS: WITHDRAWAL PERIODS AND TISSUE TOLERANCE

By

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The aim of this study was to determine withdrawal periods and tissue tolerance of intramammary antibiotics (Curaclox LC, Spectrazol Milking Cow and Rilexine 200 LC) in goats, measured in different ways, and to evaluate the effects of related factors.

#### **Method:**

Three experimental trials were conducted. Trial 1 and Trial 2 were conducted at the Faculty of Veterinary Science, Onderstepoort using the goat herd of the Onderstepoort Teaching Animal Unit (OTAU) (Herd A), while Trial 3 was conducted on a commercial goat dairy in the Limpopo Province of South Africa (Herd B). In addition, four goats with clinical mastitis from a smallholding close to the Faculty of Veterinary Science at Onderstepoort were studied (Herd C). This herd consisted of 13 lactating Saanen and Saanen/Toggenburg crossbred dairy goats.

In all trials foremilk was stripped, teats were disinfected and a milk sample was taken from each udder half of each goat (half-milk samples). In all three trials the following milk samples were taken: two sets of half samples and a composite sample (before, during and after treatment). The California Milk Cell Test (CMCT) and conductivity measurements were performed. In Trial 3 the conductivity meter became non-functional on the second day, and thus the conductivity test was eliminated from then on. Each udder half was milked separately and milk volume was recorded. The temperature of goats was taken and recorded to identify sick animals. All goats in the treatment group were treated.

In all three trials after treatment, sampling continued until SCC returned to baseline and until there were at least two consecutive negative TRIS tests for each goat, approximately 10 days. Milk production was based on the following milk production groups: low (less than 1.3L), medium (1.3L to 1.5L) and high (greater than 1.5L) daily milk production.

The antibiotics used in these trials were selected for being commonly used, broad-spectrum preparations.

Trial 1, a semi-synthetic penicillin based intramammary preparation (Curaclox LC, which contains 75mg sodium ampicillin and 200mg sodium cloxacillin per dose plus blue dye). Curaclox LC G2615, (Norbrook (Pharmacia AH) P.O. Box 10698 Centurion, 0046), cloxacillin 200mg, ampicillin 75mg, blue dye/ 4.5g syringe.

Trial 2, a cefuroxime 250mg based intramammary product (Spectrazol Milking Cow, Schering-Plough). Spectrazol milking cow, cefuroxime, 250mg, S4 Intramammary Injection 83/594, (Schering-Plough Animal Health, P.O. BOX 46, Isando, 1600).

Trial 3, a cephalexin 100mg, neomycin sulphate 100mg and prednisolone based intramammary product, Rilexine (SA) 200LC injection 83/638, (Logos Agvet (Virbac), Private bag X115, Halfway House, 1685). Curaclox LC G2615, Norbrook (Pharmacia AH), cloxacillin 200mg, ampicillin 75mg, blue dye/ 4.5g syringes.



In the clinical mastitis cases (Herd C); Goat 1 was treated with Spectrazol milking cow (as above), Goat 2 was treated with Curaclox LC (as above), Goat 3 was treated with Curaclox LC in the left udder half and Goat 4 was treated with Curaclox LC in the right udder half (as above).

### **Results:**

#### **Trial 1: Curaclox LC**

The mean withdrawal periods for the product Curaclox LC (intramammary) as measured by Thermo Resistant Inhibitory Substances (TRIS), colour dye, Parallax testing for cloxacillin and ampicillin, on eight relatively low producing Saanen dairy goats (Trial 1) were 74h  $\pm$  19.21; 90h  $\pm$  16.97; 99h  $\pm$  9.07 and 93h  $\pm$  11.41 respectively. The withdrawal period for Curaclox LC recommended for use in cattle (72h) was significantly shorter than the withdrawal periods as measured by colour dye ( $P < 0.001$ ), Parallax testing for cloxacillin ( $P < 0.001$ ) and Parallax testing for ampicillin ( $P < 0.05$ ) in Trial 1. There was a significant difference of withdrawal periods as measured by TRIS ( $P < 0.05$ ) and colour dye ( $P < 0.05$ ) between goats with and without clinical mastitis in Trial 1.

#### **Trial 3: Curaclox LC**

The mean withdrawal periods for Curaclox LC as measured by TRIS, colour dye, Parallax testing for cloxacillin and ampicillin, on 12 relatively high producing Saanen and Saanen-Toggenburg crossbreed dairy goats (Trial 3) were 42h  $\pm$  7.08; 65h  $\pm$  60.26; 77h  $\pm$  13.56 and 71h  $\pm$  12.65 respectively. The withdrawal period for Curaclox LC recommended for use in cattle (72h) was significantly longer than the withdrawal periods as measured by TRIS ( $P < 0.001$ ) and colour dye ( $P < 0.001$ ) in Trial 3.

#### **Curaclox LC: Trials 1 & 3 combined**

The mean withdrawal periods for Curaclox LC as measured by TRIS, colour dye, Parallax testing for cloxacillin and ampicillin, for Trials 1 & 3 combined were 59h  $\pm$  24.31; 76h  $\pm$  17.70; 87h  $\pm$  16.10 and 80h  $\pm$  16.23 respectively. The withdrawal period for Curaclox LC recommended for use in cattle (72h) was significantly longer than the withdrawal periods as measured by TRIS ( $P < 0.001$ ) in Trials 1 & 3 combined.

#### **Trial 2: Spectrazol Milking Cow**

The mean withdrawal periods for Spectrazol Milking Cow (intramammary) as measured by TRIS on seven relatively low producing Saanen dairy goats (Trial 2) was 95h  $\pm$  17.23. The withdrawal period for Spectrazol Milking Cow recommended for use in cattle (60h) was significantly shorter than the withdrawal period as measured by TRIS ( $P < 0.001$ ) in Trial 2.

#### **Trial 3: Rilexine 200 LC**

The mean withdrawal periods for Rilexine 200 LC (intramammary) as measured by TRIS on 20 relatively high producing Saanen and Saanen-Toggenburg crossbreed dairy goats (Trial 3) was 37h  $\pm$  9.94. The withdrawal period for Rilexine 200 LC recommended for use in cattle (96h) was significantly longer than the withdrawal period as measured by TRIS ( $P < 0.001$ ) in Trial 3.

The regression model for goats with clinical mastitis was:

$$\begin{aligned} \text{Withdrawal period as measured by TRIS} &= 30.21 + 4.692 (\text{sampling time}) \\ &+ 22.11 (\text{udder palpation}) - 13.6 (\text{flocules}) - 0.00649 (\text{volume}) \\ (R^2 &= 95.7\%, \text{ standard error of regression} = 3.41) \end{aligned}$$

There was great variation in Somatic Cell Count (SCC) between trials, ranging from 1928 X 10<sup>3</sup>cells/mL to 9274 X 10<sup>3</sup>cells/mL for infected udder halves and from 1817 X 10<sup>3</sup>cells/mL to 3639 X 10<sup>3</sup>cells/mL for non-infected udder halves, at the morning milking. At the evening milking SCC ranged from 1927 X 10<sup>3</sup>cells/mL to 6415 X 10<sup>3</sup>cells/mL for infected udder halves and from 2103 X 10<sup>3</sup>cells/mL to 3304 X 10<sup>3</sup>cells/mL for non-infected udder halves. SCC of udder halves with clinical mastitis ranged from 7053 X 10<sup>3</sup>cells/mL to 7948 X 10<sup>3</sup>cells/mL for udder halves in which bacteria could not be isolated and from 6476 X 10<sup>3</sup>cells/mL to 8479 X 10<sup>3</sup>cells/mL in udder halves from which bacteria was isolated. Most of the variation in SCC was unexplained. In this research all SCC values were determined using the Fossomatic 90 counter and the arithmetic means were reported. The factors valid for

determining clinical mastitis were the presence of floccules in the milk and high SCC, with or without udder damage and/ or bacteria. Intramammary infection (IMI) was determined by the presence or absence of bacteria only.

**Conclusions and Recommendations:**

The variability in SCC was largely unexplained, and an increased SCC did not necessarily indicate an intramammary infection in goats, as it does in cows. Therefore further, research is required to assess SCC and all possible factors affecting it. Further research is also required to find a more reliable method for mastitis diagnosis apart from SCC, for example, NAGase. The “Goatside” tests used (California Milk Cell Tests, CMCT) and SCC on their own were not reliable methods of mastitis diagnosis and should be accompanied by microbiological tests. However, CMCT and SCC were indicators of tissue tolerance and udder irritation. Tissue irritation is considered to indicate the limit of tissue tolerance. In healthy goats Spectrazol Milking cow caused the least tissue irritation, followed by Rilexine 200 LC, and Curaclox LC. However, for goats with clinical mastitis Rilexine 200 LC caused the least tissue irritation followed by Curaclox LC; and Spectrazol Milking cow caused the most tissue irritation in goats with clinical mastitis. Withdrawal periods of healthy goats and goats with clinical mastitis also differed for each product. Further research is necessary to determine withdrawal periods and tissue irritation of different intramammary products on goats with clinical mastitis.

Withdrawal period was affected by volume of milk produced, due to the dilution factor of continuous milk secretion. High producers had shorter withdrawal periods than low producers. However, treatment with intramammary antibiotics did not significantly affect the volume of milk produced. Further research is required to assess the effect of milk production volume on withdrawal periods when comparing withdrawal periods of different products. Antibiotic withdrawal periods on goat milk were different from those recommended for use in cattle for each of the products used and for the different intramammary antibiotics used. The withdrawal periods recommended for use cattle have a 24h safety margin added to the longest withdrawal period in the trial. In this research 24h safety margins were not added in the original tables. Therefore, in practice 24h safety margins should be added to all withdrawal periods in this research. Later the 24h safety margins were subtracted from the withdrawal periods recommended for use in cattle in order to obtain a rough estimate of the actual withdrawal periods in cattle. In this analysis all withdrawal periods measured by different methods for goats were significantly different from withdrawal periods recommended for use in cattle (-24h safety margin). However, in the original tables not all withdrawal periods for goats as measured by different methods were significantly different from those in cattle (with 24h safety margin).

Conductivity was found to be an unreliable “Goatside” test.

## CHAPTER 1: INTRODUCTION

### 1.1 Introduction

The aim of this study was to determine withdrawal periods and tissue tolerance of intramammary antibiotics (Curaclox LC, Spectrazol and Rilexine) measured in different ways and to evaluate the effects of related factors.

The demand for goat milk production is steadily increasing. This is due to two major factors:

- i) The production of specific cheeses from goat milk, with the distinct goat milk flavour (Jaubert & Kalantzopoulos, 1996).
- ii) The use of goat milk for babies which are intolerant to other sources of milk (Fisberg *et al.*, 2000).

An increasing need for high-quality protein to reduce malnutrition, especially in children, is a result of the rapidly growing population of Southern Africa. One source of high-quality protein that should be developed is milk production from dairy goats (Donkin, 1997). The use of dairy goats rather than cows for subsistence production by householders and smallholder farmers has many advantages. Dairy goats are more appropriate to the needs of subsistence production and their use would be in harmony with the concept of household economy (Low, 1986). Goats are cheaper; require less food; produce appropriate quantities of milk; breed at a younger age; have multiple births; are more easily handled by women and children; represent a smaller loss in the event of death; and produce a carcass of appropriate size for a household needs (Devendra & Burns, 1983). In contrast, dairy cows, as the traditional source of milk, are expensive, require sophisticated feeding and management to be productive, and may produce more milk than required for the household.

In animal production systems, the perceived value of a species increases in relation to its adaptation, capacity to make socio-economic contributions, response of the owners to market opportunities and the potential for an increase in productivity. In this context, the role and potential contribution of goats for increased productivity merits an improved understanding of their many attributes and functional values (Devendra & Morand Fehr, 2000). In the search for the efficiency in the improved use of goats, more enlightened thinking is necessary than in the past about their attributes. This should be backed by increasing resource use and interdisciplinary systems to increase productivity from goats, and by so doing, to enhance livelihoods of the poor and protect the environment (Devendra, 1999).

Goats account for about 30% of Africa's ruminant livestock and produce only 17% and 12% of its meat and milk respectively. Notwithstanding, goats have received relatively less attention in terms of research and development compared to cattle and sheep. As a result the impact of research of goat production in Africa so far has been minimal (Lebbie, 2000). Thus there is scope for goat research that could have an impact on productivity in Africa.

One of the problems identified in developing the use of dairy goats is their susceptibility to disease (Donkin, 1997).

One of the most threatening diseases to dairy goat production is mastitis (Jackson, 1980; Youzhang, 1996). At present mastitis in goats is treated with antibiotics used for treating bovine mastitis. Initially, assumptions were made that withdrawal times for antibiotic residues in milk are the same for goats as for cows (Debackere, 1995). However, limited research has shown that residues persist for a longer period in goat milk than in milk from cows (Bangen *et al.*, 1992). The milk may not be used during and after mastitis treatment, due to the long, probable withdrawal times of antibiotic residues in goat milk. Antibiotic residues in goat milk may pose a serious health hazard for humans consuming goat milk, as anaphylactic and allergic reactions may occur due to these residues, as well as the danger of development of resistant strains of bacteria. The production of cheese is also seriously affected by the presence of antibiotic residues in the milk. Therefore, the consequences of mastitis and antibiotic treatment lead to economic losses in the goat dairy farming industry (Youzhang, 1996).

Thus, research is necessary to determine the most appropriate antibiotic preparations used for the treatment of mastitis in goats as well as to establish appropriate withdrawal times for these

preparations. The most common mastitis bacteria that have been reported in goats are *Staphylococcus aureus* and *Staphylococcus epidermidis* (Pelant, 2000).

This research will assist in management of dairy goats in developing communities and also for commercial farmers. Milk is an ideal supplement to reduce malnutrition (Davidson *et al.*, 1984; Malentlema, 1987). Families may not be able to buy fresh milk or powder milk because of the cost or because it is not available. The obvious solution for people in rural areas is for them to increase milk production and utilization from the animals already available (Donkin, 1997).

## **1.2 The Main Problem Statement and Sub Problems**

The main problem to be investigated in this research is, to determine the withdrawal periods of antibiotic residues in goat milk. Mastitis in dairy goats affects milk production and use, thus affecting the goat dairy industry as a whole. It may be that the withdrawal period for antibiotic residues in goat milk is different from that in cow milk.

### **1.2.1 Measurement of Antibiotic Withdrawal Periods**

Goat milk may have different antibiotic residue withdrawal periods than those of cow's milk and this may be substantiated by the excretion of the blue dye included in a mastitis preparation such as Curaclox LC. Withdrawal periods will be calculated according to the Thermo Resistant Inhibitory Substances (TRIS) test and the Parallax test, (testing for cloxacillin and ampicillin residues) and compared with the withdrawal period of the blue dye in the milk.

### **1.2.2 Effect of The Presence of Bacteria**

Goats with and without intramammary infection may have different withdrawal periods for the same antibiotic residue in the milk. Further research is necessary to determine the distinction between clinical and sub clinical mastitis in goats.

### **1.2.3 "Goatside" Milk Tests**

"Goatside" tests (California Milk Cell Test (CMCT), conductivity) and TRIS, Somatic Cell Count (SCC) and microbiology may be influenced by the following factors: stage of lactation; lactation age (parity); udder health history; environmental factors (temperature and rainfall) and volume of milk production.

### **1.2.4 Effect of Milk Volume**

The withdrawal period of antibiotic residues may be affected by the volume of milk produced by goats, due to dilution.

### **1.2.5 Effect of Udder Irritation and Tissue Tolerance**

Udder irritation may differ in treated and untreated udder halves of goats with or without intramammary infection. This will be measured by SCC, CMCT and conductivity results of all samples from infected and non-infected goats. Udder irritation may differ in treated and untreated udder halves of goats with clinical mastitis compared to goats with healthy udders.

## **1.3 Hypothesis**

Antibiotic withdrawal periods for goat milk are different from those recommended for use in cattle.

## **1.4 Objectives**

The objectives of this project are the following:

- To compare if withdrawal times approved for cattle are applicable for goats.
- To establish whether the dye excretion of Curaclox LC, indicating the passage of antibiotic residues for cows, is the same for goats and whether this substantiates the hypothesis that goats have a longer withdrawal period than cows.



- To establish whether withdrawal times differ between goats with clinical mastitis and goats with healthy udders.
- To establish withdrawal times for three different intramammary preparations in dairy goats with clinical mastitis, in goats with intramammary infection and in goats without intramammary infection.
- To establish whether the withdrawal periods of antibiotic residues in goat milk differ according to stage of lactation, parity, volume of milk produced, udder irritation.
- To establish the most effective method for diagnosing intramammary infections in dairy goat herds before treatment, e.g.: SCC, CMCT and conductivity.
- To evaluate the degree of tissue irritation or tissue tolerance in the goat udder after administering each of these intramammary preparations.
- To establish if the treatment of intramammary antibiotic preparation affects the volume of milk produced and or the compositional quality of the goat milk produced.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Introduction

The production of cheese from goat milk has a very long history. In Homer's *Odyssey*, there is a vivid description of the manufacture of cheese by the beastly Cyclops, Polyphemos, perhaps the oldest recorded cheese maker in the world. Ipcrates (460-356) also mentioned the production of cheese from goats' milk (Jaubert & Kalantzopoulos, 1996).

The composition of goat milk varies widely and is influenced by: breed, nutritional and environmental factors, stage of lactation, parity and season (Tziboula-Clarke, 2002).

The composition of goat milk is different to that of milk from cattle or sheep. The milk of sheep is richer in fat and protein than that of dairy goats and cattle (Cross & Overby, 1988).

**TABLE 2.1: GROSS COMPOSITION OF MILK FROM GOATS, SHEEP AND COWS.**

| Species | Water (%) | Total solids (%) | Fat (%) | Total protein (%) | Casein (%) | Whey Protein (%) | Lactose (%) | Ash (%) |
|---------|-----------|------------------|---------|-------------------|------------|------------------|-------------|---------|
| Goat    | 85.2      | 14.8             | 5.6     | 3.8               | 3.1        | 0.7              | 4.8         | 0.7     |
| Sheep   | 80.7      | 19.3             | 7.4     | 5.5               | 4.6        | 0.9              | 4.8         | 1.0     |
| Cow     | 87.5      | 12.5             | 3.8     | 3.3               | 2.7        | 0.6              | 4.7         | 0.7     |

Galina *et al.*, (1996b), studied Somatic Cell Count (SCC), California Milk Cell Test (CMCT), milk acidity (pH and titratable acidity) and their relationship with artisan soft chevre-type goat cheese yield, using individual samples taken from Alpine goats over a seven year period. In this study, lactation stage affected cheese yield, CMCT and SCC although it did not affect pH or Dornic acidity (Galina *et al.*, 1996b).

Dairy goats have increased in their popularity and in their ability to produce large amounts of milk. With this increase in productivity has come a parallel increase in the prevalence and severity of mastitis and other diseases of the udder. Some goats are capable of producing 8L of milk a day. In France, some of the best does give more than 2000 L in 10-month lactation. As might be expected, udder problems in modern dairy goats parallel those seen in high-producing dairy cows (Smith & Roguinsky, 1977).

Limited information is available on goat lactation and milk production volumes, related to breeds, management and milk analysis.

In a study done on Alpine goats average milk production per doe per day was 2.69kg and milk volume was not affected by parity (Zeng & Escobar, 1995).

In a study done on Israeli Saanen goats, an average of  $1.5 \pm 0.3$  L of milk was produced per day, which contained  $18.2(\pm 2.6)$  g/L of protein,  $12.6 \pm 3.1$  g/L of casein,  $6.3 \pm 2.8$  g/L of whey proteins.

### 2.2 Mastitis

Mastitis is a serious disease affecting the milk production of the Saanen dairy goat (Al-Bassan & Hasso, 1996; De La Concha-Bermejillo *et al.*, 1998). The main causes of mastitis are poor hygiene during milking as well as inefficient use of milking machines (Joy *et al.*, 1989; Kingwill *et al.*, 1979).

In the past diagnosis of mastitis in goats has been done by using: SCC, CMCT, Conductivity meter, residue tests and screening tests (Contreras *et al.*, 1997b; Galina *et al.*, 1996a; Hart *et al.*, 1996; Morgante *et al.*, 2000; Paape, 2000; Zeng *et al.*, 1998). More research needs to be done to determine the most effective method for diagnosing both clinical and sub clinical mastitis.

Mastitis, or inflammation of the mammary gland of goats, is currently a broad diagnosis that may be based on changes in the physical characteristics of the udder or its secretion. Mastitis usually results from an infectious agent (Smith & Sherman, 1994). Goats are much less frequently affected by contagious mastitis than cattle, but mastitis may be an important sign in

many of the infectious diseases caused by major pathogens, minor pathogens, mycoplasmas and retroviruses.

### **2.3 Anatomy of the Goat Udder and Malformations**

#### **2.3.1 Normal Anatomy**

Each gland has a single large teat (Smith & Sherman, 1994), with six to nine large milk ducts joining to form the gland cistern, which ends at a single streak canal and single teat opening (Turner, 1952; Heindrich & Renk, 1967).

The glands are separated by a median suspensory ligament. The lateral laminae of the suspensory apparatus are lateral to the external pudendal vessels and attach to the symphyseal tendon caudally and the tunica flava abdominis cranially (Smith & Sherman, 1994). The mammary (superficial inguinal) lymph nodes are located deep to the lateral laminae and caudal to the external pudendal arteries (Garrett, 1988).

The major blood supply is provided by external pudendal artery. Venous return is via the external pudendal vein and the subcutaneous abdominal vein. The genitofemoral nerve, which passes through the inguinal ring, supplies most of the udder. Some skin innervation is also supplied by lumbar cutaneous nerves cranially and the mammary branch of the pudendal nerve caudally (Smith & Sherman, 1994).

The various suspensory ligaments of the udder should be strong and broad so that the udder is held tightly against the body with the floor of the udder above hock level (Considine & Timberger, 1978). Low slung udders are prone to injury and are also bruised by alternatively wrapping around one hind leg and then the other as the goat moves. Affected does and their offspring should be culled (Smith & Sherman, 1994). An enlarged, pendulous udder may be the result rather than the cause of mastitis (Addo *et al.*, 1980).

### **2.4 Immune System of the Cow**

#### **2.4.1 Defence Mechanism of the Udder**

##### **2.4.1.1 Primary Defence Mechanism: The Teat Canal**

The teat canal represents a physical barrier to the penetration of bacteria. When it is dilated the risk of ascending infection is high. The teat canal remains open after milking for approximately two hours. Therefore, animals should be allowed to stand outside and graze after milking in order to prevent them from lying down in soiled pens, which could increase the risk of an ascending bacterial infection. Post milking teat dipping disinfects the teat canal and thus reduces the risk of infection.

Epithelial desquamation and milk flow are mechanisms of the host to decrease local bacterial colonization. The keratin layer contains basic antibacterial proteins and antibacterial fatty acids. Specific immunological factors also play a role in the defence of the teat canal.

Lymphocytes and plasma cells accumulate beneath and between the epithelium of the teat canal wall, particularly around the Furstenburg rosette. This indicates local immunological activity. Neutrophil phagocytes directly penetrate the teat wall to the infected and inflamed teat canal. The infection and inflammation of the teat canal can be considered a pre-mastitis stage, at least for the staphylococci (Sandholm *et al.*, 1995).

##### **2.4.1.2 Humoral Antibacterial Factors**

Lactoferrin is a glycoprotein, which can bind two ions of ferric iron together with bicarbonate ion (Sandholm *et al.*, 1995). The lactoferrin content of milk increases markedly during mastitis.

Transferrin is the most important iron binding protein in the circulating body fluids. During inflammation, the liver increases production of transferrin, which passes from blood to milk, thus increasing the iron binding capacity of milk. (Sandholm *et al.*, 1995).

Lysozyme is a basic protein, which hydrolyses  $\beta$ -bonds within the peptidoglycan structure of the bacterial wall (Sandholm *et al.*, 1995).

Lactoperoxidase (LP) activity of Saanen goat milk is higher than that of indigenous goat milk (Seifu *et al.*, 2003). The function of the LP-system depends on the concentration of the substrate components: thiocyanate (SCN<sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Sandholm *et al.*, 1995).

Bacteria can protect themselves from various soluble and cellular antibacterial factors and from washout effects by either increasing their replication rate or by adhering to tissue linings (Sandholm *et al.*, 1995).

#### 2.4.1.3 Immunological Defence Mechanisms

The immune system of the mammary gland consists both of humoral and cellular components. Immunoglobulins, which contain specific antibody activity against antigenic stimuli, form the humoral component. The cellular component consists of several different cell groups, the most important of which are the macrophages and various lymphocyte subsets (Sandholm *et al.*, 1995).

#### **2.4.2 Humoral Immune System**

Humoral immunity is immunity mediated by antibodies contained in body fluids (humors) (Goldsby *et al.*, 2000).

The most important feature of antibodies in milk is their opsonizing ability. In addition, the antibodies neutralize toxins and are occasionally directly bactericidal. However, bacterial elimination, which takes place through phagocytosis, is considered the most important antibacterial mechanism of the udder.

The total amount of immunoglobulin varies during the stages of lactation, as does the relative proportion of the different Ig-subclasses (isotypes) (Sandholm *et al.*, 1995).

Infection by extra cellular bacteria induces production of humoral antibodies, which are ordinarily secreted by plasma cells in regional lymph nodes. The humoral immune response is the main protective response against extra cellular bacteria. Extra cellular bacteria can be pathogenic because they induce a localized inflammatory response or because they produce toxins (Goldsby *et al.*, 2000).

#### **2.4.3 Cellular Immunity or the Cell-mediated Immune Response**

Cellular immunity or the cell-mediated immune response is a host defence that is mediated by antigen-specific T cells and various non-specific cells of the immune system. It protects against intracellular bacteria, viruses and cancer and is responsible for graft rejection.

In a study on goats radial immuno-diffusion was used to determine immunoglobulin (Ig)-G concentrations in 16 mammary secretions from uninfected udder halves and in 10-14 secretions from halves sub clinically infected with coagulase negative staphylococci in goats throughout lactation. IgG concentrations in samples from uninfected halves decreased rapidly during the first week after parturition, thenceforth falling slowly up to 30 d post partum. From 30 d post partum to 180 d of lactation, IgG concentration showed a continuous decline to the end of the experiment but these changes were not statistically significant until 150 d after kidding. IgG concentration in lacteal secretions of halves harbouring coagulase negative staphylococci showed a similar pattern, from the first month of lactation, to that observed in healthy udder halves, but concentrations were always lower. These differences were significant from two months after parturition (Ferrer *et al.*, 1997). The results of the above study are in disagreement with several studies developed in bovine animals, where an increase of IgG (IgG<sub>1</sub>, IgG<sub>2</sub>) in milk from infected glands (Caffin *et al.*, 1983; Hidioglou *et al.*, 1992) and positive correlations between IgG concentrations and SCC have been reported (Caffin & Poutrel, 1988; Rogers *et al.*, 1989).

#### **2.4.4 Immune Response to Infectious Diseases**

If a pathogen is to establish an infection in a susceptible host, a series of coordinated events must circumvent the non-specific (innate) and specific (adaptive) host defences.

Depending on the number of organisms entering and their virulence, different levels of host defence are enlisted (Goldsby *et al.*, 2000).



## **2.5 Basic Caprine Immunology**

There are no reported inherited immunodeficiencies in goats. Acquired immune-mediated diseases are uncommon. The most important immunological disease of goats is that shared with other ruminant species, failure of passive transfer (FPT) of immunoglobulins to the newborn via the dam's colostrum.

Information on caprine immunology is limited and in most texts the caprine immune system is not addressed directly, but is usually covered in general discussions on the ruminant immune system (Smith & Sherman, 1994).

### **2.5.1 Immunoglobulins**

The categories and distribution of caprine immunoglobulins fit the general ruminant pattern (Butler, 1986). The major classes of immunoglobulin identified in the goat are IgG, IgA and IgM. There are two IgG subclasses: IgG<sub>1</sub> and IgG<sub>2</sub>, as for cattle and sheep (Gray *et al.*, 1969). The main immunoglobulin in goat colostrum IgG<sub>1</sub>, is transported preferentially into the mammary gland from serum (Micusan & Borduas, 1976). IgG<sub>1</sub> is also the predominant circulating serum antibody produced in response to infection (Micusan & Borduas, 1977). Caprine IgA has been isolated from serum, colostrum, milk, saliva and urine (Smith & Sherman, 1994).

### **2.5.2 Cell Mediated Immune System**

Distinct populations of B and T lymphocytes have been identified in goats and sub-populations of T lymphocytes also have been identified on the basis of reactivity and non-reactivity to peanut agglutinin (PNA) (Banks & Greenlee, 1982; Sulochana *et al.*, 1982). There are several reports on optimization, kinetics and application of the in vitro lymphocyte transformation or blastogenesis assay for the measurement of lymphocyte responses using standard mitogens, specific antigens, such as CAE virus, steroids and allogenic lymphocytes (DeMartini *et al.*, 1983; Greenlee & Banks, 1985; Staples *et al.*, 1981; Staples *et al.*, 1983; Van Dam *et al.*, 1978).

Normal caprine neutrophil function has been evaluated in female goats using a variety of indices including migration, chemotaxis, bacterial ingestion, cytochrome C reduction and antibody-dependent, cell-mediated cytotoxicity (Maddux & Keeton, 1987). Selenium deficiency in goats has been shown to have adverse effects on caprine neutrophil function (Aziz *et al.*, 1984). There is very little information available on characterization and function of caprine macrophages and non-neutrophil leukocytes (Smith & Sherman, 1994).

### **2.5.3 Cytokines**

Interleukin 1 occurs in the plasma of goats during bacterial-induced febrile episodes (Verheijden *et al.*, 1983). Other studies have demonstrated the existence and activity of neutrophil chemotactic factor, leukocyte migration inhibition factor and interleukin 2 in goats (Aziz & Klesius, 1985; Aziz and Klesius, 1986a; Aziz and Klesius, 1986b).

### **2.5.4 Major Histocompatibility Complex (MHC)**

The MHC in goats is called the goat lymphocyte antigen (GLA) system. Both serologically defined (SD) class I and lymphocyte defined (LD) class II antigens have been identified. Three distinct gene clusters appear to be involved in expression of the GLA; an SD<sub>1</sub> an SD<sub>2</sub> and LD, producing as many as 27 class I antigen specificities (Ruff & Lazary, 1987). The degree of humoral immune response has been associated with GLA type. Increased antibody responses to tetanus toxoid were demonstrated in goats with GLA-SD<sub>1-2</sub> and SD<sub>1-4</sub> specificities (Van Dam & Van Kooten, 1980).

## **2.6 Milking Machine**

### **2.6.1 Influence of Vacuum Level and Over-milking on Udder Health and Teat Thickness Changes**

Research work carried out in cows has demonstrated that faulty design and/ or use of milking machine can increase the rate of new IMI and elevate the SCC of tank milk (Hamann *et al.*, 1996; Zecconi *et al.*, 1996).

An aspect widely studied in cows is the relationship between over-milking and mastitis, although results obtained have not always been in agreement (Hamann *et al.*, 1994b; O'Shea, 1987). Over-milking causes teat tissue congestion leading to oedematation (Hamann, 1990; Isaksson & Lund, 1992) and in field conditions has been associated with a deterioration in teat end condition (Huusko *et al.*, 2002; Osters *et al.*, 1990).

Many technicians currently consider that, at field level, a milking vacuum greater than 40kPa and the presence of over milking are factors that tend to increase mastitis rates and SCC in tank milk in ewes. However according to Peris *et al.*, (2003) with ewes of medium productive level (1L/d of milk on average) and in conditions that may be considered suitable in terms of the milking machine and handling of milking, there was no significant effect of the vacuum levels and over-milking, in the short term, on IMI rates and on SCC of the milk.

Some studies on ewes have found that as vacuum level was decreased to values around 40kPa or lower, the SCC also tended to diminish (Le Du, 1983; Pazzona *et al.*, 1993), although this effect was not reported in other cases (Molina *et al.*, 1999a). It was reported that at both vacuum levels (36 and 42 kPa) the teat cup fall-off rate was similar and, under normal milking conditions (not over-milking), teat thickness changes were not affected (Marnet *et al.*, 1996). In contrast, the information available for cows indicates that in the interval from 25 to 50 kPa, as milking vacuum rises, teat thickness changes also increase, indicating a greater congestion/oedema of the teat (Hamann *et al.*, 1993).

All milking machine settings (for example, pulsation phases, liner size and cluster weight) need to be considered and not just vacuum level.

### **2.6.2 Vacuum Level**

In mechanical milking, a relevant aspect that may influence the state of udder health is the vacuum level existing in the teat (Osteras & Lund, 1988).

In cows, Hamann & Mein, (1996) concluded that changes of teat thickness up to 5% are normal. Results of Peris *et al.*, (2003) indicated that in the short term a moderate over milking does not in itself seem to have any important effect on IMI rate in goats. This conclusion is in agreement with that expressed by Hamann *et al.*, (1994a) in cows or that encountered by Molina *et al.*, (1999b) in dairy ewes. Nevertheless, bearing in mind the results and opinions of other authors studying cows (Bramley, 1992; Hamann *et al.*, 1994b; O'Shea, 1987), doubt remains concerning the effect that over-milking may have on the teat canal and the udder.

When a commercial goat dairy installs or modifies a machine milking system, attention should be given to choosing an efficient low-line system that milks goats under low vacuum with minimal vacuum fluctuation. Vacuum levels of 38.9 to 40.6 kPa are adequate for low-line systems, but higher vacuum is needed for high-line systems (Smith & Sherman, 1994). Machines available for milking goats in France are operated at comparable vacuum levels (40kPa to 51kPa) (Darracq, 1973). (Another vacuum unit in use in the literature cm Hg, 1kPa equals 0.75 cm Hg.)

The vacuum pump should have a capacity of 30 Cubic Feet per Minute (CFM) for the pipeline system, 1.5 CFM per unit and three to four CFM reserve air flow (Smith & Sherman, 1994). Bucket milker systems require 10 CFM reserve and 1 CFM per unit (East & Birnie, 1983; Spencer, 1984). (28 L/min free air is approximately equal to 1 CFM.)

### **2.6.3 Pulsation Rate**

Pulsation rates of 70 to 100 per minute and pulsation ratios of 50:50 and 70:30 (milk: rest) have been recommended for goats (Le Du, 1987). In a study that attempted to optimise milking rate and SCC, a pulsation ratio of 60:40, a pulsation rate of 90 per minute and a vacuum level of 45 to 52 kPa were judged to be optimal (Lu *et al.*, 1991).

Inflations (teat cup liners) should be replaced regularly (Smith & Sherman, 1994).

#### 2.6.3.1 Milking Procedures:

The method of udder preparation for milking is very important in determining the incidence of new cases of mastitis in goats, as it is in cows (Smith & Sherman, 1994). Hands and udders should be clean and dry to prevent bacterial transfer. Animals with mastitis or with skin lesions on the teats should be milked last, with appropriate sanitation of hands between infected animals.

Goats should be milked gently and in quiet surroundings to encourage oxytocin release and milk letdown reflex (Smith & Sherman, 1994). The biological half-life of oxytocin has been calculated to be approximately 22 minutes in goats (Homeida & Cooke, 1984). The pre-milking stimulation afforded by udder washing is not necessary for goats to milk out completely (Smith & Sherman, 1994). Research has demonstrated no effect of udder washing on milking time or milk yield (Ricoardeau & Labussiere, 1970). Vigorous stripping by hand or by machine should be avoided (Smith & Sherman, 1994). Over-milking in late lactation does may contribute to the risk of mastitis (East *et al.*, 1987). Restraining goats during milking by pulling on teats has been proposed as one reason for a high incidence of gangrenous staphylococcal mastitis in Cyprus (Petris, 1963).

Goats should be milked regularly, but equal 12 hour intervals are not necessary, but preferable. The goat has a relatively large gland cistern for holding milk (Smith & Sherman, 1994). Preliminary research in France suggests that the speed of milking is under genetic control, with the goats homozygous for recessive gene 'hd' having the most rapid milk flow (Bouillon, 1990).

### 2.7 Residues in Milk

“Drug residues” mean the total quantity of both the parent drug and all its metabolites in the edible tissues or meat from food producing animals. Residues of the parent drug and/or its metabolites may accumulate or be deposited within the cells, the tissues or secretions of an animal following its use (Debackere, 1995).

As milk is an essential foodstuff especially for babies and young children and it is widely consumed as such or in the form of various milk derivatives, attention must be given to the presence of drug residues and especially antibiotic residues in the milk because of their possible pharmacodynamic properties.

Goat milk with antibiotic residues cannot be used for human consumption as it poses a health hazard and affects the production of cheese. Thus, antibiotic residues in the goat milk leads to economic losses in the goat dairy farming industry (Debackere, 1995; Youzhang, 1996).

Antibiotic residues in goat milk affect the production of milk and milk products (Heeschen & Bluthgen, 1991, Klinger & Rosenthal, 1997).

Lower limits of detection for antibiotics (ampicillin, cloxacillin, sulfonamides and cephalosporins) in milk have been documented. The acceptable level of residues of penicillin in milk is 0.006 (mg/kg of moist substance). Withdrawal times for milk after intramammary antibiotic for  $\beta$ -lactams is: 60-96h in the USA, 72-96h in Canada and 96-144h with a safety margin of an additional one to two milking times; for aminoglycosides withdrawal times are 48h, 96h and 288h respectively. In general in countries where no regular tests for antibiotics are carried out, the percentage of inhibitor positive samples (herd milk) may reach 1-10%. This study was carried out on dairy cattle (Heeschen & Bluthgen, 1991).

Residues which remain, in the tissues and in secretions, especially in the milk and which ultimately becomes part of the human diet, are a problem to the safety of the consumer.

Adequate pharmacological and toxicological evaluation of the parent drug administered and its residues in edible products are essential.

The same antibiotics used for treatment of bovine mastitis are used for treatment in goats as well. However, research suggests that residues persist for a longer period in goat milk than in cow milk.

### 2.7.1 Withdrawal Periods: Udder Residues

Withdrawal times were introduced as a means for farmer and veterinarian to determine the excretion time of drugs applied to animals without specific analysis. The withdrawal time may be defined as the period following the last medication that is required to bring the concentration of the drug to below a tolerable value. The tolerable value of a drug depends on the Acceptable Daily Intake (ADI), which is based on pharmacological studies in experimental animals and is the lowest dose that is found to give some disorder, divided by a large safety factor, e.g. 100 or 1000. Many parameters have to be studied to establish the ADI value. From the ADI and the average amount of food taken by humans, the maximum residue level (MRL) permissible in foodstuffs from animal origin, such as meat or milk, is calculated.

**TABLE 2.2: THE MAXIMUM RESIDUE LIMITS (MRLS) AND SAFE/TOLERANCE LEVELS FOR RESIDUES OF ANTI-MICROBIALS IN MILK FIXED BY CODEX ALIMENTARIUS, EUROPEAN UNION (EU) AND FOOD AND DRUG ADMINISTRATION (USFDA). (1996) (Honkanen-Buzalski & Reybroeck, 1997)**

| Anti-microbials        | MRL Codex (mg/kg) | MRL EC (mg/kg) | Safe/tolerance FDA (mg/kg)            |
|------------------------|-------------------|----------------|---------------------------------------|
| Ampicillin             | 4                 | 4              | 10/10                                 |
| Cloxacillin            | 4                 | 30             | 10/10                                 |
| Ceftiofur              | 100               | 100*           | 50 <sup>(a)</sup> /100 <sup>(b)</sup> |
| Cephapirin             | 100               |                | 20/20                                 |
| Neomycin (+framycetin) | 500*              | 500*           | 150/150                               |

\*preliminary compounds on agenda, (a) parent drug, (b) total parent and metabolite

Companies producing veterinary medications, containing drugs, are required to carry out experimental studies to show how long residues of the active component(s) can be detected in animal tissue or fluids. Each medication should be tested separately even when the active ingredient is the same, because other ingredients used in the formulation may well have an effect on the excretion pattern. Therefore separate studies must be carried out for each animal species for which the medication is recommended. However withdrawal periods for the antibiotics used in this study are only available for use in cattle. Therefore this study was necessary to determine withdrawal periods of these antibiotics for use in goat milk after antibiotic administration.

Based on the results obtained from withdrawal period trials, withdrawal times are established and following approval by health authorities are included in the labelling and the technical documentation. These values have official status; in most countries it is stated by law that the owner of the animals should observe these withdrawal times and if this is not done is liable to a penalty (Beukers & Gist-Brocades, 1995).

In one study using commercial lactating cow infusion tubes in the United States, erythromycin, oxytetracycline, penicillin and cephapirin were administered according to labeled directions to 10 goats (Smith & Sherman, 1994). Antibiotic residues were not detected after the labeled withdrawal periods (36 h, 96 h, 60 h and 96 h respectively) except for one goat that still had detectable penicillin after 72 h (Long *et al.*, 1984). Another study found that oxytetracycline (426mg) was still detectable at 108 h and cloxacillin (200mg) at 156 h after last treatment (Hill *et al.*, 1984). A commercial combination product (200mg amoxicillin trihydrate, 50mg mg potassium clavulanate and 10mg prednisolone) labelled with a 48 h withdrawal period in dairy cows required 112 h withdrawal period to achieve acceptable amoxicillin concentrations in goat milk (Buswell *et al.*, 1989). Some authors suggest it may be prudent to at least double the recommended bovine withdrawal period when treating goats (Smith & Sherman, 1994). Antibiotic residue withdrawal periods in healthy goats after intravenous or intra-muscular treatment have been reported by Ziv (1984).

As the sensitivity of assays in use increases it can be expected that the withdrawal period will lengthen (Smith & Sherman, 1994). It is also important to remember that detectable concentrations of antibiotics appear in milk from the untreated half (Hill *et al.*, 1984). Even when the residue levels are too low to endanger any except the most allergic human consumers, a cultured cheese may fail because of antibiotics in the milk. Ideally, milk from treated does should be tested at a milk laboratory with the most sensitive antibiotic test available; milk with positive test results should not be used for human consumption (Smith & Sherman, 1994).

### **2.7.2 Disadvantages in the Use of Withdrawal Times**

- Many studies in experimental animals are required.
- It is usually not possible to carry out such studies with diseased animals, as the number of animals available with the same type of disease within a certain period of time is too limited to allow statistical evaluation. For this reason most studies are carried out with healthy animals, although it is known that certain diseases may have considerable effect on the excretion rate of the drug.
- Differences in findings of the pharmacological studies or interpretation thereof will lead to different ADI values, thus different MRL's. So, for instance, the MRL for sulpham compounds in European countries is 100ppb, but in the USA it is 10ppb. Also for the most commonly used  $\beta$ -lactams like penicillin and cloxacillin, MRL values differ. These different legal requirements in different regions will result in different withdrawal periods.
- Despite the large amount of work that is carried out, the withdrawal times as determined often do not reflect the actual situation because of the large individual variation between animals and because variations in treatment and type of disease (Beukers & Gist-Brocades, 1995).

### **2.8 The Mammary Gland and the Passage of Veterinary Drugs into Milk**

The parenchyma of the mammary gland is organized into a multi-compartment organ with aggregations of alveoli partitioned off into lobules; and these lobules, drained off by a collecting duct, form a lobe and the lobes in turn make up the gland. The walls of the alveoli and the finer ducts consist of a single layer of epithelial glandular cells. This epithelium is a membrane, which separates blood of pH 7.4 from milk, which has a lower pH value in the range of 6.5 to 6.8 (Baggot, 1977).

There are some pharmacokinetic models that permit calculation of pharmacokinetic parameters for antimicrobials in milk as a function of the dose and the chemical properties of the drug (Debackere, 1995).

Three parameters are estimated to be important: (Kaplan, 1983)

- The relation between the simultaneous concentration in the milk and the blood plasma: concentration of plasma ultra filtrate/ concentration of milk ultra filtrate. As milk has a lower pH than blood, the phenomenon of ion-trapping will also take place between these two compartments and the relation will be  $> 1$  for acids and lower than 1 for bases, i.e. bases will be more concentrated than acids in milk.
- The percentage of the dose of antibiotic found in milk, for example macrolides, which are basic substances, show a mean value of 1% whereas for penicillins and aminoglycosides, which are acids, this number is only 0.001%.
- The total amount of the antimicrobial, which appears in the milk during the whole time of treatment. This is calculated from the area under the curve (AUC). Area Under the Curve (AUC) is the total area under the plasma concentration curve and is obtained through the plasma concentration of the remedy plotted against time. The bigger this surface, the greater the amount of the drug and/or its metabolites in the milk (Ziv, 1978).

### 2.8.1 Ion-trapping

Since the passage of drugs across biological membranes is a free-diffusion process, there should be no further transmembranous movement when the concentration of non-ionised drug becomes the same on both sides of the membrane. However between most tissues there exists a pH gradient across the membrane, and therefore unequal concentrations of the drug will be attained at both sides. At equilibrium, there will be a higher total concentration of drug on the side of the membrane where the degree of ionisation is greater. This mechanism is known as ion trapping.

As there is a pH gradient between the bloodstream and the mammary gland, the mechanism of ion trapping will occur, and as the milk is consumed in the daily diet, the quantity of residues from veterinary drugs in milk will be of great importance (Debackere, 1995).

### 2.8.2 Routes of Mastitis Therapy

#### 2.8.2.1 Intramammary Mastitis Therapy

This is by far the most common method of treatment of bovine mastitis. Reasons for poor results with intramammary therapy include poor distribution due to local oedema or poor penetration of the drug, and washing out of the antimicrobial due to regular milking (Petzer & Lourens, 2003).

Research has been done on intramammary antibiotics (Penicillin, Nafcillin and Dihydrostreptomycin in dairy sheep treated with Nafpenzal<sup>R</sup> DC), used for mastitis treatment in sheep at drying off (Lohuis *et al.*, 1995a; Lohuis *et al.*, 1995b).

**TABLE 2.3: ANTIMICROBIAL DRUGS CLASSIFIED ACCORDING TO THEIR POTENTIAL DISTRIBUTION THROUGH THE UDDER AFTER INTRAMAMMARY ADMINISTRATION (Petzer & Lourens, 2003).**

| Good Distribution | Limited Distribution | Poor Distribution |
|-------------------|----------------------|-------------------|
| Ampicillin        | Cloxacillin          | Neomycin          |
| Cephalexin        |                      |                   |
| Cefuroxime        |                      |                   |

The following influences on the persistence of antibiotic residues in the mammary gland and the milk after intra-mammary administration have to be taken into account.

1. The velocity of the release of the antibiotic from the excipient. This is much quicker when using aqueous solutions in comparison to oily preparations (Mercer *et al.*, 1976).
2. The binding of antibiotics to the udder tissues. This can reach a very high percentage for antibiotics such as spiramycin, neomycin and colistin (Ziv, 1978).
3. The percentage of antibiotic into the cell. However there must be a distinction made between passive diffusion and accumulation (Tulkens, 1991).
  - Aminoglycosides penetrate very slowly and accumulation follows some days later so that the intracellular concentration reaches three to four times the extracellular one.
  - $\beta$ -lactam antibiotics penetrate rather quickly but do not accumulate.
  - For the lincosamides there is a big difference between lincomycin that does not accumulate and clindamycin that reaches 15 to 20 times higher intracellular than extra cellular concentrations, just as for pirlimycin.
  - Macrolides also show a significant intracellular accumulation.

Factors with a possible influence on the excretion of antibiotics after intramammary application can be:

- The dose administered.
- The number of quarters treated.
- The number of treatments.
- The milk production.
- The condition of the udder.

- The excipient.
- Combination of treatments (e.g. anti-inflammatory and antibiotic).
- Health status of animal.

#### 2.8.2.2 Parenteral Mastitis Therapy

The presence of residues in milk after administration of oxytetracycline and ampicillin preparations via the intramuscular route has been researched in dairy goats (Fagiolo *et al.*, 2000a; Fagiolo *et al.*, 2000b).

The pharmacokinetics in ruminants is complicated by the fact that the volume of the Gastro-Intestinal Tract (GIT) exceeds the extra-cellular volume in the dairy cow. This leads to antibiotic disappearing in the GIT or being inactivated by the liver and not reaching the infectious foci when administering via the parenteral route.

The extent to which a drug gains access into the milk, via circulation, depends on the lipid solubility, the degree of ionisation and the extent of protein binding with serum.

The ideal antibiotic intended for parenteral mastitis therapy should have the following characteristics:

- Low Minimal Inhibitory Concentration (MIC) against the major udder pathogens
- High bio-availability from the intra-muscular injection site
- Chemically a weak base
- Sufficient lipid solubility
- Low degree of serum binding
- Long half-life in the body
- Little or no drug accumulation in specific organs
- Currently no single antibiotic meets all these requirements (Petzer & Lourens, 2003)

#### 2.9 Antimicrobials

Antibiotic contamination from intramammary or systemic medication of the goat or from consumption of “medicated” feeds may lead to allergic reactions in sensitive people, interference with cultured products, or regulatory action against the producer. Because withdrawal periods have rarely been established for goat milk, especially for sick animals, veterinarians should advise exaggerated withdrawals (longer than for dairy cattle) to allow for different rates of metabolism or excretion. Toxic parasiticides that have not been approved for dairy cows should not be used for dairy goats either (Smith & Sherman, 1994).

Sulfonamides behave as weak organic acids (Booth & McDonald, 1988). It is normally accepted that sulfonamides show half-lives of excretion in cattle ranging from 2 to 10 hours (Vree & Heckstern, 1985). Sulfonamides have a poor penetration of the blood udder barrier (Paulson *et al.*, 1994).

Bactericidal and bacteriostatic antibiotics when combined might be antagonistic (Table 2.4). Penicillin therapy should not be applied together with sulfonamides or tetracyclines: the bacteriostatic effect of sulfonamide slows down the bacterial cell wall synthesis and decreases the effect of penicillins. Penicillins are active against rapidly multiplying bacteria, which actively synthesize their cell wall (Sandholm *et al.*, 1995).

## 2.9.1 Classification of Antibiotics

**TABLE 2.4: ANTIBIOTICS USED IN TRIALS 1, 2 AND 3.**

| Group                          | Active Ingredients                       | Product    | Bacterial Affinity | Working Mechanism                             | Time or Dose dependant |
|--------------------------------|--|------------|--------------------|---|------------------------|
| Beta Lactam:<br>Penicillins    | Cloxacillin                              | Curaclox   | Bactericidal       | Inhibit synthesis of cell wall.               | Time                   |
|                                | Ampicillin                               | Curaclox   | Bactericidal       | Inhibit synthesis of cell wall.               | Time                   |
| Beta Lactam:<br>Cephalosporins | Cephalexin<br>1 <sup>st</sup> Generation | Rilexine   | Bacteriostatic     | Inhibit synthesis of cell wall.               | Time                   |
|                                | Cefuroxime<br>2 <sup>nd</sup> Generation | Spectrazol | Bacteriostatic     | Inhibit synthesis of cell wall.               | Time                   |
| *Aminoglycoside                | Neomycin                                 | Rilexine   | Bactericidal       | Inhibit synthesis of protein at ribosome 30S. | Dose                   |

\* Aminoglycosides should not be used as they may cause kidney damage.

### 2.9.1.1 Classification of Antibiotics according to Frequency of Dosing(Le Roux, 2004c).

The dosage and frequency of antibiotic therapy must be planned in such a way that effective concentrations are reached for long enough periods of time, in order to eliminate or inhibit the pathogen so that the immune system can control the infection.

The Minimal Inhibitory Concentration (MIC) and the amount of time at which this concentration must be kept in order to eliminate the pathogen, must be evaluated to determine frequency of dosing.

$C_{max}$  is the highest concentration of antibiotic reached in plasma or a specific tissue.

Area Under the Curve (AUC) is the total area under the plasma concentration curve and is obtained through the plasma concentration of the remedy plotted against time. The AUC to IV administration of a remedy represent “the total amount” in the systemic circulation and is equal to a 100% bioavailability. This should be compared to the AUC for intramammary, IM, SC, or oral administration. Bioavailability is the percentage of the remedy available after administration through specific routes (different to IV) comparable with IV-administration of the same amount.

Time-dependent remedies require a dosing interval that will allow an extended time period in which the remedy concentrations are above the MIC levels of the pathogen in order to be effective. All the bacteriostatic antimicrobial remedies and the  $\beta$ -lactams fall in this group.

The  $\beta$ -lactams are also classified in this group because the cell wall of the bacteria becomes saturated with the  $\beta$ -lactams and increased concentrations do not have a stronger effect.

Increasing the in-vitro concentration of a  $\beta$ -lactam to as high as 4 x the MIC does not increase the effectiveness of the remedy. The plasma concentration of the remedy must remain above the MIC-levels for at least 80% of the dosing interval.

Post Antibiotic Effect (PAE) is the temporary suppression of bacterial growth after antibiotic treatment. It is measured as the time necessary for a bacterial culture to regain normal logarithmic growth after antibiotic treatment. After treatment, antibiotics have a characteristic effect on susceptible organisms even though they are no longer present, or only present at concentrations lower than the MIC. Increased PAE is characteristic of Gram-positive organisms and explains why bacterial growth does not resume immediately when the concentration of the therapeutic agent falls under the MIC. PAE has clinical relevance in relation to dosing intervals (Le Roux, 2004c).



### 2.9.1.2 Classification of Antibiotics according to Water-lipid Solubility

**Water-soluble compounds:** The concentration of the compounds in the extra cellular liquid compartment is approximately equal to the intravascular concentration. A water-soluble product is usually not the remedy of choice for intracellular agents such as *Mycobacterium*, *Brucella*, *Chlamydia*, *Rickettsia* and *Bartonella* or facultative intracellular agents such as Staphylococci. Water-soluble remedies typically have a weak penetration capacity in the prostate gland, the udder, central nervous system, bronchial secretions and in the eye. An exception in this group is a remedy that is highly protein bound like the third generation cephalosporins (Le Roux, 2004c).

**Lipid soluble compounds:** These compounds tend to move quickly through a tissue and to reach high tissue concentrations. Homogenised tissue concentrations should be compared directly with MIC's when trying to measure if the effectiveness is accurate. It cannot be assumed that the concentration of the extra cellular liquid is equal to the "tissue concentration". It could be the case that these remedies are less concentrated where they have the greatest effect. A lipid soluble compound has a preference for intracellular pathogens. Antibiotics that reach high concentrations within leukocytes and other cells are: fluoroquinolone, lincosamides, macrolides and azithromycin (Le Roux, 2004c). Antibiotics with high lipid solubility are macrolides, fluoroquinolone, florfenicol and doxycycline (Le Roux, 2004c).

Those with intermediate lipid solubility are tetracyclines, sulphas, trimethoprim, and lincosamides.

Those with low lipid solubility are aminoglycosides,  $\beta$ -lactams (Le Roux, 2004c).

### 2.9.1.3 Additional Factors that Affect the Choice of Remedies: (Le Roux, 2004c).

- The micro-environment around the site of infection.
- Physiological changes present in the patient.

Conditions to keep in mind in pharmacokinetic functioning of antibiotics:

- Changes in liver function.
- Changes in kidney function.

**TABLE 2.5: DIFFERENT GENERATIONS OF CEPHALOSPORINS (Le Roux, 2004d).**

| Generation | Examples  |
|------------|---|
| First      | <i>Oral:</i> cephadrine, cefadroxil, cephalixin, cephaloglycin.<br><i>Parenteral:</i> cephacetrila, cefapirin, cefazolin, cephalothin.                                    |
| Second     | <i>Oral:</i> cefachlor, cefuroxime axetil.<br><i>Parenteral:</i> cefamandole, cefonicid, ceforanide, cefuroxime, the cephamycins: cefoxitin, cefotetan, cefmetazole.      |
| Third      | <i>Oral:</i> cefixime, cefpodoxime, ceftibuten.<br><i>Parenteral:</i> cefmenoxime, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, latamoxef, ceftiofur. |
| Fourth     | <i>Parenteral:</i> cefepime, cefsulodin, cefpirome, cefpiramide, cefquinome.  |

### **2.9.2 Influence on Factors Inherent to the Application of Antibiotics**

A number of factors influence the quantities excreted and the duration times of excretion (Debackere, 1995).

1. **The Nature of the Antibiotic Involved:** The acid or basic character of the antibiotic can be of especial importance. Amino glycosides, which are also basic, show relatively low milk concentrations as they have a high polarity and low lipid solubility.
2. **The Effect of the Dose Administered:** For procaine penicillin an increase from 3000 000 U to 6000 000 U showed a 30% longer excretion time. Doses of 20 000U/kg showed a withdrawal period of 3.5days whereas 30 000U/kg extended this period to 4.5 days. This seems to have an effect on the excretion time, although it is only to a small degree (Debackere, 1995).

3. The Influence of the Excipient: Aqueous solutions take longer to excrete compared to oil suspensions. For penicillin this amounts to an increase of 125%. This longer excretion time is due to the slower absorption of water-soluble remedy at the injection site, and not at all to the process of excretion. Once absorbed, the excipient has no more influence on the excretion (Debackere, 1995). Active ingredients and carrier substances of the products used in this experiment were as follows:
  - Curaclor LC:
  - Semi-synthetic penicillin based intramammary preparation (Curaclor LC, which contains 75mg sodium ampicillin and 200mg sodium cloxacillin per dose plus blue dye). Curaclor LC G2615, (Norbrook (Pharmacia AH) P.O. Box 10698 Centurion, 0046), cloxacillin 200mg, ampicillin 75mg, blue dye/ 4.5g syringe. The carrier substances for the above product were: liquid paraffin, soft white paraffin and Tween 80, which therefore form an oil solution.
  - Spectrazol:
  - A cefuroxime 250mg based intramammary product (Spectrazol Milking Cow, Schering-Plough). Spectrazol milking cow, cefuroxime, 250mg, S4 Intramammary Injection 83/594, (Schering-Plough Animal Health, P.O. BOX 46, Isando, 1600). The carrier substances were, a succilynated fatty acid and triglycerides, which is therefore an oil solution.
  - Rilexine:
  - A cephalixin 100mg, neomycin sulphate 100mg and prednisolone based intramammary product, Rilexine (SA) 200LC injection 83/638, (Logos Agvet (Virbac), Private bag X115, Half-Way House, 1685). The carrier substances for this product are, Butylated hydroxyanisole and benzyl alcohol.
4. Number of Milkings per day: This factor seems to have no important influence on the excretion of most antibiotics (Debackere, 1995).
5. The Plasma-Protein Binding: Systemic Route: The passage of each antibiotic into milk is also determined by the plasma-protein binding. As the binding of antibiotics in bovine serum varies considerably, the passage through the milk-barrier will also be very different. These factors will perhaps decrease the peak concentrations of residues in milk but extend the duration of their excretion.
6. The Physio-Pathological Condition of the Udder: In the case of mastitis, the pH of the milk ultra filtrate will be more alkaline than the normal conditions, where the pH is lower. Hence the following differences can be seen between normal milk and mastitis udder secretion (Barragry, 1994; Debackere, 1995).
  - Procaine-penicillin shows a three times higher concentration after 24 hours and the duration of the excretion period increases by 17%.
  - Macrolide antibiotics, with a basic character, show a lower concentration in ultra filtrate from goats with mastitis.

Conclusions that can be drawn include the following: (Barragry, 1994)

- Weakly acidic antibiotics are present in milk at lower concentrations than in serum, but their distribution is shifted toward the udder in cases of mastitis.
- Weakly basic drugs can achieve higher concentrations in milk, but this effect is reduced in mastitis.

Factors, which can change the period of excretion and the amount excreted, are predominantly limited to:

- The dose.
- The excipient.
- The condition of the udder.
- The route of administration (Debackere, 1995).

### 2.9.3 Antibiotic Resistance (Le Roux, 2004a; Le Roux, 2004d).

It has been suggested that banning of antibiotics in food animals may harm both human and animal health. Surveys have shown variation in data concerning resistant bacteria in Europe and the USA. This implies that it is unlikely that there is or has been widespread transference of resistant bacteria via the food supply.

Bacterial resistance against penicillins develops through degradation of bacterial enzymes, change in penicillin binding places and in decreased penetration ability.

**TABLE 2.6: GRAM-POSITIVE BACTERIA IDENTIFIED WITH ANTIBIOTIC RESISTANCE (Le Roux, 2004d).**

| Aerobic                             | Anaerobic          |
|-------------------------------------|--------------------|
| <i>Actinomyces pyogenes</i>         | <i>Clostridium</i> |
| <i>Listeria monocytogenes</i>       |                    |
| <i>Erysipelothrix rhusiopathiae</i> |                    |
| <i>Bacillus anthracis</i>           |                    |
| <i>Campylobacter</i>                |                    |

Staphylococci and  $\beta$ -haemolytic Streptococci produce increased activity against penicillinase.

**TABLE 2.7: GRAM NEGATIVE BACTERIA IDENTIFIED WITH ANTIBIOTIC RESISTANCE (Le Roux, 2004d).**

| Aerobic   | Anaerobic   |
|---|---|
| <i>E.coli</i>   | <i>Fusobacterium</i> with increased activity against <i>Bacteriodes</i> |
| <i>Haemophilus</i>  |   |
| <i>Moraxella</i>  |   |
| <i>Salmonella</i>   |   |
| <i>Pasteurella</i>  |   |
| <i>Mannheimia haemolytica</i>   |   |
| Increased activity against <i>Bordetella</i> ,<br><i>Haemophilus</i> , <i>Pasteurella</i> , <i>Actinobacillus</i> |   |

#### 2.9.3.1 Development of Bacterial Resistance: (Le Roux, 2004b).

Some bacteria have natural resistance, but they can also develop resistance against antibiotics by making use of one or two main mechanisms:

1. Bacterial chromosome mutation
2. Acquired resistance usually through plasmids.

#### 2.9.3.2 Practical Aspects on the Management of Antimicrobial Resistance

Recommended procedures (Le Roux, 2004b).

1. Use antibiotics sparingly and only in specific therapeutic cases.
2. Implement the correct dose and type of therapy.
3. Where possible select a product that shows activity against the specific organism in question.
4. Rather use narrow spectrum antibiotics in cases where the ethology is specified.
5. Use antimicrobial combinations when it is known that the combination works against development of resistance.
6. Use antibiotics only as prophylaxis under specific conditions where it will be of great importance and for as short a time period as possible.
7. Avoid environmental contamination with antimicrobial products.

8. To avoid the spreading of resistant strains, follow aseptic techniques and wash and disinfect hands properly.
9. Make use of quarantine procedures to isolate patients with resistant organisms and use protective procedures to prevent spreading.
10. Monitor resistant organisms where possible and increase the strength of management procedures in case of a problem.
11. Limit the use of antibiotics for non-therapeutic purposes.

#### **2.9.4 Antibiotic Treatment**

Techniques for treatment and control of mastitis in goat does can be adapted from those used for cattle. Laboratory cultures and antibiotic sensitivity examinations can be used to decide which antibiotics are appropriate. Antibiotic residue tests for screening bovine milk adequately identify goat milk that is free of antibiotic residues (Contreras *et al.*, 1997b).

##### 2.9.4.1 Antibiotic Treatment during Lactation

Treatment should begin as soon as clinical signs are noted, to prevent further destruction of milk secreting tissue or the development of gangrenous (*Staphylococcus aureus*) mastitis. Treatment during lactation of sub clinical mastitis detected by culture or somatic cell determination is not usually economically feasible, except possibly for *Streptococcus agalactiae*, which is uncommon in goats (Smith & Sherman, 1994).

##### 2.9.4.1.1 Choice of Antibiotics

The range of antibiotics available for intramammary or parenteral treatment of mastitis varies from country to country. Practitioners should evaluate antibiotic sensitivity of isolated bacteria relative to drugs that are available, are legal and are not prohibitively expensive. As STA is the most frequent cause of clinical mastitis in many herds, initial treatment (in the absence of sensitivity results) should ideally be with a drug this organism is normally sensitive to; tetracycline and cephalosporins are frequently effective *in vitro*. Resistance to penicillin is common, while ampicillin and amoxicillin have a broader spectrum of efficacy. Unless the animal is systemically ill, treatment is often limited to intramammary infusion with a full bovine tube administered two to three times at 12 or 24-hour intervals (Smith & Sherman, 1994). When extensive swelling or tissue penetration by bacteria such as *Staphylococcus aureus* has occurred, parenteral administration of an antibiotic with good bioavailability for five to seven days is recommended (Ziv, 1980).

##### 2.9.4.1.2 Infusion Techniques

Most commercial bovine infusion tubes have an applicator tip that is too large to be inserted into an average goat's teat. It is currently recognized that full insertion, even in a cow, damages the lining of the streak canal (Smith & Sherman, 1994).

##### 2.9.4.2 Supportive Therapy for Mastitis

Stripping of foremilk is beneficial because it serves to remove both bacteria and toxins. Oxytocin (5 to 10 units) and hot compresses assist in achieving milk let down from a painful gland. Anti-inflammatory agents are frequently administered to animals with symptoms of discomfort or toxemia. Systemic antibiotics are commonly administered when the mastitis is severe or chronic (Smith & Sherman, 1994).

##### 2.9.4.3 Treatment of Gangrenous Mastitis

Treatment is unsuccessful as the gangrenous udder sloughs off (Petris, 1963; Smith & Sherman, 1994). In the early stages when the affected gland is warmer than normal and painful and the secretion is blood tinged, successful medical treatment has been reported. In addition to treatment, the secretion was drained with a sterile teat cannula, a diuretic was used daily for five days and topical treatment on the udder with an antiseptic cream were used (Abu-Samra, 1988).

Amputation of the udder can be a life-saving procedure if the goat is showing signs of toxicity with gangrenous mastitis or if the goat has chronic abscesses in the udder and is in poor body condition. The reason for saving the goat's life should be evaluated against economic viability in the case of a commercial animal or sentimental value in the case of a pet. In selected cases, when surgery is not an option, the gangrenous gland can be de-activated (infused once with 60ml of 10% formalin) (Smith & Sherman, 1994).

#### 2.9.4.4 Dry Period Therapy

In one survey, 76% of udder half infections caused by major pathogens (*Staphylococcus aureus* or streptococci) and 55% of infections caused by coagulase negative staphylococci persisted through the dry period to the next lactation. Goats were sampled at the end and beginning of lactation (Lerondelle & Poutrel, 1984). Infusions of an appropriate long-lasting dry cow preparation (1 tube per udder half) at the time of drying off is recommended to increase the cure rate during the dry period while simultaneously preventing some new infections during this period. Teat dipping before and after infusion and partial insertion of the tube are recommended (Smith & Sherman, 1994).

##### 2.9.4.4.1 Drying-off Procedures

Dairy goats are commonly allowed two to three months non-lactating ("dry") period before parturition occurs. Provision of this rest period increases milk production in the next lactation. It also permits production of colostrum for protection of neonates. In some goats it is possible to milk daily for several years with a reasonable milk yield if production of kids is not desired or if the doe fails to conceive.

During drying off, animals should be kept clean and dry and observed for inordinate udder swelling. Udders that become severely distended should be milked out and treated again five to seven days later (Smith & Sherman, 1994). In one report, approximately two thirds of infections were eliminated in goats receiving dry treatment (Plommet, 1973).

## **2.10 Pharmacokinetics and Pharmacodynamics of Antimicrobials in Relation to Residues in Milk**

### **2.10.1 Pharmacokinetics**

The parent compound has to pass a long way through the body from the site of administration to the place of excretion before becoming a residue in milk, for all antibiotic treatments except for intramammary infusion. This process is influenced by the route of administration as well as, by many physical and metabolic processes.

Pharmacokinetics may be defined as the mathematical description of concentration changes of drugs within the body (Baggot, 1977). It is the study of the rate processes associated with absorption, tissue distribution, metabolism and excretion of a drug. It involves quantification of the drug and/or metabolite concentrations in the body fluids, tissues and excreta at any point in time from the moment of administration until elimination from the body is complete. The body absorbs the free drug. Before being excreted: the free drug may undergo biotransformation and then be excreted in the form of metabolites; the free drug may enter the systemic circulation and the locus of action "receptors", or the tissue reservoirs and then be excreted (Debackere, 1995).

This process is abbreviated as (A.M.D.E) Absorption, Metabolism, tissue Distribution, Elimination or Excretion.

Certain cephalosporins reach therapeutic levels in the somatic cells of humans: cefuroxime, ceftazidime and ceftriaxone. Elimination half-lives and volumes of distribution are the same as for prednisolone. One exception is ceftiofur, which has a considerably longer elimination half-life in veterinary species (le Roux, 2004d).

### **2.10.2 Pharmacodynamics**

The reason for scientific and regulatory residue surveillance lies in the potential effects of these residues for the consumer and for the manufacturer of dairy products, and their potential influence on the health of the consumer (Debackere, 1995).

The purpose of the administration of a drug is to obtain the proposed therapeutical effect in target tissue. The proposed effect can be twofold: either to correct or influence the function of well-defined organs or physiological systems or to prevent or treat diseases provoked by invading micro-organisms or parasites which can harm these organs and their functions (Debackere, 1995).

The pharmacodynamic effects can be divided into aspects of health and economics. The economic objective is to prevent accidents during the production of milk derivatives obtained by fermentation. The contribution to health aspects can be classified in three groups:

1. Possible occurrence of resistance by specific strains of bacteria to the antibiotics administered in human therapy.
2. Disorders of the intestinal flora.
3. Possible occurrence of allergic symptoms. (This is well-known for the penicillins.)

### **2.10.3 Pharmacokinetic-Pharmacodynamic Modelling**

Pharmacokinetic-pharmacodynamic modelling (PK/PD) can be used to study antibiotic residues in milk. The quantity of antibiotic residues in milk is a function of quantity administered related to time. A time-concentration curve can be plotted indicating a maximal concentration ( $C_{max}$ ), the time of the maximal concentration ( $T_{max}$ ) and the area under the curve and from this the total amount excreted can be calculated. Antimicrobial effect can be measured in vitro by determining their minimal inhibitory concentrations against the micro-organisms in question and by extrapolating these from in vitro data to the in vivo situation. Antibiotic residues in the milk of lactating goats could pose a problem to public health. Therefore, it is of importance to know the antecedents of the parent compound and its metabolites (Debackere, 1995). This knowledge is gained by experimental pharmacokinetic studies. From these pharmacokinetic data some pharmacodynamic aspects can be derived.

## **2.11 Diagnosis of Mastitis**

Physical examination of signs of inflammation and culture tests of the mammary secretion are the most commonly used techniques for identifying clinical or sub clinical mastitis. Monthly somatic cell determinations on all herd members are valuable for monitoring increased cell counts (and thereby satisfying government regulations) but are rarely worth the expense regarding mastitis control in goats (Smith & Sherman, 1994).

The diagnosis of mastitis in dairy goats is based on a clinical examination of the udder; strip-cup examination of the mammary secretion; the results of the California Milk Cell Test (CMCT) when the strip-cup examination is negative or inconclusive; and cytological and bacteriological examinations of the milk (Lewter *et al.*, 1984).

### **2.11.1 Clinical**

Clinical mastitis in goats is similar to that in cattle, with sub clinical, chronic, and acute and peracute gangrenous forms occurring. In goats the diagnosis of sub clinical mastitis, and the distinction between sub clinical and clinical mastitis is difficult. Particular care is needed in the clinical examination of the goat milk because of its apparent normality when there are severe inflammatory changes in the udder (Radostits *et al.*, 2000).

Clinical mastitis may occur with palpable nodules in the udder parenchyma and floccules in the milk (Dubois, 1911).

The predisposing factors and pathogenesis of udder infection and inflammation in goats are similar to those in cattle and sheep in which the route of infection via the teat canal is the most important (Blood *et al.*, 1983; Renk, 1963; Smith & Roguinsky, 1977). Injuries to the teats invariably result in severe mastitis. The clinical signs of mastitis in goats resemble those in cattle and sheep suffering from the disease (Blood *et al.*, 1983; Smith & Roguinsky, 1977). Early indications of clinical mastitis include a decrease in milk production by one gland or lameness on the affected side as the goat attempts to avoid contact of the hind limb with

tender half of the udder (Smith & Sherman, 1994). Nursing kids may appear to be hungry, and mastitis is associated with increased kid mortality rates (Addo *et al.*, 1980). Visual inspection of the udder from behind and from the sides may reveal asymmetry whereby the affected gland is swollen (acute) or atrophied (long term inflammation) (Smith & Sherman, 1994). Teat end lesions predisposing goats to mastitis (Kapur & Singh, 1978) (wounds, contagious ecthyma and warts) also may be identified. Palpation may disclose the presence of heat, tenderness and swelling (acute), induration or atrophy, (chronic mastitis), or even multiple abscesses. Goats with acute mastitis may be systematically ill, having signs of fever, anorexia and depression. If the teat is cold and oedematous or the secretion red and watery, gangrenous mastitis should be expected (Smith & Sherman, 1994).

## 2.11.2 Somatic Cell Counts

### 2.11.2.1 Cell Enumeration

An increase in the number of somatic cells in milk is used as an indication of mastitis, including sub clinical mastitis in cows. The application to dairy goats of tests and regulations developed for cattle frequently has led to panic in the commercial producer who interprets “high” cell counts as evidence of a serious mastitis problem or who is threatened by an inspector with loss of a milk market (Smith & Sherman, 1994).

### 2.11.2.2 Cytoplasmic Particles and Epithelial Cells

In the discussions on bovine mastitis, the number of somatic cells per ml of milk is generally assumed to correlate directly with the severity of mastitis or the degree of irritation to the mammary gland. The relationship of SCC to caprine mastitis is limited, unless tests appropriate to caprine milk are used. This is partly because goat milk differs from cow milk due to the presence of cytoplasmic particles and epithelial cells (Smith & Sherman, 1994). The caprine mammary gland produces milk by a process called apocrine secretion and cellular tissue appears in milk as DNA-free particles similar in size to leukocytes (Dulin *et al.*, 1982). Also present in variable numbers in goat milk are intact epithelial cells sloughed from acini and ducts (Smith & Sherman, 1994). Milk from normal goats has a higher cell count than that from normal cows (Grootenhuis, 1980; Hinckley & Williams, 1981), and a diagnosis of udder infection is based more on leukocyte count than on total somatic cell count (East & Birnie, 1983; Hinckley & Williams, 1981). The normal SCC varies between 700 000 and 1000 000/ mL. The diagnosis of mastitis is based on the premise that 1.5 million or more leukocytes per milliliter of milk are indicative of inflammation of mammary tissue and possibly of udder infection (Peterson, 1981; Smith & Roguinsky, 1977). Much of the variation in SCC was not due to intramammary infection. Non-infected goats may have a SCC of greater than  $1 \times 10^6$ . These variations mean that their value as a guide to diagnosis in this species is controversial (Radostits *et al.*, 2000). A physiological threshold of  $500 \times 10^3$  has been suggested (Contreras *et al.*, 1996), but a count of greater than 1 million cells/mL has been said to be positive for mastitis (Kalogridou-Vassiliadou *et al.*, 1992). Other observations indicate that the most discriminating threshold for diagnosis of infection is  $0.8 \times 10^6$  (Lerondelle *et al.*, 1992).

### 2.11.2.3 Reference for SCC

The Levovitz-Weber modification of the Newman-Lampert stain is commonly used to stain somatic cells for counting (Schalm, *et al.*, 1971). This stain is inappropriate for goat milk because staining is similar for cytoplasmic particles and cells (Dulin *et al.*, 1982). Currently the stain preferred for determining SCC in goat milk is the pyronin Y-methyl green stain (Dulin *et al.*, 1982), often referred to simply as the green stain. Methyl green is specific for DNA and pyronin Y is specific for RNA. Chromosomes, will stain blue-lavender while cytoplasmic particles and the cytoplasm of epithelial cells stain red (Smith & Sherman, 1994). Neutrophils do not contain pyronin Y-positive material (Paape *et al.*, 1963). Unfortunately,

this staining technique is difficult to do and potentially toxic to laboratory staff (Smith & Sherman, 1994). Leukocytes and epithelial cells in goat milk have also been differentiated by a modified Wright's stain technique (Hinkley & Williams, 1981). Others have reported that many leukocytes are masked by the background smears prepared with Wright's stain (Paape *et al.*, 1963).

### **2.11.3 Sampling for Mastitis Diagnosis**

#### 2.11.3.1 Type of Sample and the Role of Oxytocin in Taking Milk Samples

Foremilk samples have been used for detection of mastitis. It has been proven for cattle that the Somatic Cell Count (SCC) of residual milk was twice that of foremilk in healthy cows. In chronic bovine mastitis, samples from residual milk have usually four times more somatic cells than foremilk. This is due to the fact that in cattle pharmacological doses of oxytocin increase the somatic cell and sodium content of milk and decrease the potassium and lactose contents. This suggests that oxytocin opens up tight junctions, permitting migration of cells from the interstitial space into milk. This phenomenon can be used as a diagnostic aid to indicate presence of phagocytes at the alveolar level. In the case of chronic staphylococcal mastitis, the residual milk (released by oxytocin) is rich in phagocytic bacteria. Bacteria are in these cases often intracellular and can be released for bacteriology by freezing and thawing. Oxytocin may be pharmacologically administered to remove inflammatory secretions (containing pus and bacteria) from the udder in connection with mastitis therapy. This should be done before applying antibacterial therapy (Sandholm *et al.*, 1995). Therefore representative samples must be taken of all the milk in the udder.

### **2.11.4 Factors Affecting SCC**

#### 2.11.4.1 Breed

In a study done with Alpine milking does SCC increased with intramammary infection (IMI) (Wilson *et al.*, 1995). SCC varies for infected and uninfected goats of different breeds. Marked herd differences in SCC of uninfected goats have been reported in several studies (Smith & Sherman, 1994).

#### 2.11.4.2 Stage of Lactation

The number of cells and distribution of cell types are not constant throughout lactation. Epithelial cells and macrophages are most numerous in late lactation (Smith & Sherman, 1994). When many goats are stressed by oestrus, the percentage of neutrophils in the milk increases (Atherton, 1992). Cytoplasmic particles in the milk change little with stage of lactation (Dulin *et al.*, 1983).

Increased stage of lactation was also found to be associated with increased SCC in goats with or without diagnosis of IMI (Wilson *et al.*, 1995).

SCC in milk of goats are higher than in cattle or sheep but vary widely (Zeng & Escobar, 1996). In one study, increased SCC was clearly linked to advancing lactation and decreased production. In a seasonal dairy goat herd, where most animals are in late lactation simultaneously, cell counts determined by whatever method will often exceed regulatory standards for cow milk even with low prevalence of mastitis (Smith & Sherman, 1994). Change in cell concentration and types of cells through lactation were studied in milk of Verata breed goats (Rota *et al.*, 1993). In this study the proportions of cell types were analysed: During 210 days of lactation; as a function of number of lactations (first to fourth parity; in relation to total cell count (Rota *et al.*, 1993). Average percentages of the various cell types in milk of all the goats used in this study were: 63.0% for polymorphonucleates, 16.2% macrophages, 7.2% lymphocytes, 13.0% remainder. Through the lactation the polymorphonucleates increased, while the rest of the cell types decreased.

Polymorphonucleates increased from the first to the fourth lactation, and macrophages, lymphocytes and degenerated cells decreased. In this study correlation coefficients reached



statistical significance in all cases and cell counts were done by direct microscopy (Rota *et al.*, 1993).

Coagulase negative staphylococci are the most frequent infectious agents prior to the dry period, whereas after calving the different hygienic conditions influence the infection rate and SCC. This study confirmed that infection risk is higher during lactation and in particular, during peak lactation, at which time the somatic cell counts also increase (Moroni *et al.*, 2000).

The specificity of a given threshold as an indicator of mastitis is greatly decreased at or near drying off (Lerondelle & Poutrel, 1984). A threshold of 1 million cells has been proposed for detecting major pathogens in early and mid-lactation, in the USA. A marked difference in the cell count (by whatever test) between halves is a very good indicator of infection in the gland with the higher count (Smith & Sherman, 1994).

In a study by Galina *et al.*, (1996a) SCC was high (50% above  $500 \times 10^3$  and 35% above  $1000 \times 10^3$ ) in the first 45 days of the lactation and again at the end of the milking period, from days 170-210 (23% above  $500 \times 10^3$  and 15% above  $1000 \times 10^3$ ), but low (90% less than  $500 \times 10^3$ ) during mid-lactation.

#### 2.11.4.3 Parity (Number of Lactations)

Lower mature equivalent milk production and increased parity were also associated with higher cell counts (Wilson *et al.*, 1995). Parity of milking goats has been found not to affect SCC, standard plate count, and major milk components (Zeng & Escobar, 1995).

#### 2.11.4.4 Season

The SCC has been found to be highest in lactating does during October, December and January (Northern Hemisphere) (Wilson *et al.*, 1995).

#### 2.11.4.5 Management/ Farming Systems

It is very difficult to establish a threshold cell count for the diagnosis of mastitis. Future research efforts should be directed towards enumeration of leukocytes alone because these cells are more likely than macrophages and epithelial cells to indicate mastitis.

Several management and environmental factors can affect SCC such as milking routine, procedures in the dairy parlour and design of the dairy parlour and housing facilities. The environment should be examined for sources of trauma to the udder (causing increased SCC), including high doorsills and rough milkers, their hands, machines or butting kids (Smith & Sherman, 1994).

#### 2.11.4.6 Effect of Micro-organisms on SCC

In a study non-infected goats had SCC greater than 1000 000 per ml (Wilson *et al.*, 1995). In the same study, approximately 90% of the difference in goats SCC was not due to IMI, 77% of the variation in SCC among does was unexplained (Wilson *et al.*, 1995). In another study geometric means of SCC for uninfected halves, halves infected by coagulase negative staphylococci and halves infected by major pathogens were 272 000 cells/ml, 932 000 cells/ml and  $2443 000 \times 10^3$  cells/ml, respectively. In the above study it was concluded that systematic treatment of goats at drying-off is an efficient method for the cure of sub clinical mastitis and control of SCC at the beginning of the following lactation and that effectiveness of post milking teat disinfection remains to be demonstrated (Poutrel *et al.*, 1997).

Most (but not all) goats with sub clinical *Staphylococcus aureus* infection show elevated cell count (Lerondelle & Poutrel, 1984; Nesbacken, 1978a). The nucleated cell count in the milk from an udder half chronically infected with STA can fluctuate widely from week to week (Nesbacken, 1978b). A decrease from 10 million to 1 million per mL cannot be used as evidence of elimination of the infection (Smith & Sherman, 1994).

Some studies have shown increased SCC in goats infected with coagulase negative staphylococci as compared with non-infected herd mates, while others have shown no difference (Hunter, 1984; Manser, 1986; Sheldrake *et al.*, 1981). In one study, the proportion of cells that were neutrophils was increased (approximately 75% compared with

approximately 50%) in milk from goats with coagulase negative staphylococci when compared with milk from goats with negative culture test results (Dulin *et al.*, 1983). It has been proposed that strain differences in pathogenicity are responsible for variation in inflammatory response in different herds (Smith & Sherman, 1994).

Infections with coliforms or other bacteria producing endotoxin can result in increased nucleated cell counts, and specifically neutrophils, in goats (Smith & Sherman, 1994). Various species of mycoplasma have also been associated with increased leukocyte counts in goat milk (Prasad *et al.*, 1985).

Some researchers have considered that Caprine Arthritis Encephalitis (CAE) virus infection leads to higher cell counts in goats and accounts for part of the difference between SCC of “normal” goats and cows (Smith & Sherman, 1994). French workers have noted an increase in proportion of mononuclear cells in milk from goats with mastitis caused by CAE (Lerondelle, 1988; Lerondelle *et al.*, 1989). In one study, goats serologically positive for CAE had increased cell counts but also had more sub clinical infections with staphylococci than did CAE-negative herd mates (Smith & Cutlip, 1988).

Many factors were found to affect somatic cell numbers in goat milk: coagulase negative staphylococci or CAEV mammary infection, but also vaccination or alimentary stress. The weak increase of cell numbers due to CAEV infection and the susceptibility of goat udders must be taken into account before using cell count as diagnostic of mammary infections (Lerondelle *et al.*, 1992). CAE has not yet been reported in goats in South Africa.

#### 2.11.4.7 Miscellaneous

The feeding of avocado leaves (*Persea americana*) of the Guatemalan, but not the Mexican variety to goats caused an increase in SCC (more than 700 000), decrease in milk production, udder oedema and grossly curdled milk (Craigmill *et al.*, 1992).

#### 2.11.4.8 Infusion Products/ Intramammary Treatment

Some intramammary infusion products can cause a marked increase in SCC, swelling and tenderness of the udder and flakes or clots in the milk occur when given to healthy goats (Smith & Sherman, 1994). Oxytetracycline increased the SCC by an average of 42 times 12 hours after infusion, erythromycin by an average of 23 times, and penicillin and cephalixin by six times pre-treatment cell counts (Ziv, 1984).

### **2.11.5 Determination of SCC in Goat Milk**

#### 2.11.5.1 California Milk Cell Test (CMCT)

##### 2.11.5.1.1 CMCT and SCC Correlation

Arithmetic and geometric means of SCC per micro litre for each CMCT score has been found to be, respectively:  $312 \times 10^3$  and  $172 \times 10^3$  for score 0 and traces;  $1014 \times 10^3$  and  $531 \times 10^3$  for score 1;  $2912 \times 10^3$  and  $2051 \times 10^3$  for score 2 and  $4950 \times 10^3$  and  $4436 \times 10^3$  for score 3. The CMCT scores 2 and 3 discriminated between infected and uninfected glands. Both tests (SCC and CMCT) could be used in the period of study to detect a high percentage of true uninfected glands but the percentage of false positives was high (Contreras *et al.*, 1996).

##### 2.11.5.1.2 CMCT in Mastitis Diagnosis

The CMCT is more useful for ruling out than for diagnosing mastitis in goats (Smith & Sherman, 1994). Caution must be exercised when interpreting the results of CMCT in goats. A negative result is a good indicator of the absence of infection, but a positive test does not always indicate infection (Lewter *et al.*, 1984).

Positive correlations between CMCT results and age and negative correlations between CMCT results and milking hygiene and technique were found. The CMCT was evaluated as an indicator of mastitis diagnosis. Therefore clinical examination of the udder, bacteriological examination of milk samples (aseptically collected) and the determination of somatic cell count were carried out in this study. The results showed that CMCT is not specific for

infected udder halves. STA and coagulase negative staphylococci were found at the same level of importance of udder pathogens. This has shown that CMCT can be used as an additional diagnostic tool concerning goat mastitis, but it should not be overestimated because of different factors, which influence the cell count. For the control of udder health additional diagnostic measures are of utmost necessity (Winter & Baumgartner, 1999).

### 2.11.5.1.3 Factors Affecting CMCT

#### 2.11.5.1.3.1 Breed, Lactation Stage, Parity, Micro-organisms and Management

In a study by Boscos *et al.*, (1996), parity, breed and stage of lactation differences were not found to have any effect on mean CMCT scores. The presence of bacteria in caprine milk elevated CMCT scores in primiparous and multiparous goats. This elevation was associated with the type of bacteria; STA elevated CMCT scores in milk more than coagulase negative staphylococci did. The results of this investigation lead to the conclusion that CMCT could only be appropriate for the prediction of the presence of major pathogen such as STA in goat milk, even if their application was restricted to only one breed, one parity or to a specific stage of lactation (Boscos *et al.*, 1996).

High scores at the end of lactation (Maisi, 1990a; Maisi 1990b), or in systemically ill goats with drastically reduced milk production occur in the absence of mastitis. A sick goat with a negative or trace CMCT reaction is probably not sick because of mastitis. If there is a marked difference between the scores of two halves of a goat, mastitis is very likely. The usefulness of CMCT (or any other test) for diagnosing sub clinical mastitis depends on the prevalence of mastitis in a herd (Smith & Sherman, 1994).

In a well-managed herd, the predictive value of a positive CMCT test is unacceptably low (Hueston *et al.*, 1986).

#### 2.11.5.1.3.2 Intramammary Infection

Sensitivity of the test is indicated by the smallest measurable amount that can be distinguished. The specificity of the test is good when it only measures the intended indicator (Sandholm *et al.*, 1995). (For instance the cell counter should not count cream or casein micelles.)

In one research project the overall sensitivity of CMCT for detecting intramammary infection (IMI) in goats was lower than the specificity of the CMCT (Hueston, 1986). When only infections by major pathogens were considered, the sensitivity increased and the specificity decreased to even further. Positive CMCT scores were recorded for all samples from udder halves infected with major pathogens. The CMCT scores for samples from udder halves infected with coagulase negative staphylococci were variable. Sensitivity is shown by the likelihood of a positive CMCT score in the presence of IMI. Specificity is shown by the likelihood of a negative CMCT score in the absence of IMI (Hueston *et al.*, 1986).

Significantly increased positive CMCT readings occurred when streptococci, other staphylococci and micrococci were present. In another study infection within one half was reflected as an increase in the inflammatory parameters in the milk of the infected half as well as a slight increase in the inflammation parameters in the adjoining half (Maisi & Riipinen, 1988).

### 2.11.5.2 Wisconsin Mastitis Test (WMT)

The WMT uses diluted CMCT reagent (Smith & Sherman, 1994). It is more objective than CMCT as the viscosity of the milk-reagent mixture is estimated from volume remaining in a special tube after draining through a standard-sized hole for 15 seconds (Schalm *et al.*, 1971). The WMT is considered to be DNA specific (Smith & Sherman, 1994).

### 2.11.5.3 Electrical Conductivity

Sub clinical infections by minor pathogens that do not damage the mammary epithelium may be of little or no concern to udder health or milk production (Smith & Sherman, 1994). With this in mind, researchers have tried to use electrical conductivity of the milk as an indicator of

the severity of a mastitis infection (Linzell & Peaker, 1975; Sheldrake *et al.*, 1983). A convincing increase of detecting bovine mastitis, relative to somatic cell counts, has not been demonstrated.

Preliminary work has not shown electrical conductivity to be useful in screening for sub clinical mastitis in goats (Smith & Sherman, 1994). One group failed to find a correlation between SCC (Fossomatic) and electrical conductivity (Park & Nuti, 1985; Park, 1991). They noted little variation between conductivity of foremilk and strippings of the same goat but demonstrated a negative correlation between electrical conductivity and butterfat percentage (Smith & Sherman, 1994).

In one study French-Alpine and Anglo-Nubian does in mid-lactation at 150-180 days of milking, were tested for interrelationships between levels of SCC, electrical conductivity, standard plate count, staphylococcal counts, coliform counts, percent fat and protein in goat milk. Mean cell counts of the above combined breed data for SCC, standard plate count and staphylococcal counts in goat milk were 682, 38.9 and  $4.03 \times 10^3$  cells/ml, respectively. Mean electrical conductivity overall for Alpine and Nubian breeds were 4.75, 5.80 and 3.76, respectively. Results from the above study suggest that SCC and electrical conductivity by Fossomatic 215 (Foss Electric, Hillerod, Denmark) and a portable hand-held conductivity meter (MD-18, MAS-D-TEC, Wescor, Inc, Logan, UT, USA) may not be good indicators for bacterial count in goat milk. Further studies are necessary to find optimal unit measures of an electrical conductivity instrument for caprine milk (Park, 1991).

#### 2.11.5.4 Fossomatic

The Fossomatic method (Foss-O-Matic, Foss Electric, Hillerod, Denmark) of determining SCC is an automated fluorescent technique that uses a dye that specifically binds to the DNA of cell nuclei. With Fossomatic equipment currently used by Dairy Herd Improvement Associations in the USA, both epithelial cells and leukocytes are counted, but counts are not confounded by cytoplasmic particles (Smith & Sherman, 1994).

The Fossomatic instruments and infrared milk analysers must be calibrated with goat milk standards for more reliable and accurate analysis of milk.

Another research project showed that results from a Fossomatic-300 and DairyLab II (for analysis of SCC and fat, protein respectively) calibrated with goat milk standards differed. Somatic cell counts (SCC) of goat milk were 27% lower when the Fossomatic was calibrated with goat milk standards than with cow milk standards. When the DairyLab II was calibrated with goat milk component standards, the levels of fat and protein in milk samples were 0.04% and 0.27% higher respectively than with cow milk component standards. Thus the above study concluded that Fossomatic instruments and infrared milk analyzers must be calibrated with goat milk standards for more reliable and accurate analysis of goat milk (Zeng, 1996).

#### 2.11.5.5 Coulter Counter

The Coulter counter enumerates particles as milk flows past an electronic eye. Because cytoplasmic particles are similar in size to leukocytes, they too are counted in goat milk (Smith & Sherman, 1994). Certain counters with channels that permit categorizing cells by cell diameter may improve differentiation of mastitic from non-mastitic samples (Smith & Roguinsky, 1977). Coulter counter cell counts tend to be approximately double the counts in goat milk determined by Fossomatic (Poutrel & Lerondelle, 1983; Lerondelle, 1984).

#### 2.11.5.6 Additional Tests: Diagnostic

The chloride content of goat milk is greater than in cow milk, with means in various studies ranging from 121 to 204 mg/ 100mL. Bacterial infections in the udder alter cell wall permeability and permit an increased flow of sodium and chloride into milk (Smith & Sherman, 1994). Lactose and potassium concentrations decrease (Linzell & Peaker, 1972), but these substances have not been used for diagnoses of caprine mastitis.

According to a study by Zeng *et al.*, (1996), the Penzyme test can be used (as a goatside test for screening of antibiotic residues in goat milk), because of its high sensitivity and specificity and its quick results (20-25min). In the above study Thermo Resistant Inhibitory Substances

(TRIS) test or *Bacillus stearothermophilus* disc assay (BsDA) were used as standard references. The Delvo test P yielded approximately 7% false positive results among milk samples. In contrast to cow milk, the increased somatic cell counts of goat milk did not elevate the false positive results of Delvo test P (Zeng, *et al.*, 1996).

#### 2.11.5.6.1 NAGase

N-acetyl- $\beta$ -D glucosaminidase (NAGase) has received attention as a possible marker for inflammation in both bovine and caprine milk (Smith & Sherman, 1994). Several studies have confirmed that NAGase is elevated in milk from halves with major pathogens and with coagulase negative staphylococci (Maisi & Riipien, 1988; Maisi & Riipien, 1991; Timms & Schultz, 1985; Vihan, 1989). However the test has been reported to be less sensitive than CMCT for detecting infection and there is no significant difference between NAGase levels in infected and uninfected halves of the same goat (Maisi, 1990a; Maisi, 1990b). Others have concluded that NAGase is superior to cell counting techniques for diagnosing sub clinical mastitis in goats (Vihan, 1989).

#### 2.11.5.6.2 Delvo Test

Although laboratory procedures such as the Delvo test have been available since the 1980's, little research has been done to investigate the time that antibiotic residues will be detected in the milk of goats after intra-mammary applications of antibiotics. The few research projects investigating this problem differed in the results that were achieved as well as for the withdrawal times set for certain antibiotics in intra mammary preparations (Buswell *et al.*, 1989; Hill *et al.*, 1984; Long *et al.*, 1984).

Normal goat milk appears to have some antibacterial action (Smith & Sherman, 1994). In one study, 24% of pre-antibiotic treatment milk samples from 75 healthy goats showed false-positive Delvo test results, and 11% of the 75 animals the natural inhibitors were heat stable (140°F or 60°C for 20 min) (Ziv, 1984). Small zones of inhibition (halos) are sometimes noted with the *Bacillus stearothermophilus* disc assay, especially in late lactation and this naturally attracts the attention of regulatory officials (Smith & Sherman, 1994). Iron-binding protein (lactoferrin) in goat milk has been shown to be bacteriostatic for *Bacillus stearothermophilus* (Oram & Reiter, 1968). Both the bacteriostatic effect and the lactoferrin concentration are increased in dry period secretion (Smith & Sherman, 1994). Rancidity (lipolysis) is also increased in late lactation, and heat treatment would not affect bacterial inhibition due to fatty acids (Atherton, 1992). The *Bacillus stearothermophilus* test may be appropriate for goat milk (Klima, 1980).

Incubation of bovine milk samples at warm temperatures, with resulting increase in bacterial numbers and decrease in pH levels, has been shown to cause inhibitory zones in disc assays (Smith & Sherman, 1994). Some of these inhibitory zones, like those caused by antibiotics, persisted after raw milk had been heated to 180°F (82.2°C) for 5 minutes (Kosikowski, 1963). Thus, improper handling of milk samples may contribute to false-positive residue test reactions.

Non-antibiotic inhibitors in milk may have an importance that transcends regulatory considerations (Smith & Sherman, 1994). Residues of disinfectant in milk and iodine originating from excessive dietary levels may interfere with bacterial acidification necessary for cultured products (Le Jaouen, 1987).

### **2.12 Control and Prevention of Bacterial Mastitis:**

#### **2.12.1 Culling**

Culling of affected animals is often an economically sound alternative. Culling serves to decrease exposure of other does to contagious organisms (Smith & Sherman, 1994).

#### **2.12.2 Prevention of Bacterial Mastitis through Management**

Management decisions that decrease the risk of injury to the udder or teat by selecting for improved udder conformation and controlling skin lesions on the teats should decrease the

risk of mastitis. Attention to hygiene, teat dipping and dry period antibiotic therapy is less beneficial (Smith & Sherman, 1994).

### **2.12.3 Nutrition**

Although it seems reasonable to assume that nutritional deficiencies should increase an animal's susceptibility to mastitis and other infectious diseases, studies in goats are not available (Smith & Sherman, 1994). However, it has been shown that selenium deficiency is associated with reduced neutrophil function in goats (Aziz *et al.*, 1984; Aziz & Klesius, 1986b). Also, somatic cell count (by Fossomatic) was decreased and milk production increased in Finn goats with a high glutathione peroxidase activity when compared with goats with decreased selenium status judged by this enzyme's activity (Atroshi *et al.*, 1985).

### **2.12.4 Vaccination**

Vaccination against *Streptococcus agalactiae* in goats may provide a degree of immunity (Radostits *et al.*, 2000). A mastitis vaccine is available from Onderstepoort and should be used strictly according to the instructions (Bath & De Wet, 2000). This vaccine is effective against the following organisms: *Pasteurella haemolytica*, types two, seven and nine and *Staphylococcus aureus*, two strains, in sheep. Staphylococcal toxoid vaccinations have been used in herds experiencing clinical mastitis caused by *Staphylococcus aureus* (Lerondelle & Poutrel, 1984; Petris, 1963). An adjuvanted cell-toxoid vaccine has been used in studies using goats as models for cows (Derbyshire, 1960). In contrast, a polyvalent somatic agent vaccine did not prevent mastitis or decrease severity of clinical signs (Lepper, 1967). It is very important to distinguish between the various forms of mastitis because each has its own vaccine (Bath & De Wet, 2000; Smith & Sherman, 1994). Goats previously inoculated with *M. putrefaciens* showed resistance to subsequent challenge for at least one year (Brooks *et al.*, 1981), suggesting that a vaccine might help to control this organism as well.

### **2.12.5 Teat Dips**

Although few studies have been conducted in goats, it is generally accepted that teat dipping or spraying with properly mixed and uncontaminated solution is of economic benefit via prevention of bacterial mastitis (Plommet, 1973). Products with evidence of efficacy for prevention of mastitis in dairy cows (e.g., 0.5% iodine or 0.5% chlohexidine) are commonly used (Smith & Sherman, 1994).

## **2.13 The Effect of Freezing on Goat Milk**

There is no information available on the ability of frozen goat milk to be used for antibiotic residue tests and whether or not the freezing and thawing of the milk affects these tests. The goat milk output is seasonal; to maintain the production throughout the year, storage of frozen milk for the commercialization as liquid milk or to manufacture milk products has been proposed. Physicochemical changes in pasteurised milk (63°C for 30min) stored at –18°C for 90 days were not observed in some studies (Calvo, 2000; Gomes *et al.*, 1997). However the sensorial characteristics were altered significantly and the authors suggested that homogenizing the milk before the heat treatment might minimize this problem. The use of frozen ultra filtration retentates has also been reported. However, problems of oxidation during storage were found, and the authors proposed the addition of the antioxidant Tenox-6 to avoid this problem (Calvo, 2000).

## **2.14 Micro-organisms**

An IMI has been defined as “true and persistent” when the same pathogen was isolated two or more times consecutively from the same half of the udder (Contreras *et al.*, 1997a). In this study, statistically significant relationships were found between staphylococci and true positive diagnosis and between corynebacteria and false-positive diagnosis. Two or more consecutive negative culture test results are often presumed to indicate absence of infection. Most mastitis isolates can be identified on the basis of a few simple tests such as colony characteristics, haemolysis on blood agar, Gram stain and catalase tests.

In a study by Boscós *et al.*, (1996) no breed or parity differences were observed with regard to the type of bacteria isolated.

In another study by Vega *et al.*, (2000), carried out to determine prevalence and aetiology of non-clinical IMI in dairy goats, IMI was present in 22.6% of the glands and 32.5% of the animals. In the above study 85 micro organisms were isolated: Major pathogens; staphylococci (50.6%), minor pathogens; Enterobacteria (7.1%), Gram-negative, non-enterobacteria (3.5%), streptococci (3.5%), Corynebacteria (2.3%), micrococci (2.3%), Mycoplasmas (29.4%) and fungi (1.1%). Among the staphylococci isolated were: *Staphylococcus xylosus* (25.6%), *Staphylococcus caprae* (20.9%), *Staphylococcus epidermidis* (STE) (16.3%) and *Staphylococcus chromogenes* (11.6%); and more infrequently *Staphylococcus aureus* (STA) (6.9%) was isolated. Among the mycoplasma isolates, 23 cases were identified as *Mycoplasma agalactiae* (92%) and 2 as *Mycoplasma mycoides* subsp. *mycoides*, large colony (8%).

### 2.14.1 Major Pathogens

Major pathogens that play a role in mastitis in goats are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*.

STA can cause either gangrenous or non-gangrenous mastitis (Freeman & Clark, 1977).

#### 2.14.1.1 Bovine Staphylococcal Mastitis

This is common and of great economic importance (Carter *et al.*, 1995). Staphylococcal mastitis, usually caused by *Staphylococcus aureus*, is a common form of bovine mastitis worldwide. It may be sub clinical, acute or chronic. The majority of infections are sub clinical. Peracute and gangrenous forms are associated with severe systemic reactions and can be life threatening. In gangrenous mastitis, seen in post-parturient cows, the affected quarter becomes cold and blue-black and eventually sloughs off. Tissue necrosis is attributed to the alpha-toxin, which causes lysosomal disruption in leukocytes and also affects smooth muscle, leading to constriction, paralysis and finally necrosis of the smooth muscle cells of the walls of blood vessels (Quin *et al.*, 2002).

#### 2.14.1.2 Staphylococcal Mastitis in Goats: *Staphylococcus aureus*

This is the most common cause of mastitis in goats. In the experimentally produced disease caused by *Staphylococcus aureus* in goats the pathogenesis is very similar to that in the cow except that there is a marked tendency for the staphylococci to invade and persist in foci in interacinar tissue. As in cattle, some staphylococci in goat milk produce enterotoxins and the toxic shock syndrome toxin and are likely to cause food poisoning in humans. Latex agglutination tests are available for the identification of the enterotoxins (Radostits *et al.*, 2000). In a study by Kyozaire *et al.*, (2005) in South Africa, 13.4% of the infections were due to STA. In the above study, there was no significant relationship between the SCC and the presence of bacterial infection in goat milk ( $p=0.2$ ) (Kyozaire *et al.*, 2005).

The Staphylococci are Gram-positive cocci that tend to be arranged in irregular clusters or “bunches of grapes” formation. They are aerobic (Carter *et al.*, 1995) and facultative anaerobes (fermentative), catalase positive, oxidase-negative and non-motile. Growth occurs on nutrient and blood agars but not on MacConkey agar. The pathogenic staphylococci (STA) produce toxins and enzymes, but the significance of many of them in the pathogenesis of the disease is not fully understood.

Enterotoxins (A-E) are involved in human food poisoning and they act by reflex stimulation of the emetic centre. Protein A, a surface component of most strains of virulent STA binds to the Fc region of IgG and may play a part in the pathogenesis of staphylococcal diseases (Jarp *et al.*, 1989).

Some of these enzymes are able to increase the invasive powers of the organism and possibly protect it from body defence mechanisms (Carter *et al.*, 1995): coagulase, enterotoxins A, B, C1, C2, C3, D, E, haemolysins  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  haemotoxins, cytolysins, lipase, staphylokinase, leukocidin, exfoliative toxins A and B (exfoliatin), toxic shock syndrome toxin, hyaluronidase, penicillinase, lysostaphin, protein A, slime, other toxins and enzymes. Most

strains produce both alpha-haemolysin and beta haemolysin (Roguinsky & Grandemy, 1978). Colonies are large on blood agar and usually surrounded by a zone of incomplete haemolysis or complete haemolysis.

As is the case with cows the organism resides in micro abscesses in chronically infected goats and they are very difficult to cure (Derbyshire, 1958a; Derbyshire, 1958b). Transmission to other goats occurs during milking. Animals that are culture positive for *Staphylococcus aureus* should be culled or milked last. In larger herds with machine milking a *Staphylococcus aureus* unit can be identified and used to milk only *Staphylococcus aureus* affected goats. If these animals are retained in the herd, they should certainly be dry-treated and then should remain in the group of *Staphylococcus aureus* infected goats into the beginning of the next lactation. If cultures are repeatedly negative and somatic cell counts remain low, the animal can be returned to the main herd. Ideally, the milk of infected goats should be pasteurised before it is fed to the kids (Smith & Sherman, 1994).

STA causes sub clinical, clinical or peracute mastitis and staphylococcal dermatitis in goats (Quin *et al.*, 2002). The most severe, acute form is gangrenous mastitis (Smith & Sherman, 1994).

In gangrenous mastitis, half the udder will suddenly become swollen, hot to the touch, red and painful. Milk production will stop and only a thin pale fluid with white flecks or a thicker pus-like mixture will be excreted from the teat. The reddish colour of the udder soon changes to purplish and the tissue sloughs off, even if the goat survives. Gangrenous mastitis in goats is most frequently due to *Staphylococcus aureus* in animals lacking adequate concentrations of antitoxin against the narcotising alpha-toxin produced by the organism. The condition is usually restricted to the period of lactation. Death may occur within 24 hours (Smith & Sherman, 1994). In one report from Cyprus in 1961, almost 9% of 8000 goats were affected with gangrene (Petris, 1963). Histologically, there is a venous thrombosis and the initial inflammatory changes are replaced by necrosis and sloughing of epithelial cells (Derbyshire, 1958b). Because of its dramatic presentation and associated great economic loss, highest SCC in milk and the highest prevalence of clinical udder alterations were associated with coagulase-positive *Staphylococcus aureus* (Deinhofer & Pernthaner, 1995).

#### 2.14.1.2.1 Resistance Immunity and Treatment of Staphylococci

Pus is protective, and organisms may remain viable in dried pus. Some staphylococci can survive a temperature of 60°C for 30 minutes. Staphylococci are susceptible to common disinfectants but are resistant to other disinfectants including phenolic compounds and high salt concentrations; the latter resistance is taken advantage of in the selective medium mannitol salt agar.

Strains of *Staphylococcus aureus* possessing capsular and certain surface antigens are most immunogenic. Immunity is both cell-mediated and humoral and in the humoral reaction, it is antibacterial as well as antitoxic.

Penicillin is the drug of choice; however penicillin resistant strains have been found in cattle and dogs. New synthetic penicillinase resistant penicillins are of value. Tetracyclines, bacitracin, nitrofurans, erythromycin, trimethoprim-sulfamethoxazole, vancomycin, cephalosporins and clindamycin have been effective against *Staphylococcus aureus* infections. Newer drugs such as Augmentin (amoxicillin and clavulanic acid) and enrofloxacin have been shown to be effective. Treatment may be ineffective because of pathogens and because *Staphylococcus aureus* can survive in phagocytes (Carter *et al.*, 1995).

#### 2.14.1.3 Bovine Streptococcal Mastitis

*Streptococcus agalactiae* and *Streptococcus dysgalactiae* are the principle pathogens involved in streptococcal mastitis. *Streptococcus agalactiae* colonizes the milk ducts and produces persistent infection with intermittent bouts of acute mastitis. *Streptococcus dysgalactiae*, which is found in the buccal cavity and genitalia and on the skin of the mammary gland, causes acute mastitis. Clinical signs include inflammation of the mammary tissue and clots in the milk. Milk samples should be collected carefully and be cultured on blood agar, Edwards's medium and MacConkey agar and incubated aerobically at 37°C for 24 to 48



hours. Differentiation of mastitis-producing streptococci is done by: haemolysis on blood agar, CAMP test, sugar fermentation tests, Aesculin hydrolysis (Edwards medium), growth on MacConkey agar and Lancefield group (Quin *et al.*, 2002).

*S. pyogenes* (group A) may infect the bovine udder and be disseminated to humans in milk (Carter *et al.*, 1995).

#### 2.14.1.4 Streptococcal Mastitis

*Streptococcus agalactiae* may cause chronic mastitis in the milk ducts of cattle, goats and sheep and may produce beta-haemolysis with and or alpha or gamma haemolysis on bacterial cultures (Quin *et al.*, 2002). Most reports are in the older literature (Heindrich & Renk, 1967) or from India (Mukherjee & Das, 1957).

Goats are uniformly susceptible to *Streptococcus agalactiae*; mastitis caused by it does occur but to a lesser extent than in cattle. In flocks of milk goats the infection is passed from infected quarters to others by means of the milker's hands, the teat cups of milking machines, and washcloths used to disinfect the udder before milking (Radostits *et al.*, 2000).

The organism does not appear to be a problem in goats in the USA.

The goat is not systemically ill, but induration of the udder and loss of secretory tissue may occur (Smith & Sherman, 1994). *Streptococcus agalactiae* has been associated with severe stromal proliferation and fibrosis in goat udders (Addo, 1984). Introduction of a disease to a herd can be avoided by screening bulk tank milk from the herd of origin or culturing of the milk of the individual purchased doe before it joins the milking herd. Most isolates are sensitive to penicillin. Colonies are not sufficiently characteristic in blood agar cultures to permit differentiation from other streptococci, as *Streptococcus agalactiae* may be accompanied by greening of the medium, beta haemolysis or no haemolysis (Smith & Sherman, 1994).

Protein X, a surface protein of *Streptococcus agalactiae*, is frequently associated with strains recovered from cases of bovine mastitis (Carter *et al.*, 1995). This protein behaves as a target of opsonins and therefore, is possibly an important protective antigen against *Streptococcus agalactiae* mastitis. *Streptococcus agalactiae* does not split aesculin. The Camp test for complete haemolysis of erythrocytes sensitised by staphylococcal beta haemolysin is frequently used to make a presumptive diagnosis of *Streptococcus agalactiae* (Smith & Sherman, 1994). *Streptococcus dysgalactiae* may cause acute mastitis and mastitis respectively. *Streptococcus dysgalactiae* causes alpha haemolysis, beta or gamma haemolysis and alpha haemolysis with beta or gamma haemolysis on blood agar (Quin *et al.*, 2002). Artificially induced infections with *Streptococcus dysgalactiae* are distinguishable from mastitis caused by *Streptococcus agalactiae* (Major pathogen) (Radostits *et al.*, 2000).

*Streptococcus uberis* may cause acute mastitis and mastitis respectively. *Streptococcus uberis* causes alpha haemolysis and /or gamma haemolysis (Quin *et al.*, 2002). Surface M protein and to a lesser degree surface hyaluronic acid are considered to be major virulence factors in streptococci. Group A streptococci produce more than 20 extra-cellular products (Carter *et al.*, 1995).

#### **2.14.2 Minor Pathogens**

Minor pathogens that play a role in mastitis in goats are: *Corynebacterium pseudotuberculosis*, *Actinomyces pyogenes*, coagulase negative staphylococci, *Proteus* spp. *Escherichia coli* and *Klebsiella* have also been isolated from the caprine mammary gland, but they are not a frequent problem (Smith & Roguinsky, 1977). Yeasts and fungi have occasionally been isolated (Heindrich & Renk, 1967).

##### 2.14.2.1 Coliforms

Coliforms, including *E. coli* and *Klebsiella* spp., occasionally cause clinical mastitis in goats (Adinarayanan & Singh 1968; Lewter *et al.*, 1984). The organisms are Gram-negative, KOH<sup>+</sup> positive, oxidase negative rods. Colonies are large, grey or yellow and moist. *E. coli* gives off a faecal odour. Infections tend to be more common in periparturient does. Clinical signs in acute cases include anorexia, fever and yellowish or reddish watery secretion with an

increased somatic cell count. The gland is warm, swollen and painful. Occasionally signs progress to gangrene. These organisms are considered to represent “environmental” mastitis. Thus control involves keeping sleeping areas clean and dry, drying teats thoroughly before milking and avoiding teat end injuries. The use of post milking teat dipping does not aid in control of coliform infections, as these are initiated between milkings except when wet udders are milked (Smith & Sherman, 1994). Infusions of the udder on a prescription basis with gentamycin or trimethoprim/sulfadiazine have been recommended for confirmed coliform mastitis (Lewter *et al.*, 1984). Recent work in cows suggests that gentamycin therapy does not affect severity or duration of *E.coli* mastitis and clearance of gentamycin from kidney tissue requires many months (Erskine *et al.*, 1991). The use of antibiotics then is best avoided (Smith & Sherman, 1994).

#### 2.14.2.2 Coagulase Negative Staphylococci

Coagulase negative staphylococci were the most common cause of IMI with a prevalence of 86.6% of the infected udder halves, in a study of goats in South Africa (Kyozaire *et al.*, 2005). Increases in milk SCC as well as pathological udder findings were observed in infections with coagulase negative staphylococci such as novobiocin-sensitive *Staphylococcus epidermidis*, *S. simulans*, *S. lugdunensis*, *S. chromogenes* and *S. warneri* (Deinhofer & Pernthaner, 1995). *S. intermedius* and *S. hyicus* (most strains) are coagulase-positive staphylococci, but they are minor pathogens (Quin *et al.*, 2002).

Minor pathogens isolated from goat milk have been: *Staphylococcus epidermidis*, *S. caprae*, *S. lentus*, *S. simulans*, *S. capitis*, *S. lugdunensis*, *S. xylosus*, *S. chromogenes*, *S. hominis*, *S. arlettae*, *S. warneri*, *S. sciuri* and *S. saprophyticus* (Deinhofer & Pernthaner, 1995). *Staphylococcus caprae* has been isolated from goats’ milk. *S. arlettae*, *S. caprae* and *S. lentus* have been recovered from goats (Carter *et al.*, 1995).

In many herd surveys, non-haemolytic or coagulase negative staphylococci are the most commonly isolated organisms other than STA (Smith & Sherman, 1994). As many as 71% udder halves in a herd were found to be infected by coagulase negative staphylococci (Poutrel, 1984a; Sheldrake *et al.*, 1981). In a large survey, coagulase negative staphylococci were isolated from 17.5% of does (East *et al.*, 1987). In four commercial herds in Australia, the prevalence was 13.3% of 896 halves tested (Ryan & Greenwood, 1990). Numerous staphylococcal species have been identified, including *Staphylococcus epidermidis*, *S. intermedius*, *S. caprae* and *S. hyicus* (Kalogridou-Vassiliadou, 1991; Maisi, 1990b; Maisi & Riipinen, 1991; Poutrel, 1984a). These infections tend to persist throughout much of lactation (Smith & Sherman, 1994). Some authors regard the coagulase negative staphylococci as major pathogens (Dulin *et al.*, 1983), while others see them as minor pathogens or incidental infections (Smith & Sherman, 1994). Some authors define an increased SCC to be equivalent to a serious mastitis (Hinckley *et al.*, 1985). The economic importance of coagulase negative staphylococci remains unclear. Generally, the practitioner should look further to explain serious illness or marked loss in production because the coagulase negative staphylococci are unlikely to be the cause (Smith & Sherman, 1994).

Milk from mastitis-free French goat herds was examined for the presence of coagulase negative staphylococci, and 165 positive isolates were found. Most isolates were identified as *Staphylococcus caprae* or *Staph. xylosus*, but members of at least nine other species were present. No clinical consequences were observed to correlate with exoprotein production, which proved to be inconstant within individual coagulase negative staphylococci species (Bedidi-Madani *et al.*, 1998).

Two commonly isolated coagulase negative staphylococci: *Staphylococcus epidermidis* and *S. saprophyticus*, occur as commensals and in the environment. They cause opportunistic infections in humans and, very occasionally, in animals although they are usually regarded as non-pathogenic (Quin *et al.*, 2002).

STE: It is an occasional opportunist of low pathogenicity. *Staphylococcus epidermidis* is coagulase negative; colonies are non-haemolytic and unpigmented but otherwise resemble those of *Staphylococcus aureus*. (Carter *et al.*, 1995). It is identified by oxidase, coagulase, haemolysis (beta), pigment, mannitol and Dnase negative tests; glucose (O-F) fermentation,

novobiocin susceptibility, maltose and purple agar base acidic test results (Carter *et al.*, 1995).

Staphylococci isolated from mastitic goat milk in 29 Brazilian dairy herds were analysed for the production of alpha, beta and delta-haemolysin. Of the total strains studied, 80% demonstrated haemolytic activity. Among coagulase negative staphylococci isolated from sub clinical mastitis, 65.2% produced alpha-haemolysin, 19% beta-haemolysin and 83.3% delta-haemolysin, either alone or combined (Da Silva *et al.*, 2004).

#### 2.14.2.3 Streptococci

The streptococci and enterococci are Gram-positive cocci that occur in pairs or chains of varying lengths. They are facultative anaerobes, catalase negative, oxidase-negative and non-motile with the exception of some of the enterococci.

The streptococci can be divided into six principal or main categories based on growth characteristics, type of haemolysis, and biochemical activities. Haemolysis produced by a streptococcal species can be variable (Quin *et al.*, 2002). The main types of haemolysis are:

- Alpha ( $\alpha$ ) –haemolysis: a zone of greening or of partial haemolysis.
- Beta ( $\beta$ ) –haemolysis: a clear zone of haemolysis around the colony.
- Gamma ( $\gamma$ ) -haemolysis: no haemolysis.
- Alpha-prime haemolysis: a small zone of partially lysed red blood cells lying adjacent to the colony followed by a zone of completely lysed red blood cells extending further into the medium (Carter *et al.*, 1995).

Most streptococci of veterinary interest live as commensals in the mucosa of the upper respiratory and lower urogenital tracts (Quin *et al.*, 2002).

*Streptococcus zooepidemicus* caused chronic mastitis in goats. Streptococcal species form colonies on blood agar that are smaller than staphylococci (Smith & Sherman, 1994).

Another important way in which the streptococci are classified is into Lancefield groups. This grouping is based on serological differences in a carbohydrate substance in the cell wall. Some of the Lancefield group may be further divided by means of the agglutination test.

#### 2.14.2.4 Actinomyces (*Corynebacterium*) pyogenes:

This organism is frequently isolated from udders containing multiple abscesses (Smith & Sherman, 1994). In experimental infections, nonlactating glands are more severely affected than lactating glands (Jain & Sharma, 1964). The organism grows slowly on blood agar. Colonies are tiny at 48hours but made visible by a narrow zone of clear haemolysis (Smith & Sherman, 1994).

#### 2.14.2.5 *Corynebacterium pseudotuberculosis*

Though caseous lymphadenitis is very common in goats, mastitis caused by the disease is relatively rare (Smith & Sherman, 1994). Occasionally goats develop mastitis or abscesses in the parenchyma of the udder (Addo *et al.*, 1980; Burrell, 1981; Schreuder *et al.*, 1990).

Abscesses can burst inside or outside the udder sinuses and milk production decreases (Bath & De Wet, 2000).

Positive catalase test results will distinguish *C. pseudotuberculosis* from *A.pyogenes* (Smith & Sherman, 1994). Non-lactating goats developed a severe mastitis, lactating animals only a moderate one (Radostits *et al.*, 2000).

#### 2.14.2.6 Listeria

It has been suggested that *Listeria monocytogenes* can cause sub clinical interstitial mastitis (Sasshofer *et al.*, 1987). Listerias are commonly shed in the milk of clinically normal goats during herd outbreaks of listeriosis or immediately after parturition (Loken *et al.*, 1982).

Listerias are more important from a food safety perspective than a cause of mastitis in goats (Pearson & Marth, 1990).

#### 2.14.2.7 Mycobacterium

Mycobacterium infections (tuberculosis) can all be associated with tubercular mastitis in goats (Murray *et al.*, 1921; Sashofer *et al.*, 1987). In regions where bovine tuberculosis is common, cows infect goats by respiratory or alimentary routes (Heindrich & Renk, 1967). The udder becomes involved during generalization of the infection to other organs such as lungs, liver and spleen (Smith & Sherman, 1994). Often, the source is infected humans. The infection is sub-acute to chronic (Soliman *et al.*, 1953). Ante mortem diagnosis is by tuberculin test. Affected goats are slaughtered because of the risk they pose to human health (Smith & Sherman, 1994).

#### 2.14.2.8 Pseudomonas

*Pseudomonas* (PSE) species are also oxidase-positive, gram-negative rods, but colonies are usually granular and dry and may be a variety of colours. The source is usually contaminated water or teat dips, old pitted inflations (teat cup liners), or wet bedding (Smith & Sherman, 1994). Goats have been experimentally infected, but inoculation of the udder with large numbers of *Pseudomonas aeruginosa* was required; clinical signs varied from mild mastitis to severe, haemorrhagic mastitis with terminal septicaemia (Lepper & Mathews, 1966).

*Pseudomonas pseudomallis* is the causative agent of melioidosis. Mastitis sometimes occurs, although abscesses are most common in lymph nodes, spleen and lungs (Smith & Sherman, 1994). Abscesses in the udder that repeatedly break and drain to the outside also have been observed (Olds & Lewis, 1954). The organism has been isolated from macroscopically normal goat milk. Treatment is often unsuccessful, and affected goats are usually destroyed because of public health concerns (Smith & Sherman, 1994). Experimental pseudomonas mastitis in goats is acute with extensive necrosis and fatal septicaemia (Radostits *et al.*, 2000).

#### 2.14.2.9 Brucella

*Brucella melitensis* (or less frequently *Brucella abortus*) and the Q-fever organism, *Coxiella burnetti*, have serious public health implications because they can be excreted in the milk of apparently healthy goats without evidence of clinical mastitis. *Brucella melitensis* is responsible for Malta or undulant fever in humans, and a clinically detectable mastitis has only been reported in a few instances (Heindrich & Renk, 1967).

#### 2.14.2.10 Miscellaneous Organisms

Miscellaneous organisms isolated from mastitic goat milk include *Yersinia pseudotuberculosis* (Cappucci *et al.*, 1978), *Nocardia* (Dafaalla & Gharib, 1958), *Cryptococcus neoformans* (Aljaburi & Kalra, 1985; Pal & Randhawa, 1976) and numerous other fungi (Pal, 1982). *Yersinia pseudotuberculosis* has caused mastitis in an aborting goat doe, which probably experienced a bout of systematic yersiniosis (Radostits *et al.*, 2000). Granulomatous lesions in the mammary glands and in internal organs have been observed in goats experimental infected with *Cryptococcus neoformans*. Fungal mastitis has also been produced experimentally in goats using numerous *Candida* species and *Rhodotorula glutinis* (Aljaburi & Kalra, 1985).

An organism recovered from mastitic goat milk was identified as *Staphylococcus sciuri* subsp *lentus*, which was found to have pathogenic potential (Poutrel, 1984b).

### **2.14.3 Mycoplasmas**

Infection of goats with *Mycoplasma mycoides* is a major problem in some countries (Allen, 1985). It is associated with mastitis, pleuritis, peritonitis, polyserositis, septicaemia and abortion (Hungerford, 1990).

Mycoplasmas also play a role in mastitis in goats: *Mycoplasma agalactiae*, *Mycoplasma mycoides*, *Mycobacterium bovis*, *Yersinia pseudotuberculosis* and *Mycoplasma capricolum* (Blood *et al.*, 1983; DaMassa *et al.*, 1983; DaMassa *et al.*, 1992; Heindrich & Renk, 1967; Hunter, 1984; Perreau *et al.*, 1972; Perreau & Breard, 1979; Picavet *et al.*, 1983; Smith & Roguinsky, 1977; Taoudi *et al.*, 1988).

When repeated efforts to isolate bacteria from udders affected with clinical mastitis yield no growth or only non-haemolytic staphylococci, the possibility of mycoplasmal involvement should be investigated (Smith & Sherman, 1994).

Contagious agalactiae is a disease primarily of goats, caused by *Mycoplasma agalactiae* (Hungerford, 1990). It has been reported that tiamulin at 10mg/kg intramuscularly can maintain concentrations in the udder inhibitory for *Mycoplasma agalactiae* for 12 hours (Ziv, *et al.*, 1983).

*Mycoplasma arginini* has produced natural cases of mastitis in goats in India (Prasad *et al.*, 1984) although it is usually considered to be non-pathogenic (DaMassa *et al.*, 1992).

*Mycoplasma putrefaciens* has caused an outbreak of mastitis, abortion and arthritis in a large California dairy (DaMassa *et al.*, 1987).

The classic mastitic milk sample from an animal with a mycoplasma infection is one that separates into granular sediment and a greenish-yellow watery supernatant. Both halves of the udder are often infected and there is no response to treatment and slaughter of all affected goats is recommended. The udder atrophies but may be completely functional after the next parturition (Smith & Sherman, 1994).

#### **2.14.4 Retroviruses**

The presence of these organisms would probably be suspected if brucellosis were diagnosed as a cause of abortion in the herd, or if humans consuming raw milk products developed undulant or Malta fever (Stiles, 1950).

##### 2.14.4.1 Retroviral Mastitis (Hard Udder)

The mastitis resolves spontaneously, but the goat should be slaughtered to avoid human infections (Smith & Sherman, 1994).

Retroviral mastitis can develop as a result of the following bacterial infections: *Pasteurella haemolytica* and *Corynebacterium pseudotuberculosis*. *Pasteurella haemolytica* has been isolated only occasionally from goat milk (Bagadi & Razig, 1976; Manser, 1986; Schroter, 1954). In Angoras in South Africa, *Pasteurella haemolytica* is reported to be a more frequent cause of acute mastitis than *Staphylococcus aureus*, typically occurring four to six weeks after parturition and seldom being accompanied by gangrenous colour changes (Van Tonder, 1975). The organism is a Gram-negative, oxidase-positive, bipolar rod. Colonies are medium grey, transparent and haemolytic on blood agar plates (Smith & Sherman, 1994).

When the acute form of retroviral mastitis appears at parturition, the udder is hot with erythema and is very firm, but the overlying skin is loose and free of oedema. What milk is obtained appears normal but has an elevated cell count (Lerondelle, 1988). Signs of systemic illness are absent, but supramammary lymph nodes are enlarged (Smith & Sherman, 1994). Clinical signs may be less severe and SCC lower in subsequent lactations (Le Guillou, 1989; Lerondelle, 1989). The cellular infiltrations may externally compress ducts or protrude into ducts, preventing the passage of milk (Post *et al.*, 1986). Many goats with CAE infection do not develop a hard udder (Smith & Sherman, 1994).

## CHAPTER 3: MATERIALS AND METHODS

### 3.1 Model System

#### **3.1.1 Herds Used in Trials**

Three experimental trials were conducted. Trial 1 and Trial 2 were conducted at the Faculty of Veterinary Science, Onderstepoort using the goat herd of the Onderstepoort Teaching Animal Unit (OTAU) (Herd A), while Trial 3 was conducted on a commercial goat dairy in the Limpopo Province of South Africa (Herd B).

##### 3.1.1.1 Trial 1

The Onderstepoort herd (Herd A), consisted of 15 lactating Saanen dairy goats. Trial 1 was conducted over a period of seven days from the 17-24 June 2003 using 14 lactating goats all of which were in early lactation except one (Herd A). Trial 1 commenced in winter when temperatures were low, with no rainfall.

##### 3.1.1.2 Trial 2

The Onderstepoort herd (Herd A) was also used to conduct Trial 2. Trial 2 was conducted over a period of eight days from 8-16 September 2003, in spring with moderate temperatures and with light rainfall in the evenings.

##### 3.1.1.3 Trial 3

Trial 3 was conducted on a commercial goat farm, near Louis Trichardt (Makhado) in Limpopo province, which had 350 lactating goats (Saanen, Saanen/Indigenous crossbreeds and Toggenburg dairy goats). Trial 3 was conducted over a period of eight days from 15-23 January 2004, using 64 lactating goats all of which were in mid lactation (Herd B). Trial 3 commenced in summer when temperatures were very hot, with high humidity and light rainfall.

##### 3.1.1.4 Clinical

In addition, four goats with clinical mastitis from a smallholding close to the Faculty of Veterinary Science at Onderstepoort were studied (Herd C). This herd consisted of 13 lactating Saanen and Saanen/Toggenburg crossbred dairy goats.

#### **3.1.2 General Herd Management Programme:**

The management system of Herd A was evaluated as “fair”. All goats were identified with ear tags, and milk production, kidding dates, dates of birth and lactation numbers were kept on record. However the kidding pattern was irregular with a long kidding period. In Herd A the goats were fed on Total Mixed Rations (TMR) and kept in an intensive system. Goats were generally milked daily at 07:30 and 14:00 except for the duration of Trials 1 and 2, during which they were milked at 12 hourly intervals, at 07:00 and at 19:00. An adaptation period of three milkings was allowed in Trial 1 before treatment commenced. In Trial 2 an adaptation period of four milkings was allowed before treatment, due to the malfunction of the conductivity meter.

The management system of Herd B was evaluated as relatively good. The goats were identified by ear tags and kept in groups according to age and stage of lactation with 20-30 goats per group, in a free-stall barn system. Accurate records were kept of milk production, kidding dates, dates of birth and stage of lactation. A regular kidding pattern and a good breeding programme were used. In Herd B the goats were fed on Total Mixed Rations (TMR). The goats were milked at 12 hourly intervals starting at 03:00 and 15:00. The goats used in the trial were kept in two separate groups and were milked at 06:00 and at 18:00 daily for the duration of the trial. An adaptation period of two milkings was allowed before treatment, for practical reasons on the commercial dairy farm.

Four additional clinical mastitis cases were sampled from Herd C. Goats in Herd C were milked by hand at 12 hourly intervals, fed on Total Mixed Rations (TMR) and kept on a semi-intensive system.

**TABLE 3.1: MILKING MACHINE CHARACTERISTICS IN TRIALS 1, 2 AND 3.**

| Trial | Herd | Milking Machine Type   | System Vacuum | Vacuum at Teat End (Second minute) | Pulsation Rate                            |
|-------|------|--|---------------|------------------------------------|---|
| 1     | A    | De Laval, Six point milking machine with a low milk line.                                  | 36kPa         | 36.3-37.4kPa                       | Set to 74 pulses per minute.              |
| 2     | A    | De Laval, Six point milking machine with a low milk line.                                  | 36kPa         | 32.5-38.4kPa                       | Set to 74 pulses per minute.              |
| 3     | B    | De Laval and Milkrite combination, Ten point, quick exit, milking machine with a low line. | 37.3kPa       | 38.4-40.3kPa                       | Ranged from 78.6-103.1 pulses per minute. |

### **3.2 Experimental Design and Procedure**

#### **3.2.1 Experimental Animals**

In all trials, results of clinical udder examination, milk production, age and stage of lactation were recorded before experimental animals were selected by the principle of pairing. Goats that showed severe damage of the udder parenchyma (determined by palpation) were not eligible for inclusion in Trials 1, 2 and 3. Goats were divided into two groups (treatment group and control group), in each trial. The goats were selected in pairs according to lactation stage and milk production and one of each pair was then allocated to either the treatment group or the control group. Goats were identified by temporary markings. In Herds A and B, milk production for selection was based on the following production groups: low (less than 1.3 L), medium (1.3 L to 1.5 L) and high (greater than 1.5 L) daily milk production.

In Trial 1 all goats were in early lactation except for one goat that was in late lactation. In Trials 1 & 2 there were two goats with lactation numbers of 2, five goats with a lactation number of 3, two goats with a lactation number of 4, two goats with a lactation number of 5, two goats with a lactation number of 7 and one goat with an unknown lactation number. In Trials 1 & 2, the goats were between two and seven years old.

In Trial 2 all goats were in mid lactation except for one goat that was in late lactation. There was one more goat, which was five years old, with a lactation number of 5 in Trial 2.

In Trial 3 all goats were in mid lactation, seven goats had a lactation number of 2 and the remaining 57 goats had a lactation number of 1. All the goats in Trial 3 were almost one year old.

**TABLE 3.2: TREATMENT AND CONTROL GROUPS OF LACTATING GOATS IN EACH TRIAL.**

| Trial | Herd | Product Used           | Treatment Group | Control Group |
|-------|------|------------------------|-----------------|---------------|
| 1     | A    | Curaclox LC            | 8               | 6             |
| 2     | A    | Spectrazol Milking Cow | 7               | 7             |
| 3     | B    | Curaclox LC            | 12              | 12            |
| 3     | B    | Rilexine 200 LC        | 20              | 20            |

Two goats with clinical mastitis in Herd C were treated 12 hours after identification of mastitis in both halves: Goat 1 was treated with Curaclox LC and Goat 2 was treated with Spectrazol Milking Cow. Two other goats (Herd C) were both treated with Curaclox LC, 24 hours after sampling in the infected halves.

### 3.2.2 Sampling

- Foremilk was stripped, teats were disinfected and a milk sample was taken from each udder half of each goat (half-milk samples).
- In all three trials the following milk samples were taken: two sets of half samples and a composite sample (before, during and after treatment).
- The California Milk Cell Test (CMCT) and conductivity measurements were performed. In Trial 3 the conductivity meter became non-functional on the second day, and thus the conductivity test was eliminated from then on.
- Each udder half was milked separately and milk volume was recorded.
- The temperature of goats was taken and recorded to identify sick animals. All goats in the treatment group were treated.
- In all three trials after treatment, sampling continued until SCC returned to baseline and until there were at least two consecutive negative TRIS tests for each goat, approximately 10 days.

In Trial 1, the first three samplings were used to determine baseline values, and both udder halves of goats in the treatment group were treated. On the fourth sampling, the treatment group was given intramammary treatment with Curaclox LC (refer to Table 3.2), for three treatments at 12 hourly intervals.

In Trial 2, the first four samplings were used to determine baseline values, and intramammary treatment with Spectrazol milking cow commenced on the fifth sampling, at 12 hourly intervals for three treatments.

In Trial 3, the first two samplings only were used to determine baseline values, due to practical reasons on the commercial dairy farm. Intramammary treatment with both Curaclox LC and Rilexine 200 LC commenced on the third sampling and at 12 hourly intervals for three treatments.

#### 3.2.2.1 Aseptic Milk Sampling Procedure

- Collection of aseptic milk samples from individual udder halves for laboratory diagnosis of mastitis (performed according to Giesecke *et al.*, (1994), modified for use in goats). (See Appendix 3.1).
- Goats' udders were cleaned with the minimum amount of water and dried.
- Teats of goats were disinfected and cleaned with cottonwool swabs soaked in methylated spirits.
- Damaged halves or halves with clinical mastitis were handled last.
- The first two or three jets of milk were milked into a strip cup and evaluated for clinical changes.
- The sample tube was held in the correct manner and filled with a single jet of milk. This procedure was repeated for each udder half. Care was taken not to contaminate the lids.
- The samples were kept on ice at approximately 4°C from collection until arrival at the laboratory.



- In Trial 3 samples were placed in a cooler bag with ice, and sent by courier to the milk laboratory at Onderstepoort for analysis. The samples were kept cold and reached the laboratory within 48 hour from the time the samples were taken.

### 3.2.2.2 Composite Samples

Composite milk samples were taken on three occasions during each trial:

1. 12 hours prior to the first intramammary treatment,
2. 12 hours after the last intramammary treatment had been administered,
3. At the end of the trial when no antibiotic residues, as measured by the TRIS test, were present in the milk.

The composite samples were not taken aseptically. A milk sample was taken from the total amount of milk produced by each goat (a whole milk sample).

In Trial 1 these composite milk samples (15ml) were preserved with potassium dichromate and in Trials 2 and 3 they were preserved with bronopol.

Composite samples were used for testing of butterfat, protein and lactose by an accredited laboratory, (Lacto Lab Pty Ltd, ARC, Irene, P.O. Box 326 Irene 0062) using a Milk Oscan System 4000.

### 3.2.2.3 Conductivity

Conductivity was measured with a MAST-O-TEST™ 2.0 (Durotec, P.O. Box 12540, 6006 Centralhil, Port Elizabeth, South Africa) conductivity meter. The MAST-O-TEST 2.0 consists of a measuring cup with terminals, electronic unit, switch and handle. The measuring cup was equipped with two graphite electrodes and one temperature sensor.

#### 3.2.2.3.1 The Principle of The Test

The MAST-O-TEST™ 2.0 sub clinical mastitis detector measured electrical conductivity, correlated to sodium chloride concentration, which increases with sub clinical mastitis. Conductivity was tested after foremilk was stripped and half milk samples were taken. A jet of foremilk was directed into the milk compartment of the conductivity meter, to cover the electrodes. A reading and a colour code indicated conductivity.

#### 3.2.2.3.2 Interpretation of Results

**TABLE 3.3: CONDUCTIVITY METER CHARACTERISTICS.**

| Colour | Conductivity | Wavelength Limit | Comment    |
|--------|--------------|------------------|------------|
| Green  | <54 units    | <5.3mS/cm        | Acceptable |
| Orange | 54-70 units  | 5.4-6.9mS/cm     | Marginal   |
| Red    | >70 units    | >7mS/cm          | Severe     |

Conductivity readings of 54 units or more should according to the manufacturer be an indication of increasing infection or of mechanical injury. Results for both halves of each animal were compared. Previous experience with the conductivity meter has shown that a 15% difference in the readings of each udder half in the same goat might indicate a development of mastitis (Petzer, I M, Faculty of Veterinary Science, Onderstepoort, Personal Communication, 2004). This difference has been shown to be more useful than the conductivity level itself (Mast O Test 2.0, P.O. Box 12540 Centralhill 6006 South Africa; (Fax) +270415855597). A flashing bar on the display of the conductivity meter indicated this 15% difference.

#### 3.2.2.3.3 Unreliability of Conductivity Meters

Conductivity meters were used and cleaned according to manufacturer's instructions, but they proved to be most unreliable.

One conductivity meter was used in Trial 1. Three conductivity meters were needed in total to complete Trial 2 due to the breakdown of the conductivity meters. However only one

conductivity meter was available in Trial 3 and due to the distance from the distributors, Trial 3 had to be completed without conductivity measurements after the breakdown of the last conductivity meter.

Conductivity measurements were not taken for clinical mastitis cases (Herd C) because meters were not available on those occasions.

#### 3.2.2.4 California Milk Cell Test (CMCT)

The CMCT is a chemical-physical technique for the evaluation of somatic cell numbers in milk. Chemically it depends on the reaction between the CMCT reagent and the DNA (from the nuclei of somatic cells and leucocytes) in the milk. This affected the viscosity of the mixture which was evaluated by sight, and which in turn was correlated to numbers of somatic cells in the milk. Increased numbers of somatic cells in milk gave rise to thicker mixtures and thus higher CMCT reaction scores. The CMCT also gave an indication of the milk pH. For example, increased udder inflammation might cause the milk to become more alkaline and the mixture would then turn a darker purple.

The CMCT paddle was calibrated in order to use equal amounts of milk and reagent for the test. The paddle was then gently swayed from side to side and in a circular motion. A non-viscous solution was given a negative CMCT score. A slightly viscous solution starting with a small slimy centre was given a +1 CMCT score. A more viscous solution with a larger slimy centre gave a +2 score. A semi-solid state was given a CMCT score of +3. CMCT was not done for clinical mastitis cases. Inter-half differences of more than two points between milk samples from udder halves of the same goat were taken as significant for identification of infection (Petzer, I M, Faculty of Veterinary Science, Onderstepoort, Personal Communication, 2004).

#### 3.2.2.5 Milk Oscan

The Milk Oscan system 4000 of an accredited laboratory (Lacto Lab, Pty, Ltd, P.O. Box 326 Irene, 0062) was used to determine the butterfat, total protein and lactose content of the composite milk samples taken during all three trials. The above criteria were not determined for clinical mastitis cases.

#### 3.2.2.6 Milk Volume

In Trials 1 and 2 (both Herd A), milk volume was measured separately for each udder half, starting with the left udder half each time. An Ehrlenmeyer flask was inserted between the milking cluster and the milk pipeline. One half of the udder was milked at a time. Milk collected in this way was measured manually in a measuring cylinder.

For clinical mastitis cases in Herd C, the goats were milked manually and milk volume of each udder half was measured separately using a measuring cylinder.

In Trial 3 (Herd B) for practical reasons on the commercial dairy farm, the volume of both udder halves combined was measured using the Waikato meters.

### **3.2.3 Clinical Examination of the Mammary Parenchyma**

#### 3.2.3.1 Clinical Procedure

Clinical examination of the mammary parenchyma was performed post milking in the lactating goats. Superficial palpation of the udder was performed to assess the degree of firmness of attachment of the skin to the parenchyma and areas of increased temperature or pain associated with touch. This was done by lightly moving four fingers of the hand in circular movements over the surface of the udder. All lesions were described and recorded. This was followed with deep palpation of the udder parenchyma.

Deep palpation was done to detect pain and texture of the udder parenchyma. Chronic cases of mastitis may have hard granular tissue or may be atrophied and or fibrotic. Two hands were used on both sides of the udder. Circular movements were executed more firmly than with superficial palpation and signs of pain and abnormalities in the texture of the parenchyma were recorded. Clinical mastitis was diagnosed based on the presence of pain,

heat and redness of the mammary gland with or without oedema present (Giesecke *et al.* 1994).

### 3.2.3.2 Body Temperature

The body temperature of the goats was taken at every milking in Trial 1, but in further trials and clinical cases only when sick animals were identified.

**TABLE 3.4: CRITERIA FOR CLINICAL EXAMINATION OF THE PARENCHYMA OF THE MAMMARY GLAND.**

| Udder Parenchyma | Description   |
|------------------|---|
| 0                | Normal pliability and elasticity of the glandular tissue, fine and soft glandular tissue. |
| F1               | Slight diffuse fibrosis with few slight coarse granular indurations.                      |
| F2               | Distinct fibrosis with multiple coarse granular indurations.                              |
| F3               | Marked fibrosis with generally coarse multiple extensive indurations.                     |
| N1               | Single distinct induration 2.5- 5 cm in diameter.   |
| N2               | Single distinct induration 5-7.5 cm in diameter.  |
| N3               | Single distinct induration larger than 7.5 cm in diameter.                                |
| A1               | Slight atrophy of a half.   |
| A2               | Distinct atrophy of a half.   |
| A3               | Marked atrophy of a half.   |

Adapted from Giesecke & Van den Heever, (1974). (F=Fibrosis; N=Nodules; A=Atrophy)

### 3.2.4 Antibiotic Treatment

#### 3.2.4.1 Products Investigated: Refer to Tables 3.2 & 3.5

- The antibiotics used in these trials were selected for being commonly used, broad-spectrum preparations.
- Trial 1, a semi-synthetic penicillin based intramammary preparation (Curaclox LC, which contains 75mg sodium ampicillin and 200mg sodium cloxacillin per dose plus blue dye). Curaclox LC G2615, (Norbrook (Pharmacia AH) P.O. Box 10698 Centurion, 0046), cloxacillin 200mg, ampicillin 75mg, blue dye/ 4.5g syringe.
- Trial 2, a cefuroxime 250mg based intramammary product (Spectrazol Milking Cow, Schering-Plough). Spectrazol milking cow, cefuroxime, 250mg, S4 Intramammary Injection 83/594, (Schering-Plough Animal Health, P.O. BOX 46, Isando, 1600)
- Trial 3, a cephalixin 100mg, neomycin sulphate 100mg and prednisolone based intramammary product, Rilexine (SA) 200LC injection 83/638, (Logos Agvet (Virbac), Private bag X115, Halfway House, 1685). Curaclox LC G2615, Norbrook (Pharmacia AH), cloxacillin 200mg, ampicillin 75mg, blue dye/ 4.5g syringes.
- In the clinical mastitis cases (Herd C); Goat 1 was treated with Spectrazol milking cow (as above), Goat 2 was treated with Curaclox LC (as above), Goat 3 was treated with Curaclox LC in the left udder half and Goat 4 was treated with Curaclox LC in the right udder half (as above).

#### 3.2.4.2 Administration of Antibiotics

In Trials 1, 2 and 3, the entire content of the syringe formulated for use in cattle was inserted into each udder half of the goat. Although the goat udder is smaller than a cow udder the same amount was inserted, since this was the only size tube available. Using part of the contents would have resulted in:

- possible contamination, if one applicator had to be used on more than one udder half,
- the difficulties of controlling the quantity of antibiotic if only part of the contents of the applicator were used.

**TABLE 3.5: TABLE OF INFORMATION OF PRODUCTS USED FOR TREATMENT.**

| Product Name           | Composition  | Indication for use   | Dosage (frequency)   | Recommended Withdrawal periods prescribed for cattle. |
|------------------------|--|--|--|---|
| Curaclox LC            | Each 4.5g syringe contains 200mg cloxacillin, 75mg ampicillin and blue dye.  | Ampicillin most active against: <i>Staphylococcus spp.</i> and <i>E.coli</i> . Cloxacillin most active for: <i>Arcanobacterium</i> and <i>Streptococcus spp.</i>   | 1 syringe per half after milking for 3 consecutive milkings 12 hours apart.                                | 72h   |
| Rilexine 200 LC        | Each 10ml disposable syringe contains cephalexin 100mg, neomycin sulphate 100mg, and prednisolone 10mg. Preservatives: Butylated hydroxyanisole 0.018%, benzyl alcohol 0.9% v/v. | Cephalosporins: <i>Streptococcus agalactiae</i> mixed Streptococcus infections and <i>Staphylococcus aureus</i> . Neomycin: <i>Streptococcus agalactiae</i> , <i>Streptococcus dysgalactiae</i> , mixed Streptococcus infections, <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Pseudomonas</i> .  | 1 syringe into each udder half, 12 hourly for 3 treatments.  | 96h   |
| Spectrazol Milking Cow | Each syringe contains cefuroxime sodium salt equivalent to 250mg cefuroxime in a rapid release oil base.   | <i>Citrobacter spp.</i> , <i>Corynebacterium spp.</i> , now called <i>Arcanobacterium pyogenes</i> , <i>Enterobacter spp.</i> , <i>E. coli</i> , <i>Klebsiella spp.</i> , <i>Micrococcus spp.</i> , <i>Staphylococcus spp.</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus spp.</i> , ( <i>S agalactiae</i> , <i>S. dysgalactiae</i> and <i>S. uberis</i> ). | 1 syringe into the teat canal of the infected half every 12 hours after each of three successive milkings. | 60h   |

### 3.2.5 Laboratory Procedures

Laboratory procedures were conducted at the milk laboratory (Department of Production Animal Studies, Faculty of Veterinary Science, Private bag X04, Onderstepoort, 0110) under

Good Laboratory Practice (GLP) conditions. All laboratory analysis was done on one sample and not in duplicate/triplicate.

Milk samples taken were analyzed in the laboratory until such time that there were no more antibiotic residues present in the milk (the TRIS results had been negative for two consecutive samplings), as indicated by the results of the Thermo-Resistant Inhibitory Substances (TRIS) test, and the Somatic Cell Counts (SCC) had returned to that of the baseline values (average results before treatment). In the laboratory, SCC was measured with the Fossomatic 90 apparatus at the Laboratory of Production Animal Studies of The Faculty of Veterinary Science, University of Pretoria. Microbiological tests were done on the milk to detect inhibitory substances in milk and determine which bacteria were causing sub clinical or clinical mastitis.

Sample containers and epindorf tubes were marked according to the sampling times and goat numbers. All data of sampling in the dairy and in the laboratory were recorded in data books. In the laboratory, Bovine blood Tryptose Agar (BTA) plates were made and numbered.

The first samples were divided into sub-samples:

- microbiological tests were performed,
- epindorf tubes were frozen and stored for subsequent tests and
- the rest of the sample was preserved for somatic cell counting.

Frozen samples were used later for quantitative evaluation of antibiotic residues in milk samples.

Second samples were used for the TRIS test.

### 3.2.5.1 Visual Inspection of Milk Samples

Milk samples were inspected visually to check normal udder function. Milk samples were inspected visually to identify discolouration e.g. blood in milk, dye in milk, changes in consistency (flocules) and composition (watery or serum-like).

#### 3.2.5.1.1 Dye Colour Changes

The colours of the samples treated with Curaclox LC, containing the blue dye (refer to Table 3.5), were matched up to colours on the Plascon Colour Expressions colour chart (Figures 3.1, 3.2 & 3.3). Each colour was matched up to a unique code and colour name on the chart, in order to standardize the different variations in the colour of the blue dye in goat milk, at every consecutive milking after intramammary treatment.



**FIGURE 3.1: COLOURS USED FROM THE PLASCON COLOUR EXPRESSIONS COLOUR CHART: A29**

Left to Right (Darkest to lightest shades of blue):

A29-7 Nightfall, A29-6 Peacock Blue, A29-5 Turquoise, A29-4 Ming Blue, A29-3 Cupid Blue, A29-2 Skylight, A29-1 Igloo



**FIGURE 3.2: COLOURS USED FROM THE PLASCON COLOUR EXPRESSIONS COLOUR CHART: A28**

Left to Right (Darkest to lightest shades of blue):

A28-7 Deep Aqua, A28-6 Aqua, A28-5 Mahone Bay, A28-4 Fjord Blue, A28-3 Blue Waltz, A28-2 Snow Shadow, A28-1 Hudson Ice



**FIGURE 3.3: COLOURS USED FROM THE PLASCON COLOUR EXPRESSIONS COLOUR CHART: A27**

Left to Right (Darkest to lightest shades of blue):

A27-7 Superior, A27-6 Huronia, A27-5 Mariposa, A27-4 Bow Valley, A27-3 Portage, A27-2 Algonquin, A27-1 Peace River

### 3.2.5.2 Microbiology on Half Milk Samples: (Sandholm *et al.*, 1995)

Milk samples were cultured on Bovine blood Tryptose Agar (BTA) (Columbia Blood Agar Base, CM331 from Oxoid plus defibrinated bovine blood), which supports the growth of most mastitogenic pathogens. Inoculated agar plates were incubated for 18-24 hours at  $37 \pm 1^\circ\text{C}$  and then evaluated for growth and re-incubated and read again for a further 24 hours if no growth was present.

Colonies were tentatively identified based on colony morphology, appearance and haemolysis.

The catalase test was performed and bacterial isolates were Gram-stained to distinguish between Gram-negative and Gram-positive microorganisms. Gram-positive cocci that were catalase negative were tested by means of the CAMP/ aesculin hydrolysis test (Columbia Blood Agar Base, CM331 from Oxoid with ferric citrate and aesculin plus defibrinated bovine blood) to distinguish between the Lancefield groups. The diagnosis of streptococci was confirmed by means of the streptococcal grouping kit (Latex agglutination test) from Oxoid (CA Milsch, P.O. Box 943 Krugersdorp, 1740).

Gram-positive cocci, which tested catalase positive, were tested for coagulase by means of the Staphylase Test from Oxoid. According to Oxoid test, there were coagulase negative staphylococci and coagulase positive staphylococci. Coagulase positive staphylococci were identified as *Staphylococcus aureus*. Gram-negative organisms were identified using the API 20E from bioMerieux (Omnimed, P.O. Box 4328, Honeydew, 2040).

### 3.2.5.3 Somatic Cell Counts

Samples were preserved with potassium dichromate, mixed and left to stand for at least four hours before counting. Samples were preheated in a water bath at  $40^\circ\text{C}$  (Van den Heever *et al.*, 1983), using a Fossomatic 90 counter (The Rhine Ruhr Group, P.O. Box 76167, Wendywood, 2144). Inter and intra laboratory milk standard samples were used to verify the accuracy of the somatic cell counts.

#### 3.2.5.4 Milk Oscan

Butterfat, total protein and lactose concentrations were evaluated by an accredited laboratory (Lacto Lab Pty Ltd, ARC, Irene, P.O. Box 236, Irene, 0062), using the Milk Oscan system 4000.

#### 3.2.5.5 Thermo-Resistant Inhibitory Substances (TRIS)

TRIS tests are carried out to determine the presence of antibiotic residues and other heat stable inhibitory substances in milk.

##### Disc Assay Procedure

Milk samples were pre-treated for five minutes at 80°C. The control organism *Bacillus subtilis* (SABS TCC No.: Bac 2= Strain Ref.: ATCC 6633) was streaked out onto a Bovine Tryptose Agar (BTA) plate. Aseptically (using a pair of forceps) a Whatman disc was saturated in the heat-treated milk, and excess milk was removed by pressing the disc against the inside of the container. The Whatman disc was then placed onto the BTA plate. All BTA plates were marked according to the corresponding milk samples used for each TRIS test. BTA plates were then incubated for 24h at 37°C. The zones of inhibition were qualitatively evaluated. The diameter of the Whatman disc was measured with a caliper and found to be 6.5mm in diameter. However from the results obtained for the baseline and for the control animals, it was evident that diameters of inhibition of 1mm (a total diameter of disc plus inhibition of 7.5mm) still indicated a negative TRIS test with insignificant inhibition. The presence of a clear zone larger than 7.5mm diameter indicates the presence of one or more TRIS substances and thus results in a positive TRIS test. Results were scored as a negative TRIS test (indicating an absence of TRIS substances) or positive TRIS test ( indicating a presence of one or more TRIS substances).

A score of 1 represents a positive TRIS test result and a score of 0 represents a negative TRIS test result, for all the graphs of TRIS versus time in Chapter 4. Thus, a TRIS test result between 0 and 1 shows that not all the udder halves treated with a particular product showed a positive TRIS test result at the particular treatment time.

#### 3.2.5.6 Quantitative Evaluation of Antibiotic Residues in Milk Samples

The PARALLUX™ Beta Lactam Assay test was said to be valid by the manufacturer for raw commingled bovine milk. (Performance tested by AOAC research institute: License number 000402.) The Parallax™ Beta Lactam Assay is a competitive solid-phase fluorescence immunoassay intended for use as a rapid detection method for Pen \* (penicillin G, amoxicillin, ampicillin, cloxacillin, ceftiofur and cephalixin) residues in raw commingled bovine milk. Personnel who had been trained by an authorized representative used this kit. For more information refer to the information booklet of the IDEXX Parallax™ Beta Lactam Assay, (IDEXX distributors). The Veterinary Diagnostic Services Laboratory, Department of Agriculture, North West Province, Botha Street, Potchefstroom performed this analysis.

##### 3.2.5.6.1 Determination of Ampicillin and Cloxacillin Concentrations in Selected Goat Milk Samples

Sixteen animals were selected from those treated with Curaclox LC in Trial 1 and both ampicillin and cloxacillin concentrations were determined. Samples treated with Curaclox LC were selected, in order to see if there was a difference in the withdrawal period for each of the individual ingredients (ampicillin and cloxacillin). In all the goats selected, only milk from the left udder half was tested for antibiotic residue concentrations, to limit costs.

The amount of samples tested in this way was limited by the high cost of the above procedure. Animals with longest withdrawal periods, treated with Curaclox LC in Trial 1 and in Trial 3 were used. In total 16 animals were used. Samples from one sampling before treatment right through until two samples with negative readings for antibiotic residues, were tested.

All samples were frozen immediately after they were taken until such time that they were used. The samples were defrosted and made into 50:50 dilutions with distilled water and

mixed well before use. The dilution factor was necessary for the samples to reach the correct viscosity, in order to be read by the machine.

This test was carried out to confirm the accuracy of the TRIS test. The TRIS test was carried out on all animals, but the Parallax™ Beta Lactam Assay test was only carried out on the left udder halves of 16 animals because of the high cost.

#### 3.2.5.6.2 Information about The Parallax Test

The following are the specifications for the design of the kit.

Sensitivity: Based on 30 samples at each milk concentration.

Selectivity: 60 negative control milk samples were evaluated in an independent laboratory on each of Pen\*, cloxacillin, cephapirin and ceftiofur channels. None were positive on the cloxacillin and ceftiofur channels. One of the negative control samples tested positive on the Pen\* channel. Pen\* represents the detection of penicillin G and/or amoxicillin and or ampicillin.

Results of selectivity evaluations on milk samples containing no antibiotic demonstrated that the Parallax™ Beta Lactam Assay met the standard of 90% selectivity with 95% confidence. This information shows that it is a reliable test for commingled bovine milk. However there is no information available on whether the Parallax test is reliable for frozen goat milk or not. The Parallax™ Beta Lactam Assay cross-reacted with the following drugs at the levels indicated by the Parallax Pen\* channel.

**TABLE 3.6: CROSS-REACTIVITY AND ANTIBIOTIC CONCENTRATIONS OF PEN\* PARALLUX CHANNEL.**

| Antibiotic    | 50ppb | 20ppb | 10ppb |
|---------------|-------|-------|-------|
| Ticarcillin   | 100%  | 100%  | 100%  |
| Dicloxacillin | 100%  | 66%   | 33%   |
| Cloxacillin   | 100%  | 100%  | >90%  |

Ppb= parts per billion

Pen\* represented the detection of penicillin G and or amoxicillin and /or ampicillin.

Cloxacillin was detected at 100% between 8ppb and 10ppb. The positive controls used were 10ppb of cloxacillin with 100% detection. At 6ppb of cloxacillin there would have only been 60% detection. This means that the reliability of this test is lower at low antibiotic concentrations.

Ampicillin was detected at 100% between 4ppb and 10ppb; the Beta-Lactam positive control used to detect ampicillin was 10ppb with 100% detection. Concentrations of less than 4ppb would not give 100% detection for ampicillin.

#### 3.2.5.6.3 The Cross-reactions of Drugs

The following antibiotics could lead to cross-reactions of drugs during Parallax testing: Cefadroxyl, sulfadiazine, sulfalinamide, sulfathiazole, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamycin, neomycin, streptomycin, ivermectin, erythromycin, pirlimycin, novobiocin, furosemide, trichlormethiazide, chlorthiazide, tilmicosin, oxytocin, phenylbutazone, dexamethasone, thiabendazole, p-Aminobenzoic Acid (PABA) and dipyrone. None of the antibiotics mentioned above were used in this research. Therefore, the significance of this is that there was no cross-reaction with the antibiotics tested for in this experiment.

#### 3.2.5.6.4 Operating Instructions

Samples were tested using the Parallax Processor and the Parallax™ Beta-lactam Assay kit.

#### 3.2.5.6.5 Storage

Parallax cartridges were stored, refrigerated at 0°- 7°C and only the number of tests to be used for the day was removed from the refrigerator and kept at 18-29°C prior to use.



#### 3.2.5.6.6 Sample Handling

This test is normally used on raw commingled bovine milk, but in this experiment, thawed goat milk was used. There was no indication if this would affect the validity of the test. The frozen goat milk samples were thawed, made into a 50:50 dilution with distilled water and tested cold, 0° to 4°C. The dilution factor was necessary for the frozen goat milk in order to bring the samples to the correct viscosity to be read by the machine. To test accuracy, one sample that was already at the correct viscosity was tested both undiluted as well as with 50:50 dilution and no obvious difference was visible. The samples were thoroughly mixed before testing. According to the instructions of the Parallax test, milk being tested for NCIMS (National Conference of Interstate Milk Shippers) purposes may not be frozen at any time during the testing process. Freezing and thawing of goat milk affects the structure of the milk (Haenlein, 2000), but nothing could be done about this as samples were already frozen and stored. This was because the methods used for the Parallax testing were only available after the samples had been stored (frozen). The sensitivity of the Parallax test has shown to be affected by milk samples that have been frozen and thawed in the case of commingled bovine milk. The Parallax test correctly identified the presence of beta-lactams in raw commingled bovine milk at or below the FDA established safe tolerance level (IDEXX instruction manual). However, the sensitivity of the Parallax test has been shown to be affected by the presence of levels of bacteria in milk at the PMO (Pasteurized Milk Ordinance) limit or greater (IDEXX instruction manual).

Refer to IDEXX instruction manual for more information on, precautions, warnings and on preparation. (Available through IDEXX Laboratories, Inc or an authorized local representative.)

#### 3.2.5.6.7 Positive and Negative Controls

1. For positive control, Parallax penicillin G/cephapirin/ceftiofur positive control, (part # 99-09305), and Parallax cloxacillin positive control, (part #99-09306) were used as directed in the descriptive inserts, which accompanied these products.
2. For negative control, only Parallax Negative Control could be used, (part #99-09207), as directed in the descriptive insert, which accompanied that product. The manufacturers of the Parallax stated other negative control products including negative, raw, commingled bovine milk as being not acceptable for analyses.

[Refer to the Parallax™ Processor Operation Manual for more details in the United States for NCIMS testing.]

The Parallax™ Negative Control was intended for use with the Parallax Milk Residue Testing System only. This control was used to monitor the performance of the Parallax apparatus. The Negative Control was run as part of a quality programme and to confirm presumptive positive samples. In the United States for NCIMS testing: Positive and Negative Controls must be run daily prior to testing samples, with each new batch of product and with a sample known to be positive.

#### 3.2.5.6.8 Test Procedure

A Negative Control was run in duplicate every 24 hours for each batch of product, which sets the standard in the Processor for the current batch of product (antibiotics). Refer to the Parallax™ Processor Operation Manual for more details.

#### 3.2.5.6.9 Interpreting The Results

A negative result was any reading less than or equal to 1.00. A positive result was any reading greater than 1.00 and “U” indicated unacceptable results.

If the results were invalid and samples were retested:

- 1 referred to the initial test results.
- 2 and 3 referred to the duplicate sample confirmation results.
- – Referred to the negative control.

- + Referred to the positive control.

Pen\* represents the detection of penicillin G and or amoxicillin and or ampicillin. The Pen\* channel cross-reacts with cloxacillin; therefore, if the Pen\* channel is positive, there may be penicillin and/or amoxicillin and/or ampicillin and/or cloxacillin present in the milk sample. In the United States for NCIMS testing, three levels of testing are considered:

- A positive result obtained from the initial testing of a milk sample from a bulk tanker is defined as presumed positive.
- A presumed positive must be reported to the State Regulatory Agency for a screening test positive (Load Confirmation) retest.
- Producer trace back/permit action test performed on all producers' samples, which contributed to all positive results of a Screening Test Positive (Load Confirmation) retest.

#### 3.2.5.6.10 Confirmation Procedure with The Beta Lactam Assay

This protocol was intended for retesting initial positive raw, commingled bovine milk (in this case frozen and thawed 50:50 diluted goat milk) samples in duplicate with Positive and Negative controls.

Confirmation options included the use of Beta Lactam or single drug assays depending on which channel was positive. The descriptive inserts, which accompanied those products under the Confirmation procedure section, were referred to. For step-by-step details of the confirmation procedure, refer to the IDEXX Parallax™ Beta Lactam Assay information manual.

### **3.2.6 Data Management**

All data were entered and stored in Microsoft Excel.

#### 3.2.6.1 Criteria for Assessing Efficacy

When TRIS test results were negative for two consecutive samplings, it was assumed that no more antibiotic residues were present and the trial was ended on those grounds. This is the usual procedure. All withdrawal period results are an indication of the first time that the antibiotic residues were negative according to one of the following tests: TRIS, colour or Parallax. In practice 24h should be added to these withdrawal periods, as is done for the recommended withdrawal periods given for use in cattle to allow a safety margin.

#### 3.2.6.2 Statistical Analyses

In each Trial graphs were drawn of the means of each variable over time using Sigma Plot 9.0 [Sold by Rock Ware, [www.rockware.com/catalog/pages/sigmaplot2.html](http://www.rockware.com/catalog/pages/sigmaplot2.html)] and Microsoft Excel.

The student's two-sample unpaired t-test was used to test for differences between goats with and without bacteria, etc. The data were generally acceptably normal in distribution, except for SCC, which were log normal and thus the SCC were transformed (to base 10). All tests were considered significant up to the 5% level of significance.

When a sample of individuals may be classified according to two attributes, this results in a two-way frequency table known as an r x c contingency table (Snedecor & Cochran, 1980). The Chi-square row-by-column test was useful to determine if there were significant differences between the two independent attributes. This test had certain limitations (Siegel, 1956), namely, that no category may have an expected frequency of less than 3 to 5. In this study, the CMCT was classified as 0 (healthy) or 1 or 2 or 3 for each udder half of each milk goat. The question to be answered was to determine whether there was a difference in the distribution of CMCT categories between treatments (T1, T2 & T3).

Data were analysed using the statistical program GenStat (2003).

The critical values in the standard statistical tables for determining significance of a correlation coefficient are dependent on the pairs of data in the sample. For example, a correlation coefficient of  $r = 0.30$  is significant ( $P < 0.05$ ) for more than 43 pairs and highly

significant ( $P < 0.01$ ) for more than 72 pairs of data. However, the coefficient of determination, or  $R^2$  is only 9%. For 20 pairs of data  $r > 0.4438$  is considered to be significant at the 5% level, with  $R^2$  of 20%.

Generally, a coefficient of about approximately 0.7 or more is regarded as indicating a fairly strong correlation and in the region of approximately 0.9 it indicates a very strong correlation. In the region of approximately 0.5, the correlation is moderate and in range  $-0.3$  to  $+0.3$  it is weak (Rayner, 1969). For example, if  $r = 0.5$ , even if it is statistically significant, the  $R^2 = 25\%$ . This indicates that 25% of the variation between the observations is accounted for by the relationship between the two variables, but 75% variation remains unexplained.

In the results tables the levels of significance were indicated as follows:

\*\*\* Significant at the 0.1% level of significance

\*\* Significant at the 1% level of significance

\* Significant at the 5% level of significance

NS Not Significant

**Appendix 3.1: Correct collection of aseptic milk samples from individual udder halves for laboratory diagnosis of mastitis (procedure by Giesecke *et al.* 1994, modified for use in goats).**

- Samples were collected with dry hands from teats with dry surfaces.
- Udders which were very soiled were washed before sampling and then dried thoroughly with individual disposable paper towels.
- The most important area to be cleaned and disinfected was the teat orifice and teat tip.
- A piece of cotton wool was moistened with methylated spirits and the excess methylated spirits was squeezed out.
- The teats of the goats were disinfected and cleaned.
- The teat was taken between the index finger and the thumb and pressed lightly so that the teat orifice opened slightly and no milk was forced out.
- The teat orifice and surrounding area was wiped lightly with a piece of cotton wool moistened in methylated spirits. Rough handling of the area was avoided.
- This process was repeated with both teats and care was taken to prevent any contact with already cleaned teats.
- Damaged teats or the halves with clinical mastitis were handled last.
- Samples were collected as follows: the right teat was taken with the right hand and the first two or three jets were milked into a strip cup to obtain a rinsing effect of the teat canal.
- The teat was released, the strip cup was put down and the identifiable sample container was taken in the right hand and the cap was twisted open with the little finger of the left hand. The container was taken with the left hand while still holding the cap with the little finger. The open end of the container was kept facing downwards until the sample was collected in order to prevent any dust or material from falling into the container. Care was also taken to prevent the open end from touching a contaminated surface.
- The teat was held in the right hand and the sample container was brought to approximately 3cm from the teat at a 45° angle to the ground. The sample container was filled with a jet of milk.
- The sample container was secured tightly immediately after collecting a sample.
- Colour coded, 5ml screw top sample containers were used.
- This procedure was repeated for both teats.
- Approximately 5ml milk was required from each half.
- Where there was an udder half with no milk, the container was not discarded.
- The ear tag number of the goat was written on the sample and if the containers did not have different coloured caps to identify the halves, each half was marked distinctly and separately. (Right = white; Left = green).
- The samples were placed upright in a cooler bag filled with ice at approximately 4°C (Giesecke *et al* 1994). The samples were kept at approximately 4°C from immediately after collection until arrival at the laboratory, regardless of the number of samples collected. Samples collected in the afternoon were brought to the laboratory in a cooler bag the next morning and placed in a refrigerator at approximately 4°C overnight.

## CHAPTER 4: RESULTS

### **4.1 Tables of Original Withdrawal Period Data**

The following tables contain the raw data of withdrawal periods as measured by different methods, using different antibiotics. These withdrawal periods did not have a 24hour safety margin added, as was the case for the withdrawal periods recommended for use in cattle.

**TABLE 4.1: WITHDRAWAL PERIODS OF TRIAL 1 AND TRIAL 3 USING CURACLOX LC.**

| Goat Number | Udder half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period of Dye Colour (h) | Withdrawal Period of Cloxacillin (Parallux) (h) | Withdrawal Period of Ampicillin (Parallax) (h) | Withdrawal Period Recommendations for Cattle (h) |
|-------------|------------|--|-------------------------------------|---|--|--|
| 9/2         | R          | 48                                       | 60                                  |   |  | 72   |
| 9/2         | L          | 96                                       | 72                                  |   |  | 72   |
| 983         | R          | 48                                       | 108                                 |   |  | 72   |
| 983         | L          | 72                                       | 108                                 | 96  | 84   | 72   |
| W6          | R          | 48                                       | 96                                  |   |  | 72   |
| W6          | L          | 72                                       | 96                                  | 96  | 84   | 72   |
| 1/9         | R          | 84                                       | 72                                  |   |  | 72   |
| 1/9         | L          | 96                                       | 72                                  | 108   | 108  | 72   |
| A74         | R          | 60                                       | 108                                 |   |  | 72   |
| A74         | L          | 84                                       | 108                                 | 84  | 84   | 72   |
| A79         | R          | 48                                       | 108                                 |   |  | 72   |
| A79         | L          | 72                                       | 108                                 | 108   | 84   | 72   |
| 1/12        | R          | 96                                       | 84                                  |   |  | 72   |
| 1/12        | L          | 72                                       | 72                                  | 96  | 96   | 72   |
| W21         | R          | 96                                       | 84                                  |   |  | 72   |
| W21         | L          | 96                                       | 84                                  | 108   | 108  | 72   |
| 20194       | R          | 36                                       | 60                                  |   |  | 72   |
| 20194       | L          | 36                                       | 60                                  | 60  | 60   | 72   |
| 20164       | R          | 36                                       | 60                                  |   |  | 72   |
| 20164       | L          | 36                                       | 60                                  | 60  | 60   | 72   |
| 20182       | R          | 36                                       | 60                                  |   |  | 72   |
| 20182       | L          | 48                                       | 72                                  | 96  | 84   | 72   |
| 10171       | R          | 48                                       | 84                                  |   |  | 72   |
| 10171       | L          | 36                                       | 84                                  | 84  | 84   | 72   |
| 20048       | R          | 36                                       | 60                                  |   |  | 72   |
| 20048       | L          | 36                                       | 72                                  |   |  | 72   |
| 20130       | R          | 36                                       | 60                                  |   |  | 72   |
| 20130       | L          | 48                                       | 60                                  | 84  | 60   | 72   |
| 20129       | R          | 36                                       | 60                                  |   |  | 72   |
| 20129       | L          | 36                                       | 60                                  |   |  | 72   |
| 20091       | R          | 48                                       | 60                                  |   |  | 72   |
| 20091       | L          | 48                                       | 60                                  | 84  | 84   | 72   |
| 20065       | R          | 36                                       | 60                                  |   |  | 72   |
| 20065       | L          | 36                                       | 60                                  |   |  | 72   |
| 10210       | R          | 48                                       | 72                                  |   |  | 72   |
| 10210       | L          | 60                                       | 60                                  | 84  | 60   | 72   |
| 20181       | R          | 48                                       | 72                                  |   |  | 72   |
| 20181       | L          | 48                                       | 72                                  | 84  | 84   | 72   |
| 20012       | R          | 48                                       | 60                                  |   |  | 72   |
| 20012       | L          | 48                                       | 60                                  | 60  | 60   | 72   |

The above table shows the withdrawal periods of both udder halves of all goats in Trials 1 & 3, using Curaclox LC, as measured by TRIS, colour dye, Parallux testing for Cloxacillin and Ampicillin.

**TABLE 4.2: WITHDRAWAL PERIODS OF TRIAL 2 USING SPECTRAZOL MILKING COW.**

| Goat Number | Udder Half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period Recommendations for Cattle (h) |
|-------------|------------|--|--|
| 1) 9        | R          | 84                                       | 60   |
| 1) 9        | L          | 84                                       | 60   |
| A79         | R          | 96                                       | 60   |
| A79         | L          | 84                                       | 60   |
| W6          | R          | 72                                       | 60   |
| W6          | L          | 84                                       | 60   |
| W10         | R          | 96                                       | 60   |
| W10         | L          | 96                                       | 60   |
| W2          | R          | 108                                      | 60   |
| W2          | L          | 108                                      | 60   |
| 99041       | R          | 96                                       | 60   |
| 99041       | L          | 60                                       | 60   |
| W18         | R          | 108                                      | 60   |
| W18         | L          | 108                                      | 60   |

The above table shows the withdrawal periods of both udder halves of all goats in Trial 2, using Spectrazol milking cow, as measured by TRIS.

**TABLE 4.3: WITHDRAWAL PERIODS OF TRIAL 3 USING RILEXINE 200 LC.**

| Goat Number | Udder half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period Recommendations for Cattle (h) |
|-------------|------------|--|--|
| 20160       | R          | 24                                       | 96   |
| 20160       | L          | 36                                       | 96   |
| 10144       | R          | 36                                       | 96   |
| 10144       | L          | 36                                       | 96   |
| 20142       | R          | 36                                       | 96   |
| 20142       | L          | 36                                       | 96   |
| 20149       | R          | 36                                       | 96   |
| 20149       | L          | 36                                       | 96   |
| 10201       | R          | 48                                       | 96   |
| 10201       | L          | 48                                       | 96   |
| 20056       | R          | 24                                       | 96   |
| 20056       | L          | 24                                       | 96   |
| 20069       | R          | 24                                       | 96   |
| 20069       | L          | 24                                       | 96   |
| 20217       | R          | 36                                       | 96   |
| 20217       | L          | 48                                       | 96   |
| 20125       | R          | 24                                       | 96   |
| 20125       | L          | 36                                       | 96   |
| 20086       | R          | 36                                       | 96   |
| 20086       | L          | 48                                       | 96   |
| 20233       | R          | 48                                       | 96   |
| 20233       | L          | 48                                       | 96   |
| 20040       | R          | 24                                       | 96   |
| 20040       | L          | 24                                       | 96   |
| 20222       | R          | 36                                       | 96   |
| 20222       | L          | 48                                       | 96   |
| 20202       | R          | 48                                       | 96   |
| 20202       | L          | 48                                       | 96   |
| 20013       | R          | 24                                       | 96   |
| 20013       | L          | 36                                       | 96   |
| 20036       | R          | 48                                       | 96   |
| 20036       | L          | 48                                       | 96   |
| 20258       | R          | 48                                       | 96   |
| 20258       | L          | 48                                       | 96   |
| 20239       | R          | 24                                       | 96   |
| 20239       | L          | 24                                       | 96   |
| 20146       | R          | 48                                       | 96   |
| 20146       | L          | 24                                       | 96   |
| 20171       | R          | 36                                       | 96   |
| 20171       | L          | 48                                       | 96   |

The above table shows the withdrawal periods of both udder halves of all goats in Trial 3, using Rilexine, as measured by TRIS.

**TABLE 4.4: BACTERIA PRESENT AND WITHDRAWAL PERIODS OF UDDER HALVES WITH CLINICAL MASTITIS USING CURACLOX LC.**

| Goat Number | Udder half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period of Dye Colour (h) | Withdrawal Period of Cloxacillin (Parallux) (h) | Withdrawal Period of Ampicillin (Parallux) (h) | Withdrawal Period Recommendations for Cattle (h) | Bacteria |
|-------------|------------|--|-------------------------------------|---|--|--|----------|
| 983         | R          | 48                                       | 108                                 |   |  | 72   |          |
| 983         | L          | 72                                       | 108                                 | 96  | 84   | 72   |          |
| W6          | R          | 48                                       | 96                                  |   |  | 72   |          |
| A79         | R          | 48                                       | 108                                 |   |  | 72   | (a) STE  |
| 20164       | R          | 36                                       | 60                                  |   |  | 72   |          |
| 20182       | L          | 48                                       | 72                                  | 96  | 84   | 72   |          |
| 10171       | R          | 48                                       | 84                                  |   |  | 72   | (a) STE  |
| 10171       | L          | 36                                       | 84                                  | 84  | 84   | 72   |          |
| 20130       | R          | 36                                       | 60                                  |   |  | 72   |          |
| 20130       | L          | 48                                       | 60                                  | 84  | 60   | 72   |          |
| 20181       | L          | 48                                       | 72                                  | 84  | 84   | 72   |          |
| 20012       | R          | 48                                       | 60                                  |   |  | 72   | (a) STE  |
| Desire      | R          | 96                                       | 96                                  |   |  | 72   |          |
| Desire      | L          | 132                                      | 96                                  |   |  | 72   |          |
| Adelle      | R          | 96                                       | 96                                  |   |  | 72   | (a) STE  |
| Heidi       | L          | 60                                       | 60                                  |   |  | 72   | (b) STA  |

(a) *Staphylococcus epidermidis* (STE), (b) *Staphylococcus aureus* (STA)

The above table shows withdrawal periods, of udder halves with clinical mastitis treated with Curaclox LC, as measured by TRIS colour dye and Parallux testing for Cloxacillin and Ampicillin. The type of bacteria present or absent, in udder halves with clinical mastitis is shown.

**TABLE 4.5: BACTERIA PRESENT AND WITHDRAWAL PERIODS OF UDDER HALVES WITH CLINICAL MASTITIS USING SPECTRAZOL MILKING COW.**

| Goat Number | Udder Half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period Recommendations for Cattle (h) | Bacteria |
|-------------|------------|--|--|----------|
| A79         | R          | 96                                       | 60   | (a) STE  |
| W10         | R          | 96                                       | 60   | (b) KLE  |
| Nella       | R          | 108                                      | 60   |          |
| Nella       | L          | 132                                      | 60   |          |

(a) *Staphylococcus epidermidis* (STE), (b) *Klebsiella* (KLE)

The above table shows withdrawal periods, of udder halves with clinical mastitis treated with Spectrazol milking cow, as measured by TRIS. The type of bacteria present or absent, in udder halves with clinical mastitis is shown.



**TABLE 4.6: BACTERIA PRESENT AND WITHDRAWAL PERIODS OF UDDER HALVES WITH CLINICAL MASTITIS USING RILEXINE 200 LC.**

| Goat Number | Udder half | Antibiotic Withdrawal Period by TRIS (h) | Withdrawal Period Recommendations for Cattle (h) | Bacteria |
|-------------|------------|--|--|----------|
| 20258       | R          | 48                                       | 96   | STE      |
| 20258       | L          | 48                                       | 96   | STE      |

The above table shows withdrawal periods, of udder halves with clinical mastitis treated with Rilexine 200 LC, as measured by TRIS. The type of bacteria present or absent, in udder halves with clinical mastitis is shown.

#### **4.2 Tables of Statistical Analysis of Withdrawal Periods and Graphs of Withdrawal Periods as Measured by TRIS**

##### **4.2.1 Trial 2: Spectrazol**

**TABLE 4.7: A TWO-SAMPLE T-TEST OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) BETWEEN UDDER HALVES WITH AND WITHOUT CLINICAL MASTITIS (SPECTRAZOL).**

| Variate | Group Factor | Size (N) | Mean (N)± Std. Deviation (h) | Size (C) | Mean (C) ± Standard Deviation (h) | Probability |
|---------|--------------|----------|------------------------------|----------|-----------------------------------|-------------|
| WP TRIS | Clinical     | 12       | 91+15.74                     | 4        | 108+16.97                         | 0.087 NS    |

The mean withdrawal period as measured by the TRIS test, between udder halves with clinical mastitis or not was not significantly different at the 5% level. There were only four goats with clinical mastitis, thus the mean for goats without clinical mastitis was more reliable.

**TABLE 4.8: A TWO-SAMPLE T-TEST OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (SPECTRAZOL).**

| Variate | Group Factor | Size (B) | Mean (B)± Std. Deviation (h) | Size (N) | Mean (N) ± Standard Deviation (h) | Probability |
|---------|--------------|----------|------------------------------|----------|-----------------------------------|-------------|
| WP TRIS | Bacteria     | 7        | 96+9.798                     | 9        | 95+22.00                          | 0.884 NS    |

There was no significant difference ( $P = 0.884$ ) in the mean withdrawal period as measured by the TRIS test, between infected and non-infected udder halves. Therefore, the presence of bacteria did not affect the withdrawal period.

**TABLE 4.9: ONE SAMPLE T-TEST OF WITHDRAWAL PERIOD (WP) TRIS COMPARED TO WITHDRAWAL PERIOD (WP) RECOMMENDED FOR USE IN CATTLE 60 (h) (SPECTRAZOL).**

| Variate             | Size | Mean ± Standard Deviation (h) | Probability |
|---------------------|------|-------------------------------|-------------|
| WP TRIS             | 16   | 95 ± 17.23                    | <0.001***   |
| Inter-half variance | 8    | 1.5 ± 17.49                   | 0.815 NS    |

95% confidence interval for mean: (86h, 104h)

Test of null hypothesis that mean of withdrawal period of TRIS is equal to 60h.

Test statistic  $t = 8.18$  on 15 Degrees of freedom (d.f.).

There was a highly significant difference ( $P < 0.001$ ) between the mean of 95h as measured by TRIS for goats and the 60h recommended for use in cattle.

The difference in withdrawal periods between left and right udder halves (inter-half variance) was not significant, because the mean difference of 1.5 did not differ significantly ( $P = 0.815$ ) from 0.

#### 4.2.2 Trial 3: Rilexine

**TABLE 4.10: TWO-SAMPLE T-TEST OF THE DIFFERENCE OF MEAN WITHDRAWAL PERIOD (WP) BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (RILEXINE).**

| Variate | Group Factor | Size (B) | Mean (B) ± Std. Deviation (h) | Size (N) | Mean (N) ± Standard Deviation (h) | Probability |
|---------|--------------|----------|-------------------------------|----------|-----------------------------------|-------------|
| WP TRIS | Bacteria     | 36       | 38 ± 9.728                    | 4        | 27 ± 6.000                        | 0.034*      |

The difference between mean withdrawal periods as measured by the TRIS test of infected and non-infected udder halves was significant at the 5% level (0.034). Therefore, the presence of bacteria did affect the withdrawal period when treating with Rilexine.

**TABLE 4.11: ONE SAMPLE T-TEST OF WITHDRAWAL PERIOD (WP) AS MEASURED BY TRIS COMPARED TO WITHDRAWAL PERIOD (WP) RECOMMENDED FOR USE IN CATTLE 96(h) (RILEXINE).**

| Variate             | Size | Mean ± Standard Deviation (h) | Probability |
|---------------------|------|-------------------------------|-------------|
| WP TRIS             | 40   | 37 ± 9.943                    | <0.001***   |
| Inter-half variance | 20   | -3.00 ± 8.596                 | 0.135 NS    |

95% Confidence interval for mean: (33.7h, 40h)

Test of null hypothesis that mean of withdrawal period as measured by TRIS was equal to 96h

Test statistic  $t = -37.59$  on 39 d.f.

There was a highly significant difference ( $P < 0.001$ ) between the mean of 37h as determined by TRIS and the 96h recommended for use in cattle. The inter-half variance was not significant ( $P = 0.135$ ).

#### 4.2.3 Curaclox LC from Trials 1 & 3 Combined

**TABLE 4.12: TWO SAMPLE T-TESTS, OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) AS MEASURED BY DIFFERENT METHODS BETWEEN UDDER HALVES WITH CLINICAL MASTITIS OR NOT (CURACLOX LC; TRIALS 1 & 3).**

| Variate        | Group Factor | Size (N) | Mean (N) ± Std. Deviation (h) | Size (C) | Mean (C) ± Standard Deviation (h) | Probability |
|----------------|--------------|----------|-------------------------------|----------|-----------------------------------|-------------|
| WP TRIS        | Clinical     | 28       | 58 ± 23.26                    | 16       | 59 ± 26.82                        | 0.901 NS    |
| WP Colour Dye  | Clinical     | 28       | 72 ± 16.00                    | 16       | 83 ± 19.04                        | 0.057 NS    |
| WP Cloxacillin | Clinical     | 11       | 86 ± 19.21                    | 5        | 89 ± 6.573                        | 0.774 NS    |
| WP Parallax    | Clinical     | 11       | 81 ± 18.66                    | 5        | 79 ± 10.73                        | 0.868 NS    |
| WP Ampicillin  | Clinical     | 11       | 81 ± 18.66                    | 5        | 79 ± 10.73                        | 0.868 NS    |
| WP Parallax    | Clinical     | 11       | 81 ± 18.66                    | 5        | 79 ± 10.73                        | 0.868 NS    |

The withdrawal periods between udder halves with clinical mastitis or not, as measured by TRIS ( $P = 0.901$ ), the colour dye indicator ( $P = 0.057$ ) and the Parallax testing for both Cloxacillin ( $P = 0.774$ ) and Ampicillin ( $P = 0.887$ ) residues were not significant.

**TABLE 4.13: TWO-SAMPLE T-TESTS OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) MEASURED BY DIFFERENT METHODS BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC; TRIALS 1 & 3).**

| Variate                 | Group Factor | Size (B) | Mean (B)± Std. Deviation (h) | Size (N) | Mean (N) ± Standard Deviation (h) | Probability |
|-------------------------|--------------|----------|------------------------------|----------|-----------------------------------|-------------|
| WP TRIS                 | Bacteria     | 27       | 55 ± 22.6                    | 17       | 65 ± 26.85                        | 0.175 NS    |
| WP Colour Dye           | Bacteria     | 27       | 76 ± 7.23                    | 17       | 76 ± 18.95                        | 0.903 NS    |
| WP Cloxacillin Parallax | Bacteria     | 9        | 87 ± 16.73                   | 7        | 87 ± 16.56                        | 0.929 NS    |
| WP Ampicillin Parallax  | Bacteria     | 9        | 83 ± 15.23                   | 7        | 77 ± 18.14                        | 0.518 NS    |

The withdrawal periods between infected and non-infected udder halves, as measured by TRIS (P = 0.175), the colour dye indicator (P = 0.903) and the parallax testing for both Cloxacillin (P = 0.929) and Ampicillin (P = 0.518) residues were not significant.

**TABLE 4.14: ONE SAMPLE T-TEST OF WITHDRAWAL PERIOD (WP) AS MEASURED BY DIFFERENT METHODS COMPARED TO WITHDRAWAL PERIOD (WP) RECOMMENDED FOR USE IN CATTLE 72(h) (CURACLOX LC; TRIALS 1 & 3).**

| Variate                 | Size | Mean ± Standard Deviation (h) | Probability |
|-------------------------|------|-------------------------------|-------------|
| WP TRIS                 | 44   | 59 ± 24.31                    | <0.001***   |
| Inter-half variance     | 22   | -7.091 ± 18.76                | 0.091 NS    |
| WP Colour Dye           | 44   | 76 ± 17.70                    | 0.160 NS    |
| Inter-half variance     | 22   | 1.091 ± 9.734                 | 0.605 NS    |
| WP Cloxacillin Parallax | 16   | 87 ± 16.10                    | 0.002**     |
| WP Ampicillin Parallax  | 16   | 80 ± 16.23                    | 0.060 NS    |

WP TRIS 95% Confidence interval for mean: (51h, 66h)

Test of null hypothesis that mean of withdrawal period of TRIS is equal to 72h. Test statistic  $t = -3.65$  on 43 d.f.

WP Cloxacillin Parallax 95% Confidence interval for mean: (78h, 96h)

Test of null hypothesis that mean of withdrawal period as determined by parallax testing for Cloxacillin was equal to 72h. Test statistic  $t = 3.73$  on 15 d.f.

There was a highly significant difference ( $P < 0.001$ ) as measured by TRIS and a significant difference ( $P = 0.002$ ) as measured by the Parallax testing for Cloxacillin residues compared to 72h recommended for use in cattle.

WP Colour Dye 95% Confidence interval for mean: (70h, 81h)

Test of null hypothesis that the mean of withdrawal period colour dye was equal to 72h. Test statistic  $t = 1.43$  on 43 d.f.

The mean withdrawal period determined by the colour dye was not significantly different ( $P = 0.160$ ) from that recommended for use in cattle (72h).

WP Ampicillin Parallax 95% confidence interval for mean: (72h, 89h)

Test of null hypothesis that mean of withdrawal period as determined by Parallax testing for Ampicillin is equal to 72h. Test statistic  $t = 2.03$  on 15 d.f.

The mean withdrawal period as determined by the Parallax testing for Ampicillin residues was not significant at the 5% level.

The difference in withdrawal periods between left and right udder halves (inter-half variance) as measured by TRIS was not significant at the 5% level ( $P = 0.091$ ).

The Inter-half variance as measured by the colour dye was not significant at the 5% level of significance ( $P = 0.605$ ).

**TABLE 4.15: ONE-SAMPLE PAIRED T-TEST ON TESTING DIFFERENCES BETWEEN WITHDRAWAL PERIODS (WP) MEASURED BY DIFFERENT METHODS (CURACLOX LC; TRIALS 1 & 3).**

| Variate  | Size | Mean $\pm$ Standard Deviation (h) | Probability |
|--|------|-----------------------------------|-------------|
| Difference between WP TRIS & WP Colour Dye           | 44   | 17 $\pm$ 21.56                    | <0.001***   |
| Difference between WP TRIS & WP Cloxacillin Parallax | 16   | 26 $\pm$ 13.30                    | <0.001***   |
| Difference between WP TRIS & WP Ampicillin Parallax  | 16   | 20 $\pm$ 13.77                    | <0.001***   |

Differences between withdrawal periods as measured by TRIS and colour dye; TRIS and Parallax testing for Cloxacillin and TRIS and Parallax testing for Ampicillin were all highly significant ( $P < 0.001$ ).

#### 4.2.4 Curaclox LC from Trial 1 Only

**TABLE 4.16: TWO SAMPLE T-TESTS, OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) AS MEASURED BY DIFFERENT METHODS BETWEEN UDDER HALVES WITH CLINICAL MASTITIS OR NOT (CURACLOX LC; TRIAL 1).**

| Variate       | Group Factor | Size (N) | Mean (N) $\pm$ Std. Deviation (h) | Size (C) | Mean (C) $\pm$ Standard Deviation (h) | Probability |
|---------------|--------------|----------|-----------------------------------|----------|---------------------------------------|-------------|
| WP TRIS       | Clinical     | 12       | 81 $\pm$ 16.28                    | 4        | 54 $\pm$ 12.00                        | 0.009**     |
| WP Colour Dye | Clinical     | 12       | 85 $\pm$ 16.55                    | 4        | 105 $\pm$ 6.000                       | 0.036*      |

Differences between mean withdrawal periods of udder halves with and without clinical mastitis as measured by TRIS ( $P = 0.009$ ) and by colour dye ( $P = 0.036$ ) were significant.

**TABLE 4.17: TWO-SAMPLE T-TESTS OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) MEASURED BY DIFFERENT METHODS BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC; TRIAL 1).**

| Variate                 | Group Factor | Size (B) | Mean (B) $\pm$ Std. Deviation (h) | Size (N) | Mean (N) $\pm$ Standard Deviation (h) | Probability |
|-------------------------|--------------|----------|-----------------------------------|----------|---------------------------------------|-------------|
| WP TRIS                 | Bacteria     | 9        | 76 $\pm$ 18.97                    | 7        | 72 $\pm$ 20.78                        | 0.694 NS    |
| WP Colour Dye           | Bacteria     | 9        | 93 $\pm$ 13.11                    | 7        | 86 $\pm$ 21.27                        | 0.391 NS    |
| WP Cloxacillin Parallax | Bacteria     | 5        | 96 $\pm$ 8.485                    | 2        | 108.0 $\pm$ 0                         |             |
| WP Ampicillin Parallax  | Bacteria     | 5        | 91 $\pm$ 10.73                    | 2        | 96 $\pm$ 16.97                        |             |

Differences between mean withdrawal periods of infected and non-infected udder halves as determined by TRIS ( $P = 0.694$ ) and by colour dye ( $P = 0.391$ ) were not significant. P values for differences in withdrawal periods between infected and non-infected udder halves as according to Parallax testing for Cloxacillin and Ampicillin could not be determined due to the small number of samples.

**TABLE 4.18: ONE SAMPLE T-TEST OF WITHDRAWAL PERIODS (WP) MEASURED BY DIFFERENT METHODS COMPARED TO WITHDRAWAL PERIOD (WP) RECOMMENDED FOR USE IN CATTLE 72(h) (CURACLOX LC; TRIAL 1).**

| Variate                 | Size | Mean ± Standard Deviation (h) | Probability |
|-------------------------|------|-------------------------------|-------------|
| WP TRIS                 | 16   | 74 ± 19.21                    | 0.646 NS    |
| WP Colour Dye           | 16   | 90 ± 16.97                    | <0.001***   |
| WP Cloxacillin Parallax | 7    | 99 ± 9.071                    | <0.001***   |
| WP Ampicillin Parallax  | 7    | 93 ± 11.41                    | 0.003**     |

WP as measured by TRIS 95% Confidence interval of mean: (64h, 85h)

Test of null hypothesis that mean withdrawal period was equal to 72h. Test statistic  $t = 0.47$  on 15 d.f. Mean withdrawal period as measured by TRIS was not significantly different from that recommended for use in cattle (72h).

WP Colour Dye 95% Confidence interval of mean: (81h, 99h)

Test of null hypothesis was that mean withdrawal period as measured by the colour dye was equal to 72h. Test statistic  $t = 4.24$  on 15d.f. The difference between mean withdrawal period according to the colour dye and 72h recommended for use in cattle was highly significant ( $P < 0.001$ ).

WP Cloxacillin Parallax 95% Confidence interval of mean: (91h, 108h)

Test of null hypothesis was that mean withdrawal period as measured by the Parallax testing for Cloxacillin was equal to 72h. Test statistic  $t = 8.00$  on 6 d.f.

The difference between mean withdrawal period according to the Parallax testing for Cloxacillin and 72h recommended for use in cattle, was highly significant ( $P < 0.001$ ).

WP Ampicillin Parallax 95% Confidence interval of mean: (82h, 103h)

Test of null hypothesis was that mean withdrawal period as measured by the Parallax testing for Ampicillin is equal to 72h. Test statistic  $t = 4.77$  on 6 d.f.

The difference between mean withdrawal period according to the Parallax testing for Ampicillin and 72h recommended for use in cattle, was highly significant ( $P < 0.001$ ).

#### 4.2.5 Curaclox LC from Trial 3 Only

**TABLE 4.19: TWO SAMPLE T-TESTS, OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) AS MEASURED BY DIFFERENT METHODS BETWEEN UDDER HALVES WITH CLINICAL MASTITIS OR NOT (CURACLOX LC; TRIAL 3).**

| Variate                 | Group Factor | Size (N) | Mean (N) ± Std. Deviation (h) | Size (C) | Mean (C) ± Standard Deviation (h) | Probability |
|-------------------------|--------------|----------|-------------------------------|----------|-----------------------------------|-------------|
| WP TRIS                 | Clinical     | 16       | 41 ± 7.550                    | 8        | 44 ± 6.211                        | 0.475 NS    |
| WP Colour Dye           | Clinical     | 16       | 62 ± 4.837                    | 8        | 69 ± 10.64                        | 0.124 NS    |
| WP Cloxacillin Parallax | Clinical     | 5        | 70 ± 13.15                    | 4        | 87 ± 6.000                        | 0.046*      |
| WP Ampicillin Parallax  | Clinical     | 5        | 65 ± 10.73                    | 4        | 78 ± 12.00                        | 0.125 NS    |

Mean withdrawal period as measured by the Parallax testing for Cloxacillin was significantly different ( $P = 0.046$ ) at 5% level, between udder halves with clinical mastitis or not.

Mean withdrawal periods as measured by TRIS ( $P = 0.475$ ), colour dye ( $P = 0.124$ ) and Parallax testing for Ampicillin ( $P = 0.125$ ) were not significantly different, between udder halves with clinical mastitis or not.

**TABLE 4.20: TWO-SAMPLE T-TESTS OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) MEASURED BY DIFFERENT METHODS BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC; TRIAL 3).**

| Variate                 | Group Factor | Size (B) | Mean (B)± Std. Deviation (h) | Size (N) | Mean (N) ± Standard Deviation (h) | Probability |
|-------------------------|--------------|----------|------------------------------|----------|-----------------------------------|-------------|
| WP TRIS                 | Bacteria     | 16       | 40 ± 5.745                   | 8        | 47 ± 7.690                        | 0.024*      |
| WP Colour Dye           | Bacteria     | 16       | 65 ± 8.729                   | 8        | 63 ± 5.555                        | 0.515 NS    |
| WP Cloxacillin Parallax | Bacteria     | 4        | 75 ± 18.00                   | 5        | 79 ± 10.73                        | 0.675 NS    |
| WP Ampicillin Parallax  | Bacteria     | 4        | 72 ± 13.86                   | 5        | 70 ± 13.15                        | 0.798 NS    |

Mean withdrawal period as measured by TRIS was significantly different ( $P = 0.024$ ) at 5% level, between infected and non-infected udder halves.

Mean withdrawal periods according to colour dye ( $P = 0.515$ ), Parallax testing for Cloxacillin ( $P = 0.675$ ) and Parallax testing for Ampicillin ( $P = 0.798$ ), were not significant between infected and non-infected udder halves.

**TABLE 4.21: ONE SAMPLE T-TESTS OF WITHDRAWAL PERIODS (WP) AS MEASURED BY DIFFERENT METHODS COMPARED TO WITHDRAWAL PERIOD (WP) RECOMMENDED FOR USE IN CATTLE 72(h) (CURACLOX LC; TRIAL 3).**

| Variate                 | Size | Mean ± Standard Deviation (h) | Probability |
|-------------------------|------|-------------------------------|-------------|
| WP TRIS                 | 24   | 42 ± 7.077                    | <0.001***   |
| WP Colour Dye           | 24   | 65 ± 7.763                    | <0.001***   |
| WP Cloxacillin Parallax | 9    | 77 ± 13.56                    | 0.272 NS    |
| WP Ampicillin Parallax  | 9    | 71 ± 12.65                    | 0.760 NS    |

WP TRIS

WP TRIS, 95% Confidence interval for means: (39h, 45h)

Test of null hypothesis that mean withdrawal period according to TRIS is equal to 72h. Test statistic  $t = -20.77$  with 23 d.f.

WP Colour Dye

WP Colour Dye 95% Confidence interval for mean: (61h, 68h)

Test of null hypothesis that mean withdrawal period according to the colour dye is equal to 72h. Test statistic  $t = -4.73$  with 23 d.f.

Mean withdrawal period as measured by TRIS and colour dye was highly significantly different ( $P < 0.001$ ) from that recommended for use in cattle (72h).

WP Cloxacillin Parallax

WP Cloxacillin Parallax 95% Confidence interval for mean: (67h, 88h)

Test of null hypothesis that mean withdrawal period as measured by the Parallax testing for Cloxacillin is equal to 72h. Test statistic  $t = 1.18$  with 8 d.f.

WP Ampicillin Parallax

WP Ampicillin Parallax 95% Confidence interval for mean: (61h, 80h)

Test of null hypothesis that mean withdrawal period as measured by the Parallax testing for Ampicillin is equal to 72h. Test statistic  $t = -0.32$  with 8 d.f.

Mean withdrawal period as measured by the Parallax testing for Cloxacillin ( $P = 0.272$ ) and Ampicillin ( $P = 0.760$ ) was not significantly different from that recommended for use in cattle (72h).

#### 4.2.6 Trial 3; Curaclox LC and Rilexine

**TABLE 4.22: TEST OF DIFFERENCE BETWEEN WITHDRAWAL PERIOD (WP) TRIS AND WITHDRAWAL PERIOD (WP) COLOUR DYE VALUES BETWEEN LEFT AND RIGHT UDDER HALVES (TRIAL 3; CURACLOX LC & RILEXINE).**

| Variate                             | Size | Mean $\pm$ Standard Deviation (h) | Probability |
|-------------------------------------|------|-----------------------------------|-------------|
| Inter-half variance (WP TRIS)       | 32   | -2.625 $\pm$ 7.910                | 0.070 NS    |
| Inter-half variance (WP Colour Dye) | 12   | -1.000 $\pm$ 6.179                | 0.586 NS    |

There was no significant difference of mean withdrawal periods between left and right udder halves as determined by TRIS (P = 0.070) and colour dye (P = 0.586).

**TABLE 4.23: TWO-SAMPLE T-TESTS OF DIFFERENCES IN WITHDRAWAL PERIOD (WP) MEASURED BY DIFFERENT METHODS BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (TRIAL 3; CURACLOX LC & RILEXINE).**

| Variate       | Group Factor | Size (B) | Mean (B) $\pm$ Std. Deviation (h) | Size (N) | Mean (N) $\pm$ Standard Deviation (h) | Probability |
|---------------|--------------|----------|-----------------------------------|----------|---------------------------------------|-------------|
| WP TRIS       | Bacteria     | 34       | 38 $\pm$ 8.928                    | 30       | 40 $\pm$ 9.628                        | 0.339 NS    |
| WP Colour Dye | Bacteria     | 9        | 61 $\pm$ 4.000                    | 15       | 66 $\pm$ 8.919                        | 0.071 NS    |

Mean withdrawal periods of infected and non-infected udder halves as measured by TRIS (P = 0.339) and colour dye (P = 0.071) are not significantly different.

#### 4.2.7 All Data for Goats with Clinical Mastitis

**TABLE 4.24: TEST OF DIFFERENCE BETWEEN WITHDRAWAL PERIOD (WP) TRIS AND WITHDRAWAL PERIOD (WP) COLOUR DYE VALUES BETWEEN LEFT AND RIGHT UDDER HALVES (CLINICAL MASTITIS).**

| Variate                             | Size | Mean $\pm$ Standard Deviation (h) | Probability |
|-------------------------------------|------|-----------------------------------|-------------|
| Inter-half variance (WP TRIS)       | 9    | 6.667 $\pm$ 31.81                 | 0.547 NS    |
| Inter-half variance (WP Colour Dye) | 5    | 4.800 $\pm$ 20.08                 | 0.621 NS    |

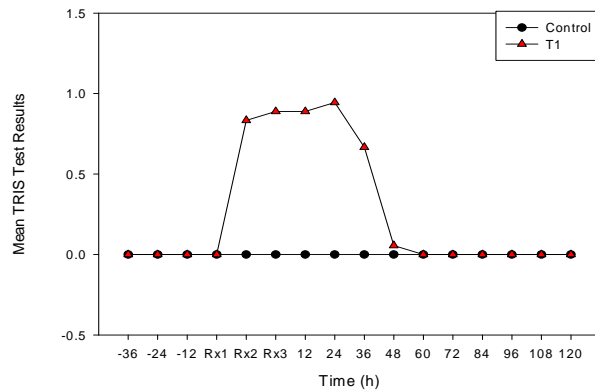
There is no significant difference of mean withdrawal periods between left and right udder halves as determined by TRIS (P = 0.547) and colour dye (P = 0.621).

**TABLE 4.25: TWO-SAMPLE T-TESTS OF MEAN WITHDRAWAL PERIODS (WP) OF UDDER HALVES WITH CLINICAL MASTITIS WHERE BACTERIAL INFECTION WAS IDENTIFIED OR NOT.**

| Variate       | Group Factor | Size (B) | Mean (B) $\pm$ Std. Deviation (h) | Size (N) | Mean (N) $\pm$ Standard Deviation (h) | Probability |
|---------------|--------------|----------|-----------------------------------|----------|---------------------------------------|-------------|
| WP TRIS       | Bacteria     | 10       | 67 $\pm$ 26.05                    | 13       | 85 $\pm$ 55.53                        | 0.324 NS    |
| WP Colour Dye | Bacteria     | 6        | 82 $\pm$ 24.49                    | 10       | 83 $\pm$ 16.44                        | 0.938 NS    |

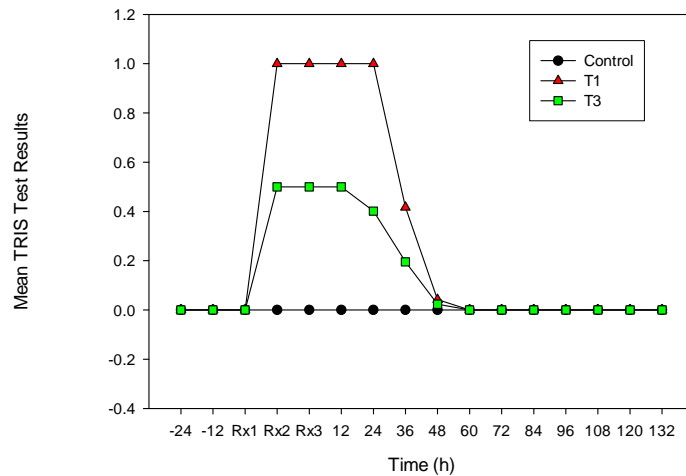
Mean withdrawal periods of infected and non-infected udder halves according to TRIS (P = 0.324) and colour dye (P = 0.938) were not significantly different.

#### 4.2.8 Graphs Showing Withdrawal Periods as Measured by Thermo Resistant Inhibitory Substances (TRIS) over Time.



**FIGURE 4.1: MEAN TRIS TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 1.**

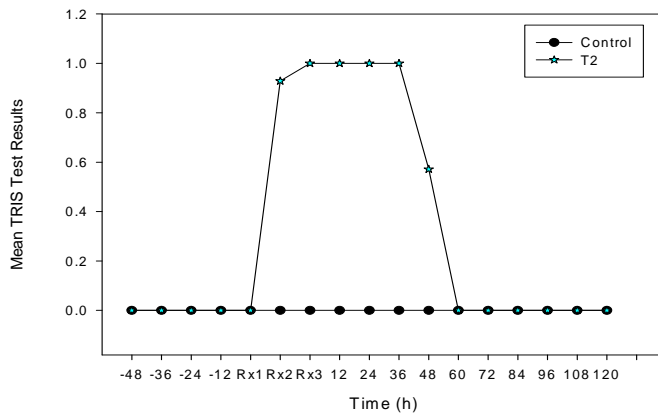
The TRIS test results of the treated group increased after treatment with Curaclox LC at Rx1 and returned to baseline of control group (negative TRIS test) at 60h.



**FIGURE 4.2: MEAN TRIS TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 3.**

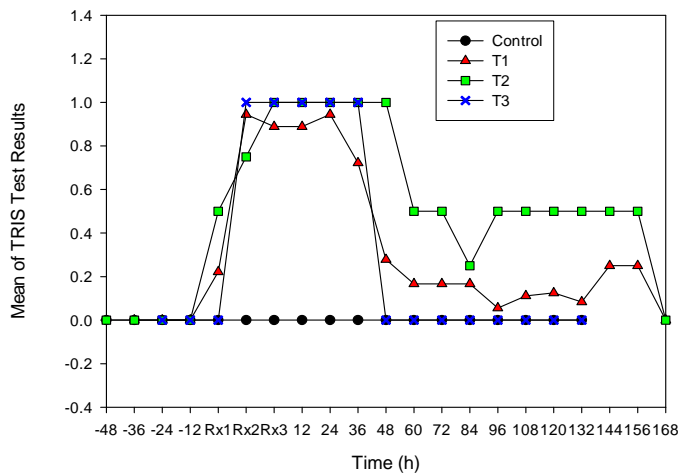
The TRIS test results of the treated group T1 (Curaclox LC) & T3 (Rilexine) increased after treatment at Rx1 and returned to baseline of control group (negative TRIS test) at 60h. A score of 1 indicated a positive TRIS test and a 0 indicated a negative TRIS test. Thus the T3 at about 0.5 show that not all the udder halves treated with Rilexine showed a positive TRIS test result after treatment.





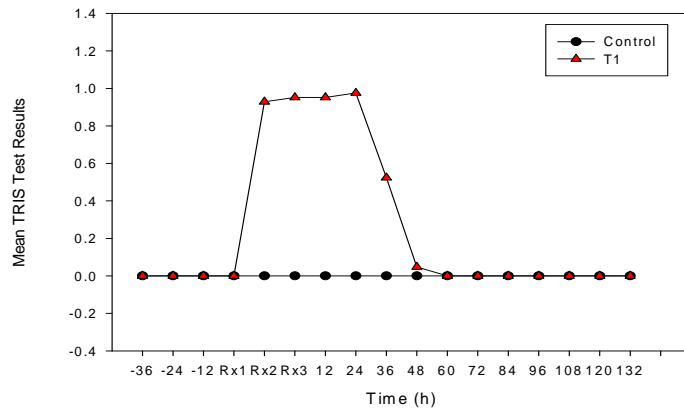
**FIGURE 4.3: MEAN TRIS TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 2 (SPECTRAZOL).**

The TRIS test results of the treated group increased after treatment with Spectrazol at Rx1 and returned to baseline of control group (negative TRIS test) at 60h.



**FIGURE 4.4: MEAN OF TRIS TEST RESULTS OF UDDER HALVES WITH CLINICAL MASTITIS OF TREATMENT GROUPS (T1=CURACLOX LC, T2= SPECTRAZOL MILKING COW, T3= RILEXINE 200 LC) VERSUS CONTROL GROUP.**

The TRIS test results of the treated group T3 (Rilexine) increased after treatment at Rx1 and returned to baseline of control group (negative TRIS test) at 60h. Thus for T3 the TRIS results are the same for healthy goats as for goats with clinical mastitis. The TRIS test results of the treated groups T1 (Curaclon LC) & T2 (Spectrazol) increased after treatment to a positive TRIS test result of 1, and decreased after 60h. However all animals with clinical mastitis treated with T1 or T2 only returned to the baseline of the control group (negative TRIS test) at 168h. Thus the withdrawal period as measured by TRIS was longer for goats with clinical mastitis than for healthy goats.



**FIGURE 4.5: MEAN TRIS TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIALS 1&3 (CURACLOX LC).**

The TRIS test results of the treated group increased after treatment with Curaclox LC at Rx1 and returned to baseline of control group (negative TRIS test) at 60h. This was the same for Curaclox LC separately in Trial 1 (see Figure 4.1) and Trial 3 (see Figure 4.3).

### 4.3 Regression Analysis of all Data from Goats with Clinical Mastitis

**TABLE 4.26: REGRESSION MODEL OF ALL DATA FROM GOATS WITH CLINICAL MASTITIS.**

| Term in model                                       | Adjusted R <sup>2</sup> | Standard Error of Regression | F probability |
|---|-------------------------|------------------------------|---------------|
| Sampling Time (006:00 & 18:00)                      | 68.1%                   | 9.30                         | <0.001***     |
| Udder palpation (damage present = 1, no damage = 0) | 90.5%                   | 5.06                         | <0.001***     |
| Floccules (Present = 1, not present = 0)            | 94.1%                   | 4.00                         | 0.035*        |
| Volume  | 95.7%                   | 3.41                         | 0.085NS       |

The above table showed that as terms were added to the regression model, the adjusted R<sup>2</sup> increased and the standard error of regression decreased. Therefore this showed that the model improved with the addition of each term, and that this was a good overall model (R<sup>2</sup> = 95.7%, standard error of regression = 3.41).

Although the regression model improves with the addition of terms, the F probability decreases from a 1% significance level (P < 0.001) to a 5% level (P = 0.035) and to a 10% level (P = 0.085).

This is a biologically meaningful model that was used to determine what factors affect the withdrawal period according to TRIS the most. Therefore this model is still meaningful even though the end significance is at the 10% level.

#### **4.3.1 Linear Regression Model**

**Withdrawal period according to TRIS = 30.21 + 4.692 (Sampling Time) + 22.11 (Udder palpation) – 13.6 (floccules) – 0.00649 (Volume)**

According to the linear model of regression shown above the withdrawal period according to TRIS increased at night (sampling time 18:00) by 4.692h.

When udder damage was present as found by palpation, the udder palpation score = 1 and the withdrawal period according to TRIS increased by 22.11h. When there was no udder damage the withdrawal period according to TRIS was not affected.

When floccules were present in the milk, the withdrawal period according to TRIS decreased by 13.6h. When there were no floccules in the milk, the withdrawal period according to TRIS was not affected.

In the presence of floccules in the milk, the adjusted  $R^2$  and the standard error of regression decreased as the volume increased at both sampling times.

At sampling time 06:00, the adjusted  $R^2$  and the standard error of regression decreased as the volume increased in goats with udder damage found by udder palpation and without floccules in the milk.

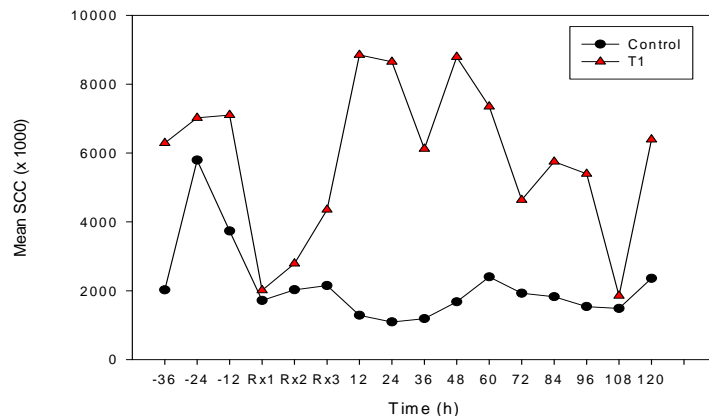
At sampling time 18:00, the adjusted  $R^2$  decreased as the volume increased in goats with udder damage found by udder palpation and without floccules in the milk. The standard error of regression decreased from volume (100ml) to volume (900ml) and then increased for volumes (1300ml & 1700ml).

At sampling time 06:00, the adjusted  $R^2$  decreased as the volume increased in goats with udder damage found by udder palpation and with floccules in the milk. The standard error of regression decreased from volume (100ml) to volume (900ml) and then increased for volumes (1300ml & 1700ml).

At sampling time 18:00, the adjusted  $R^2$  decreased and the standard error of regression increased as the volume increased in goats with udder damage found by udder palpation and with floccules in the milk.

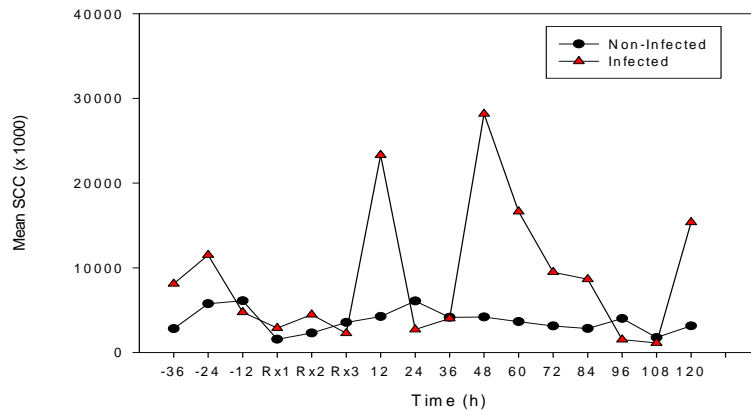
#### **4.4 Graphs and Statistical Analysis Tables Explaining Somatic Cell Count (SCC)**

##### **4.4.1 Graphs Showing Somatic Cell Counts (SCC) over Time**



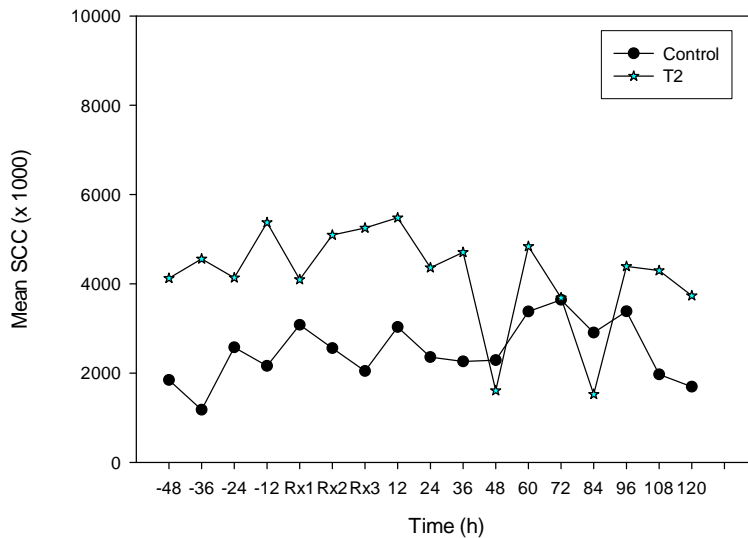
**FIGURE 4.6: MEAN SOMATIC CELL COUNT OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 1 (CURACLOX L C).**

Mean SCC of treatment and control group suddenly increased after the first sampling. This was probably due to the stress caused by the onset of the Trial procedures and the 12hourly intervals between milking times. The mean SCC of the control group increased after treatment at Rx1 and returned to baseline at 108h, only for the SCC to increase again at the last sampling of the trial. This sudden increase in SCC at the end of the trial could have been due to stress caused to the goats by the length of the trial.



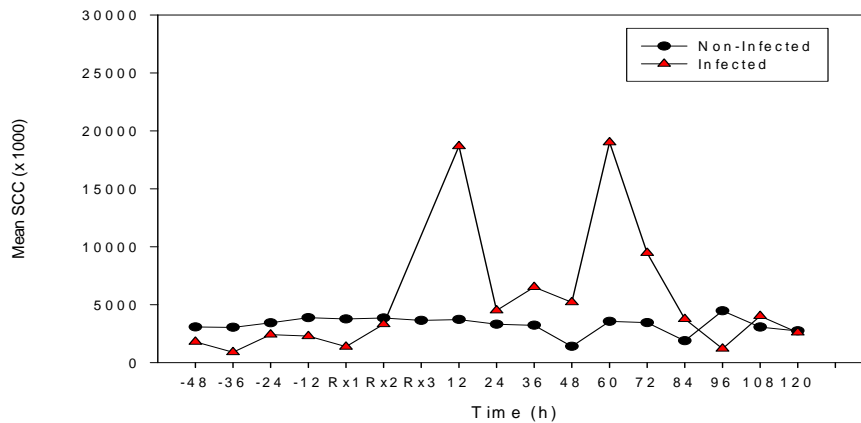
**FIGURE 4.7: MEAN SOMATIC CELL COUNT OF INFECTED UDDER HALVES VERSUS NON- INFECTED UDDER HALVES: TRIAL 1 (CURACLOX L C).**

The mean SCC of infected goats was higher than that of non-infected goats from the start of the trial. The mean SCC of infected animals decreased after treatment at Rx1, increased again at 12h and decreased again at 24h, then increased peaking at 48h, then decreasing steadily until the baseline SCC was reached at 108h, then suddenly increased again at the last sampling, see (Figure 4.6).



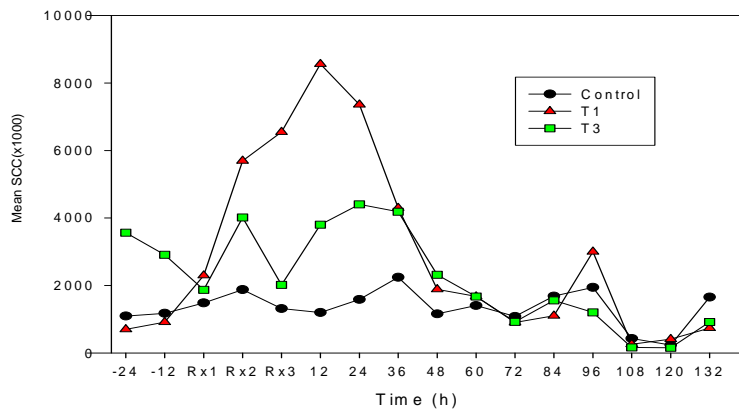
**FIGURE 4.8: MEAN SOMATIC CELL COUNT OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 2 (SPECTRAZOL).**

Figure 4.8 and Figure 4.9 illustrate the results of Trial 2 when the goats used were in mid to late lactation. SCC of the treatment group started at a higher level than that of the control group and remained higher throughout the trial except for dropping to below that of the control group on two days (Figure 4.8). The SCC of the control group remained unstable throughout the trial. The rise and fall of the SCC of (Figure 4.8) did not correspond to the administration of Spectrazol.



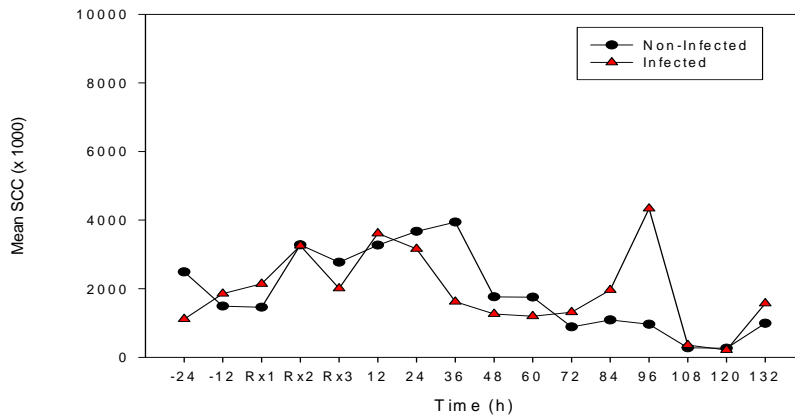
**FIGURE 4.9: MEAN SOMATIC CELL COUNT OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIAL 2 (SPECTRAZOL).**

The SCC of the non-infected udder halves started at a higher point than the SCC of the infected udder halves (Figure 4.9). SCC of infected udder halves remained unstable throughout the trial and did not correspond to administration of Spectrazol treatments.



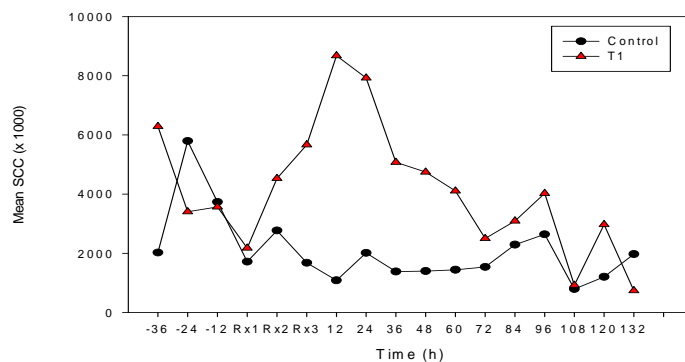
**FIGURE 4.10: MEAN SOMATIC CELL COUNT OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 3 (CURACLOX LC 7 RILEXINE).**

Mean SCC of control group remained relatively stable (baseline). Mean SCC of T1 (Curaclox LC), increased after treatment at Rx1 and returned to baseline at 72h only increasing slightly at 96h and then returning to baseline again. Mean SCC of T3 (Rilexine), increases after treatment at Rx1 and returned to baseline at 72h. However Treatment with Rilexine did not raise SCC as much as treatment with Curaclox LC did.



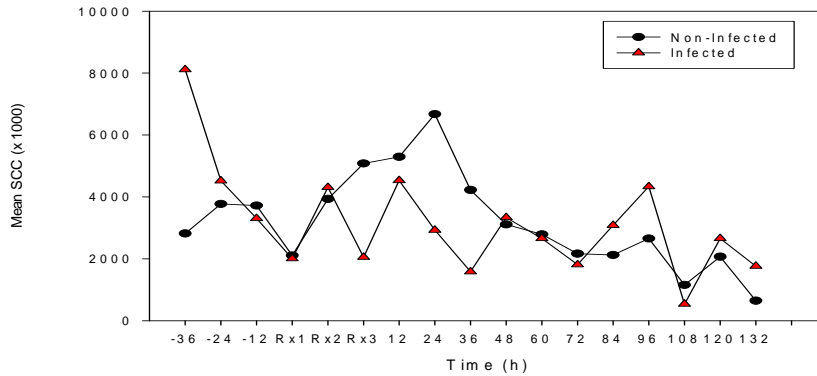
**FIGURE 4.11: MEAN SOMATIC CELL COUNT OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIAL 3 (CURACLOX LC & RILEXINE).**

Mean SCC of infected udder halves started at a lower level than mean SCC of non-infected udder halves. Mean SCC of both infected and non-infected udder halves increased after treatment at Rx1 and returned to baseline at 72h. However the mean SCC of the infected udder halves increased again to reach a peak at 96h and then decreased to baseline again.



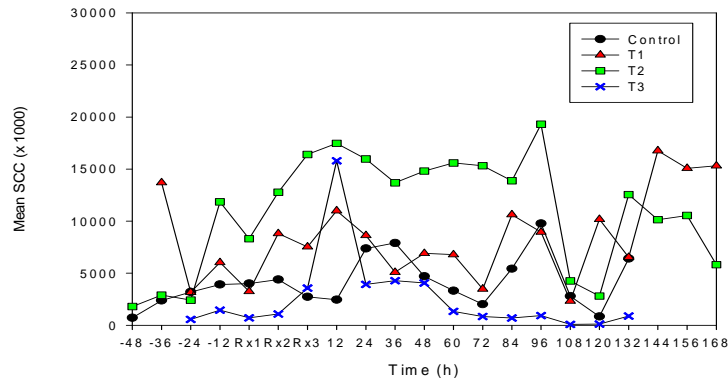
**FIGURE 4.12: MEAN SOMATIC CELL COUNT OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIALS 1&3 (CURACLOX LC).**

Mean SCC of T1 (goats treated with Curaclox LC in Trials 1 & 3) started at a high level, then reached baseline at Rx1 after which it increased after treatment and returned to baseline at 108h only to increase slightly again at 120h and then decrease again at 132h.



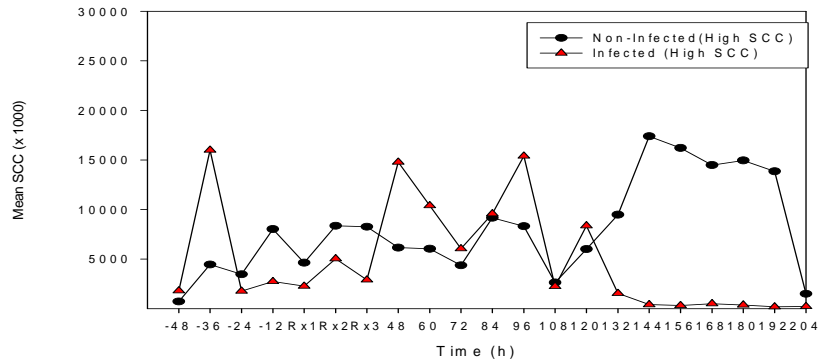
**FIGURE 4.13: MEAN SOMATIC CELL COUNT OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIALS 1&3 (CURACLOX LC).**

Mean SCC of infected udder halves started at a higher level than that of non-infected udder halves and reached a baseline at Rx1. After treatment at Rx1, mean SCC of non-infected udder halves increased and returned to baseline at 72h. Mean SCC of infected udder halves remained unstable throughout the trial.



**FIGURE 4.14: MEAN SOMATIC CELL COUNT OF UDDER HALVES WITH CLINICAL MASTITIS OF TREATMENT GROUPS (T1=CURACLOX LC, T2= SPECTRAZOL MILKING COW, T3= RILEXINE 200 LC) VERSUS CONTROL GROUP.**

Mean SCC of control group of clinical udder halves remained unstable throughout the trial. Mean SCC of T1 started at a high level, decreased and then increased after treatment at Rx1 and returned to baseline at 120h, only to increase again until the end of the trial (168h). Mean SCC of T2 increased after treatment at Rx1 and reached a baseline at 120h, only to increase again until the end of the trial. Mean SCC of T3 began relatively lower than those of T1, T2 and control increased after treatment t Rx2 and returned to baseline again at 72h and remained at the baseline for the remainder of the trial.



**FIGURE 4.15: MEAN SOMATIC CELL COUNT OF UDDER HALVES WITH CLINICAL MASTITIS WHERE BACTERIAL INFECTION WAS IDENTIFIED OR NOT.**

Mean SCC of infected and non-infected udder halves of goats with clinical mastitis remained unstable throughout the trial.

#### 4.4.2 Analysis of Variance of Somatic Cell Counts (SCC) of Curaclox LC from Trial 1 Only

**TABLE 4.27: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN TREATMENT GROUPS (CURACLOX LC; TRIAL 1).**

| Variate           | Sampling Time | Curaclox LC (T1) Mean $\pm$ Standard Error | Size (n) | Control (C1) Mean $\pm$ Standard Error | Size (n) | F probability |
|-------------------|---------------|--|----------|--|----------|---------------|
| Log SCC           | 07:00         | 3.508 $\pm$ 0.0358                         | 144      | 3.183 $\pm$ 0.0438                     | 96       | < 0.001***    |
| SCC $\times 10^3$ | 07:00         | 6298 $\pm$ 10566                           |          | 1998 $\pm$ 1460                        |          |               |
| Log SCC           | 19:00         | 3.558 $\pm$ 0.0346                         | 143      | 3.219 $\pm$ 0.0424                     | 95       | < 0.001***    |
| SCC $\times 10^3$ | 19:00         | 6415 $\pm$ 9788                            |          | 2288 $\pm$ 2086                        |          |               |

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC values between treatment (T1) and control (C1) udder halves at both treatment times.

**TABLE 4.28: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC; TRIAL 1).**

| Variate           | Sampling Time | Infected (B) Mean $\pm$ Standard Error | Size (n) | Non-infected (N) Mean $\pm$ Standard Error | Size (n) | F probability |
|-------------------|---------------|--|----------|--|----------|---------------|
| Log SCC           | 07:00         | 3.465 $\pm$ 0.0722                     | 40       | 3.360 $\pm$ 0.0323                         | 200      | 0.186 NS      |
| SCC $\times 10^3$ | 07:00         | 9274 $\pm$ 17602                       |          | 3639 $\pm$ 4534                            |          |               |
| Log SCC           | 19:00         | 3.494 $\pm$ 0.0826                     | 29       | 3.413 $\pm$ 0.0308                         | 209      | 0.355 NS      |
| SCC $\times 10^3$ | 19:00         | 9839 $\pm$ 17690                       |          | 4064 $\pm$ 5083                            |          |               |

There was no significant difference of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment times 007:00 ( $P = 0.186$ ) and 19:00 ( $P = 0.355$ ).



**TABLE 4.29: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN STAGES OF LACTATION (CURACLOX LC; TRIAL 1).**

| Variate             | Sampling Time | Early Lactation (1)<br>Mean $\pm$ Standard Error | Size (n) | Late Lactation (3)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|---|----------|---------------|
| Log SCC             | 07:00         | 3.402 $\pm$ 0.0315                               | 208      | 3.219 $\pm$ 0.0802                              | 32       | 0.035*        |
| SCCx10 <sup>3</sup> | 07:00         | 4944 $\pm$ 9039                                  |          | 2202 $\pm$ 1901                                 |          |               |
| Log SCC             | 19:00         | 3.450 $\pm$ 0.0307                               | 20       | 3.245 $\pm$ 0.0778                              | 32       | 0.015*        |
| SCCx10 <sup>3</sup> | 19:00         | 5143 $\pm$ 8439                                  |          | 2353 $\pm$ 2342                                 |          |               |

There was a significant difference in mean log SCC values of the milk between early (1) and late (3) lactation udder halves at treatment times 07:00 (P = 0.035) and 19:00 (P = 0.015).

**TABLE 4.30: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN LACTATION NUMBERS (CURACLOX LC; TRIAL 1).**

| Variate             | Sampling Time | Lactation Numbers<br>Means $\pm$ Standard Error |                    |                    |                    |                    | F probability |
|---------------------|---------------|---|--------------------|--------------------|--------------------|--------------------|---------------|
|                     |               | (2)   | (3)                | (4)                | (5)                | (7)                |               |
| Log SCC             | 07:00         | 3.453 $\pm$ 0.0771                              | 3.215 $\pm$ 0.0487 | 3.262 $\pm$ 0.0771 | 3.625 $\pm$ 0.0629 | 3.550 $\pm$ 0.0771 | <0.001***     |
| SCCx10 <sup>3</sup> | 07:00         | 4157 $\pm$ 3330                                 | 2556 $\pm$ 2606    | 2291 $\pm$ 1742    | 9978 $\pm$ 16938   | 5582 $\pm$ 4745    |               |
| Log SCC             | 19:00         | 3.536 $\pm$ 0.0754                              | 3.270 $\pm$ 0.0477 | 3.320 $\pm$ 0.0754 | 3.652 $\pm$ 0.0622 | 3.566 $\pm$ 0.0766 | <0.001***     |
| SCCx10 <sup>3</sup> | 19:00         | 4945 $\pm$ 3755                                 | 3073 $\pm$ 3711    | 2662 $\pm$ 2291    | 9235 $\pm$ 15331   | 5813 $\pm$ 5736    |               |

There was a highly significant difference (P < 0.001) of mean log SCC values between udder halves of lactation numbers, 2, 3, 4, 5 & 7 at both treatment times. At 07:00 lactation numbers 2, 4 and 7 each had a sample size of 32 udder halves and lactation numbers 3 and 5 had a sample size of 80 and 48 udder halves respectively. At 19:00 lactation numbers 2 and 4 each had a sample size of 32 udder halves and lactation numbers of 3, 5 and 7 had a sample size of 80, 47 and 31 udder halves respectively.

#### 4.4.3 Analysis of Variance of Somatic Cell Counts (SCC) of Trial 2 (Spectrazol)

**TABLE 4.31: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN TREATMENT GROUPS (SPECTRAZOL; TRIAL 2).**

| Variate             | Sampling Time | Spectrazol (T2)<br>Mean $\pm$ Standard Error | Size (n) | Control (C2)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|---|----------|---------------|
| Log SCC             | 07:00         | 3.155 $\pm$ 0.0457                           | 126      | 3.077 $\pm$ 0.0457                        | 126      | 0.227 NS      |
| SCCx10 <sup>3</sup> | 07:00         | 3933 $\pm$ 9592                              |          | 2546 $\pm$ 3655                           |          |               |
| Log SCC             | 19:00         | 3.191 $\pm$ 0.0485                           | 112      | 3.069 $\pm$ 0.0485                        | 112      | 0.078 NS      |
| SCCx10 <sup>3</sup> | 19:00         | 4484 $\pm$ 11471                             |          | 2431 $\pm$ 3557                           |          |               |

There was no significant difference of mean log SCC values between treatment (T2) and control (C2) udder halves at treatment times 07:00 (P = 0.227) and 19:00 (P = 0.078).

**TABLE 4.32: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (SPECTRAZOL; TRIAL 2).**

| Variate             | Sampling Time | Infected (B)<br>Mean $\pm$ Standard Error | Size (n) | Non-infected (N)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|---|----------|---|----------|---------------|
| Log SCC             | 07:00         | 3.218 $\pm$ 0.1071                        | 23       | 3.106 $\pm$ 0.0339                            | 229      | 0.320 NS      |
| SCCx10 <sup>3</sup> | 07:00         | 2845 $\pm$ 3057                           |          | 3279 $\pm$ 7575                               |          |               |
| Log SCC             | 19:00         | 3.440 $\pm$ 0.1274                        | 16       | 3.106 $\pm$ 0.0353                            | 208      | 0.012**       |
| SCCx10 <sup>3</sup> | 19:00         | 5456 $\pm$ 6396                           |          | 3304 $\pm$ 8671                               |          |               |

There was no significant difference (P = 0.320) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 07:00.

There was a significant difference (P = 0.01) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 19:00.

**TABLE 4.33: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN STAGES OF LACTATION (SPECTRAZOL; TRIAL 2).**

| Variate             | Sampling Time | Mid Lactation (2)<br>Mean $\pm$ Standard Error | Size (n) | Late Lactation (3)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|---|----------|---------------|
| Log SCC             | 07:00         | 3.172 $\pm$ 0.0337                             | 216      | 2.776 $\pm$ 0.0825                              | 36       | <0.001***     |
| SCCx10 <sup>3</sup> | 07:00         | 3651 $\pm$ 7782                                |          | 769 $\pm$ 619                                   |          |               |
| Log SCC             | 19:00         | 3.200 $\pm$ 0.0352                             | 192      | 3.709 $\pm$ 0.0861                              | 32       | <0.001***     |
| SCCx10 <sup>3</sup> | 19:00         | 3912 $\pm$ 9139                                |          | 730 $\pm$ 685                                   |          |               |

There was a highly significant difference (P <0.001) of mean log SCC values between udder halves of mid (2) and late (3) lactation at both treatment times.

**TABLE 4.34: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN LACTATION NUMBERS (SPECTRAZOL; TRIAL 2).**

| Variate             | Sampling Time | Lactation Numbers          |                    |                    |                    |                    | F probability |
|---------------------|---------------|----------------------------|--------------------|--------------------|--------------------|--------------------|---------------|
|                     |               | Means $\pm$ Standard Error |                    |                    |                    |                    |               |
|                     |               | (2)                        | (3)                | (4)                | (5)                | (7)                |               |
| Log SCC             | 07:00         | 2.936 $\pm$ 0.0739         | 3.131 $\pm$ 0.0468 | 2.614 $\pm$ 0.0739 | 3.388 $\pm$ 0.0739 | 3.551 $\pm$ 0.0739 | <0.001***     |
| SCCx10 <sup>3</sup> | 07:00         | 1180 $\pm$ 1041            | 4757 $\pm$ 11245   | 447 $\pm$ 183      | 2943 $\pm$ 1858    | 5645 $\pm$ 5178    |               |
| Log SCC             | 19:00         | 2.938 $\pm$ 0.0786         | 3.187 $\pm$ 0.0497 | 2.596 $\pm$ 0.0786 | 3.405 $\pm$ 0.0786 | 3.512 $\pm$ 0.0786 | <0.001***     |
| SCCx10 <sup>3</sup> | 19:00         | 1142 $\pm$ 839             | 5528 $\pm$ 13441   | 453 $\pm$ 236      | 2904 $\pm$ 1553    | 5308 $\pm$ 5261    |               |

There was a highly significant difference (P <0.001) of mean log SCC values between udder halves of lactation numbers, 2, 3, 4, 5 & 7 at both treatment times. At 07:00 lactation numbers 2, 4, 5 and 7 each had a sample size of 36 udder halves and lactation number 3 had a sample size of 90 udder halves. At 19:00 lactation numbers 2, 4, 5 and 7 each had a sample size of 32 udder halves and lactation number 3 had a sample size of 80 udder halves.

#### 4.4.4 Analysis of Variance of Somatic Cell Counts (SCC) of Trial 3 (Curaclox LC (T1) & Rilexine (T3))

**TABLE 4.35: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN TREATMENT GROUPS (CURACLOX LC & RILEXINE; TRIAL 3).**

| Variate             | Sampling Time | Control (C)<br>Mean $\pm$<br>Standard<br>Error | Size (n) | Curaclox LC (T1)<br>Mean $\pm$<br>Standard<br>Error | Size (n) | Rilexine (T3)<br>Mean $\pm$<br>Standard<br>Error | Size (n) | F probability |
|---------------------|---------------|--|----------|---|----------|--|----------|---------------|
| Log SCC             | 06:00         | 2.728 $\pm$<br>0.0274                          | 476      | 2.953 $\pm$<br>0.0433                               | 191      | 2.933 $\pm$<br>0.0335                            | 318      | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 1312 $\pm$<br>3379                             |          | 2864 $\pm$<br>5480                                  |          | 2068 $\pm$<br>4420                               |          |               |
| Log SCC             | 18:00         | 2.743 $\pm$<br>0.0277                          | 510      | 3.001 $\pm$<br>0.0452                               | 192      | 3.009 $\pm$<br>0.0351                            | 318      | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 1459 $\pm$<br>2954                             |          | 2905 $\pm$<br>6213                                  |          | 2413 $\pm$<br>4262                               |          |               |

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC values between treatment groups (T1), treatment group (T3) and control udder halves at both treatment times.

**TABLE 4.36: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC & RILEXINE; TRIAL 3).**

| Variate             | Sampling Time | Infected (B)<br>Mean $\pm$<br>Standard Error | Size (n) | Non-infected (N)<br>Mean $\pm$<br>Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|--|----------|---------------|
| Log SCC             | 06:00         | 2.890 $\pm$ 0.0319                           | 360      | 2.808 $\pm$ 0.0242                               | 625      | 0.041*        |
| SCCx10 <sup>3</sup> | 06:00         | 1928 $\pm$ 4772                              |          | 1817 $\pm$ 3901                                  |          |               |
| Log SCC             | 18:00         | 2.8748 $\pm$ 0.0308                          | 432      | 2.8745 $\pm$ 0.0264                              | 588      | 0.993 NS      |
| SCCx10 <sup>3</sup> | 18:00         | 1927 $\pm$ 3593                              |          | 2103 $\pm$ 4589                                  |          |               |

There was a significant difference ( $P = 0.041$ ) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 06:00.

There was no significant difference ( $P = 0.993$ ) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 18:00.

**TABLE 4.37: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN LACTATION NUMBERS (CURACLOX LC & RILEXINE; TRIAL 3).**

| Variate             | Sampling Time | Early Lactation (1)<br>Mean $\pm$ Standard<br>Error | Size (n) | Mid Lactation (2)<br>Mean $\pm$ Standard<br>Error | Size (n) | F probability |
|---------------------|---------------|---|----------|---|----------|---------------|
| Log SCC             | 06:00         | 2.838 $\pm$ 0.0205                                  | 879      | 2.842 $\pm$ 0.0590                                | 106      | 0.937 NS      |
| SCCx10 <sup>3</sup> | 06:00         | 1872 $\pm$ 4366                                     |          | 1738 $\pm$ 2984                                   |          |               |
| Log SCC             | 18:00         | 2.876 $\pm$ 0.0212                                  | 910      | 2.866 $\pm$ 0.0609                                | 110      | 0.877 NS      |
| SCCx10 <sup>3</sup> | 18:00         | 2066 $\pm$ 4377                                     |          | 1717 $\pm$ 2147                                   |          |               |

There was no significant difference of mean log SCC values between udder halves of early (1) and mid (2) lactation at treatment times 06:00 ( $P = 0.937$ ) and 18:00 ( $P = 0.877$ ).

#### 4.4.5 Analysis of Variance of Somatic Cell Counts (SCC) of Curaclox LC in Trials 1 & 3 Combined

**TABLE 4.38: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN TREATMENT GROUPS (CURACLOX LC; TRIALS 1 & 3).**

| Variate             | Sampling Time | Curaclox LC (T1)<br>Mean $\pm$ Standard Error | Size (n) | Control (C)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|---|----------|--|----------|---------------|
| Log SCC             | 06:00         | 3.192 $\pm$ 0.0328                            | 335      | 2.805 $\pm$ 0.0251                       | 572      | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 4340 $\pm$ 8233                               |          | 1428 $\pm$ 3150                          |          |               |
| Log SCC             | 18:00         | 3.239 $\pm$ 0.0339                            | 335      | 2.818 $\pm$ 0.0252                       | 605      | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 4403 $\pm$ 8113                               |          | 1589 $\pm$ 2850                          |          |               |

There was a highly significant difference at the 0.1% level of significance of mean log SCC values between udder halves of treatment (T1) and control (C) groups at both treatment times.

**TABLE 4.39: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CURACLOX LC; TRIALS 1 & 3).**

| Variate             | Sampling Time | Infected (B)<br>Mean $\pm$ Standard Error | Size (n) | Non-infected (N)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|---|----------|---|----------|---------------|
| Log SCC             | 06:00         | 2.979 $\pm$ 0.0349                        | 324      | 2.930 $\pm$ 0.0260                            | 583      | 0.257 NS      |
| SCCx10 <sup>3</sup> | 06:00         | 2870 $\pm$ 8079                           |          | 2300 $\pm$ 3921                               |          |               |
| Log SCC             | 18:00         | 2.951 $\pm$ 0.0345                        | 356      | 2.978 $\pm$ 0.0270                            | 584      | 0.546 NS      |
| SCCx10 <sup>3</sup> | 18:00         | 2652 $\pm$ 6518                           |          | 2555 $\pm$ 4815                               |          |               |

There was no significant difference of mean log SCC values between infected (B) and non-infected (N) udder halves at both treatment times.

**TABLE 4.40: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN STAGES OF LACTATION (CURACLOX LC; TRIALS 1 & 3).**

| Variate             | Sampling Time | Early Lactation (1)<br>Mean $\pm$ Standard Error | Size (n) | Mid Lactation (2)<br>Mean $\pm$ Standard Error | Size (n) | Late Lactation (3)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|--|----------|---|----------|---------------|
| Log SCC             | 06:00         | 3.402 $\pm$ 0.0397                               | 208      | 2.793 $\pm$ 0.0221                             | 667      | 3.219 $\pm$ 0.1011                              | 32       | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 4944 $\pm$ 9039                                  |          | 1757 $\pm$ 4148                                |          | 2202 $\pm$ 1901                                 |          |               |
| Log SCC             | 18:00         | 3.450 $\pm$ 0.0414                               | 206      | 2.814 $\pm$ 0.0225                             | 702      | 3.245 $\pm$ 0.1052                              | 32       | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 5143 $\pm$ 8439                                  |          | 1854 $\pm$ 4155                                |          | 2353 $\pm$ 2343                                 |          |               |

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC values between udder halves of early, mid and late lactation at both treatment times.

**TABLE 4.41: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN LACTATION NUMBERS (CURACLOX LC; TRIALS 1 &3).**

| Variate             | Sampling Time | Lactation Numbers          |                    |                    |                    |                    |                    | F probability |
|---------------------|---------------|----------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------|
|                     |               | Means $\pm$ Standard Error |                    |                    |                    |                    |                    |               |
|                     |               | (1)                        | (2)                | (3)                | (4)                | (5)                | (7)                |               |
| Log SCC             | 06:00         | 2.787 $\pm$ 0.0238         | 3.018 $\pm$ 0.0557 | 3.215 $\pm$ 0.0647 | 3.262 $\pm$ 0.1024 | 3.625 $\pm$ 0.0836 | 3.550 $\pm$ 0.1024 | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 1744 $\pm$ 4261            | 2537 $\pm$ 3359    | 2556 $\pm$ 2606    | 2291 $\pm$ 1742    | 9978 $\pm$ 16939   | 5582 $\pm$ 4745    |               |
| Log SCC             | 18:00         | 2.810 $\pm$ 0.0243         | 3.041 $\pm$ 0.0572 | 3.270 $\pm$ 0.0677 | 3.320 $\pm$ 0.1070 | 3.652 $\pm$ 0.0883 | 3.566 $\pm$ 0.1087 | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 1859 $\pm$ 4337            | 2708 $\pm$ 3124    | 3073 $\pm$ 3711    | 2662 $\pm$ 2291    | 9235 $\pm$ 15331   | 5813 $\pm$ 5736    |               |

There was a highly significant difference (P <0.001) of mean log SCC values between udder halves of lactation numbers, 1, 2, 3, 4, 5 & 7 at both treatment times. At 07:00 lactation numbers 4 and 7 each had a sample size of 32 udder halves and lactation numbers 1, 2, 3, and 5 had a sample size of 591, 108, 80 and 48 udder halves respectively. At 19:00 lactation numbers 1, 2, 3, 4, 5 and 7 had a sample size of 622, 112, 80, 32, 47 and 31 udder halves respectively.

#### 4.4.6 Analysis of Variance of Somatic Cell Counts (SCC) of all Data from Goats with Clinical Mastitis

**TABLE 4.42: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN TREATMENT GROUPS (CLINICAL MASTITIS).**

| Variate             | Sampling Time | Treatment groups           |                    |                    |                    | F probability |
|---------------------|---------------|----------------------------|--------------------|--------------------|--------------------|---------------|
|                     |               | Means $\pm$ Standard Error |                    |                    |                    |               |
|                     |               | Control (C)                | Curaclox LC (T1)   | Spectrazol (T2)    | Rilexine (T3)      |               |
| Log SCC             | 06:00         | 3.202 $\pm$ 0.0975         | 3.379 $\pm$ 0.0547 | 3.715 $\pm$ 0.1108 | 2.968 $\pm$ 0.1707 | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 4249 $\pm$ 9121            | 8136 $\pm$ 13177   | 11121 $\pm$ 12523  | 1839 $\pm$ 2266    |               |
| Log SCC             | 18:00         | 3.316 $\pm$ 0.0960         | 3.432 $\pm$ 0.0539 | 3.803 $\pm$ 0.1108 | 3.046 $\pm$ 0.1662 | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 4335 $\pm$ 6572            | 8111 $\pm$ 12785   | 11655 $\pm$ 11184  | 3201 $\pm$ 5337    |               |

There was a highly significant difference (P <0.001) of mean log SCC between treatment groups, (T1) Curaclox LC, (T2) Spectrazol, (T3) Rilexine and Control group (C) at both treatment times. At 06:00 Control group (C), (T1) Curaclox LC, (T2) Spectrazol and (T3) Rilexine had a sample size of 49, 156, 38 and 16 udder halves respectively. At 18:00 Control group (C), (T1) Curaclox LC, (T2) Spectrazol and (T3) Rilexine had a sample size of 48, 152, 36 and 16 udder halves respectively.

**TABLE 4.43: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN INFECTED AND NON-INFECTED UDDER HALVES (CLINICAL MASTITIS).**

| Variate             | Sampling Time | Infected (B)<br>Mean $\pm$ Standard Error | Size (n) | Non-infected (N)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|---|----------|---|----------|---------------|
| Log SCC             | 06:00         | 3.274 $\pm$ 0.0827                        | 72       | 3.406 $\pm$ 0.0513                            | 187      | 0.173 NS      |
| SCCx10 <sup>3</sup> | 06:00         | 8479 $\pm$ 16563                          |          | 7053 $\pm$ 10051                              |          |               |
| Log SCC             | 18:00         | 3.196 $\pm$ 0.0853                        | 62       | 3.517 $\pm$ 0.0487                            | 190      | 0.001***      |
| SCCx10 <sup>3</sup> | 18:00         | 6476 $\pm$ 13373                          |          | 7948 $\pm$ 10780                              |          |               |

There was no significant difference ( $P = 0.173$ ) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 06:00.

There was a highly significant difference ( $P = 0.001$ ) of mean log SCC values between infected (B) and non-infected (N) udder halves at treatment time 18:00.

**TABLE 4.44: TESTING DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN STAGES OF LACTATION (CLINICAL MASTITIS).**

| Variate             | Sampling Time | Early Lactation (1)<br>Mean $\pm$ Standard Error | Size (n) | Mid Lactation (2)<br>Mean $\pm$ Standard Error | Size (n) | Late Lactation (3)<br>Mean $\pm$ Standard Error | Size (n) | F probability |
|---------------------|---------------|--|----------|--|----------|---|----------|---------------|
| Log SCC             | 06:00         | 3.617 $\pm$ 0.0932                               | 48       | 3.105 $\pm$ 0.0552                             | 137      | 3.699 $\pm$ 0.0751                              | 74       | <0.001***     |
| SCCx10 <sup>3</sup> | 06:00         | 10305 $\pm$ 16977                                |          | 3517 $\pm$ 7438                                |          | 12877 $\pm$ 13146                               |          |               |
| Log SCC             | 18:00         | 3.641 $\pm$ 0.0895                               | 48       | 3.173 $\pm$ 0.0532                             | 136      | 3.826 $\pm$ 0.0752                              | 68       | <0.001***     |
| SCCx10 <sup>3</sup> | 18:00         | 9165 $\pm$ 15176                                 |          | 3799 $\pm$ 7607                                |          | 14046 $\pm$ 11862                               |          |               |

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC values between udder halves of early, mid and late lactation at both treatment times.

**TABLE 4.45: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT AND ACTUAL SOMATIC CELL COUNT VALUES BETWEEN LACTATION NUMBERS (CLINICAL MASTITIS).**

| Variate             | Sampling Time | Lactation Numbers          |                    |                    |                    |                    |                    | F probability |
|---------------------|---------------|----------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------|
|                     |               | Means $\pm$ Standard Error |                    |                    |                    |                    |                    |               |
|                     |               | (1)                        | (2)                | (3)                | (4)                | (5)                | (7)                |               |
| Log SCC             | 06:00         | 3.339 $\pm$ 0.0608         | 2.999 $\pm$ 0.1108 | 3.052 $\pm$ 0.1092 | 3.782 $\pm$ 0.1077 | 4.152 $\pm$ 0.1616 | 3.417 $\pm$ 0.157  | <0.001<br>*** |
| SCCx10 <sup>3</sup> | 06:00         | 7978 $\pm$ 12324           | 2753 $\pm$ 4327    | 1463 $\pm$ 1155    | 11290 $\pm$ 11174  | 24672 $\pm$ 23430  | 3798 $\pm$ 4436    |               |
| Log SCC             | 18:00         | 3.405 $\pm$ 0.0610         | 3.104 $\pm$ 0.1118 | 3.164 $\pm$ 0.1136 | 3.855 $\pm$ 0.1071 | 4.062 $\pm$ 0.1606 | 3.426 $\pm$ 0.1606 | <0.001<br>*** |
| SCCx10 <sup>3</sup> | 18:00         | 8069 $\pm$ 11401           | 2849 $\pm$ 2954    | 2055 $\pm$ 2186    | 12615 $\pm$ 11685  | 20531 $\pm$ 22350  | 3492 $\pm$ 2869    |               |

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC values between udder halves of lactation numbers, 1, 2, 3, 4, 5 & 7 at both treatment times. At 06:00 lactation numbers 1, 2, 3, 4, 5 and 7 had a sample size of 113, 34, 35, 36, 16 and 17 udder halves respectively. At 18:00 lactation numbers 1, 2, 3, 4, 5 and 7 had a sample size of 111, 33, 32, 36, 16 and 16 udder halves respectively.

#### **4.5 Statistical Analysis and Graphs of Peak Somatic Cell Count (SCC) After Treatment**

##### **4.5.1 Analysis of Variance of Somatic Cell Count (SCC) Peak Values**

**TABLE 4.46: DIFFERENCES OF TRANSFORMED LOG SOMATIC CELL COUNT VALUES AT PEAK SOMATIC CELL COUNT BETWEEN TREATMENT GROUPS.**

| Variate | Size (n) | Curaclox LC (T1)              | Size (n) | Spectrazol (T2)               | Size (n) | Rilexine (T3)                 | F probability |
|---------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|---------------|
|         |          | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |               |
| Log SCC | 42       | 3.964 $\pm$ 0.3723            | 9        | 3.559 $\pm$ 0.5376            | 40       | 3.794 $\pm$ 0.3127            | 0.006**       |

There was a significant difference ( $P = 0.006$ ) between peak log SCC values after treatment between treatments, (T1) Curaclox LC, (T2) Spectrazol and (T3) Rilexine.

**TABLE 4.47: DIFFERENCES OF TIME (h) FROM START TO PEAK IN SOMATIC CELL COUNT BETWEEN TREATMENT GROUPS.**

| Variate                 | Size (n) | Curaclox LC (T1)              | Size (n) | Spectrazol (T2)               | Size (n) | Rilexine (T3)                 | F probability |
|-------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|---------------|
|                         |          | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |               |
| Start to peak time (hr) | 42       | 71.43 $\pm$ 18.918            | 9        | 77.33 $\pm$ 26.907            | 40       | 67.80 $\pm$ 15.742            | 0.340 NS      |

There was no significant difference ( $P = 0.340$ ) between the time in hours from start to peak in SCC between treatments, (T1) Curaclox LC, (T2) Spectrazol and (T3) Rilexine.

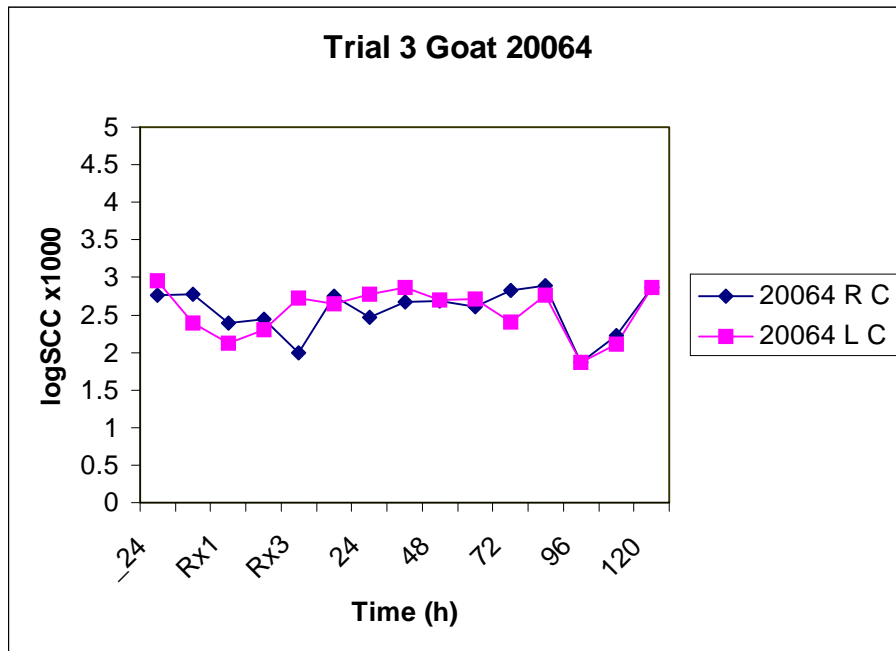
**TABLE 4.48: DIFFERENCES OF TIME IN (H) FROM PEAK SOMATIC CELL COUNT TO END BETWEEN TREATMENT GROUPS.**

| Variate               | Size (n) | Curaclox LC (T1)<br>Mean $\pm$ Standard Deviation | Size (n) | Spectrazol (T2)<br>Mean $\pm$ Standard Deviation | Size (n) | Rilexine (T3)<br>Mean $\pm$ Standard Deviation | F probability |
|-----------------------|----------|---|----------|--|----------|--|---------------|
| Peak to end time (hr) | 42       | 108.6 $\pm$ 18.918                                | 9        | 114.7 $\pm$ 26.907                               | 40       | 112.2 $\pm$ 15.742                             | 0.543 NS      |

There was no significant difference ( $P = 0.543$ ) between the time in hours from peak in SCC to the end between treatments, (T1) Curaclox LC, (T2) Spectrazol and (T3) Rilexine.

#### 4.5.2 Graphs of Somatic Cell Count (SCC) for Selected Goats

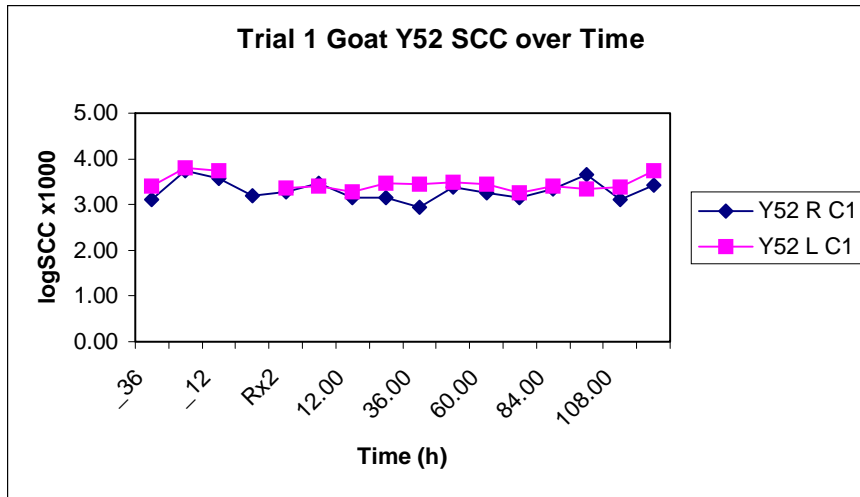
These are examples of SCC of goats from each trial illustrating the peak SCC.



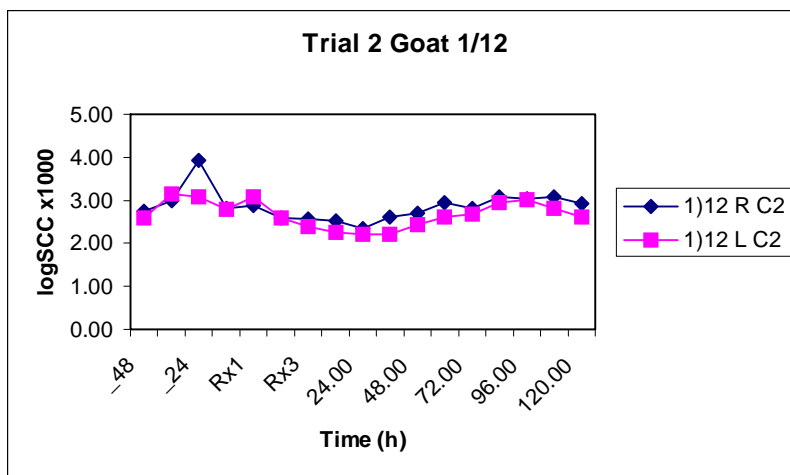
**FIGURE 4.16: LOG SOMATIC CELL COUNT OVER TIME FOR LEFT AND RIGHT UDDER HALVES SEPARATELY FOR CONTROL GOAT 20064 IN TRIAL 3.**

The above graph shows an example of a control goat from Trial 3 (Curaclox LC & Rilexine). As with all other goats in the control group of Trial 3, this goat was not treated. Therefore SCC could not peak after treatment and SCC remained relatively stable throughout the trial, with peaks in SCC not corresponding to any treatment.

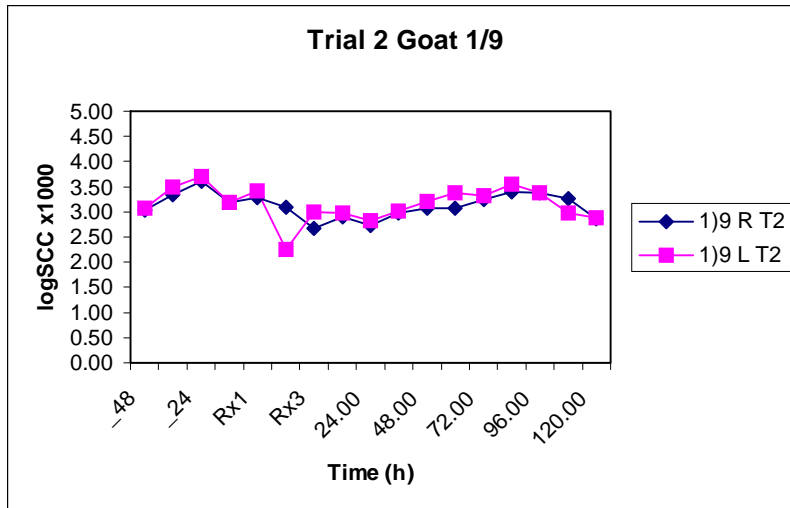




**FIGURE 4.17: LOG SOMATIC CELL COUNT OVER TIME FOR LEFT AND RIGHT UDDER HALVES SEPARATELY FOR CONTROL GOAT Y52 IN TRIAL 1.** The above graph shows an example of a control goat from Trial 1 (Curaclox LC). As with all other goats in the control group of Trial 1 this goat was not treated. Therefore SCC could not peak after treatment and SCC remained relatively stable throughout the trial, with peaks in SCC not corresponding to any treatment.



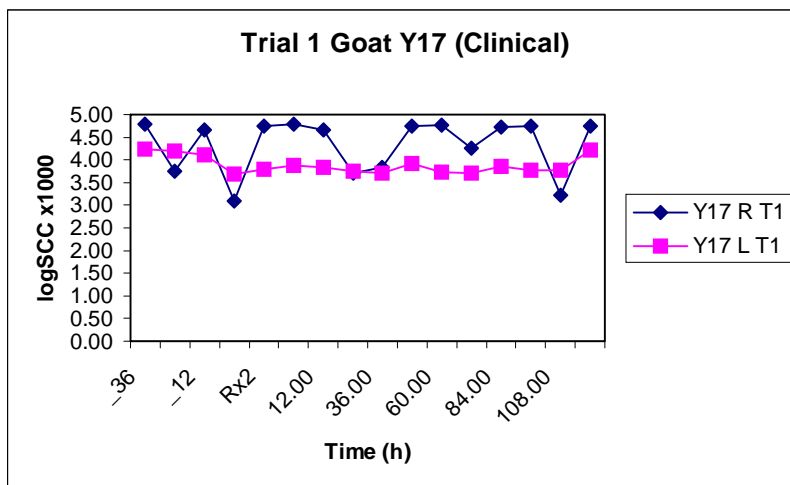
**FIGURE 4.18: LOG SOMATIC CELL COUNT OVER TIME FOR LEFT AND RIGHT UDDER HALVES SEPARATELY FOR CONTROL GOAT 1/12 IN TRIAL 2.** The above graph shows an example of a control goat from Trial 2 (Spectrazol). As with all other goats in the control group of Trial 2 this goat was not treated. Therefore SCC could not peak after treatment and SCC remained relatively stable throughout the trial, with peaks in SCC not corresponding to any treatment.



**FIGURE 4.19: LOG SOMATIC CELL COUNT OVER TIME FOR LEFT AND RIGHT UDDER HALVES SEPARATELY FOR GOAT 1/9 TREATED WITH SPECTRAZOL IN TRIAL 2.**

The above graph shows SCC for a goat, which was typical of 35.7% of the udder halves in Trial 2. This group of udder halves did not have a peak in SCC after treatment with Spectrazol. Instead this graph shows the same pattern as (Figure 4.18) for the control animals of Trial 2.

The remaining 64.3% of udder halves treated with Spectrazol in Trial 2 showed a peak in SCC after treatment. This explains the low numbers of Trial 2 udder halves in the statistical analysis in (Tables 4.46, 4.47 & 4.48).



**FIGURE 4.20 LOG SOMATIC CELL COUNT OVER TIME FOR LEFT AND RIGHT UDDER HALVES SEPARATELY FOR GOAT Y17 WITH CHRONIC MASTITIS IN RIGHT UDDER HALF, TREATED WITH CURACLOX LC IN BOTH UDDER HALVES IN TRIAL 1.**

The above graph shows log SCC values over time for a goat with chronic mastitis and udder damage in the right udder half. This goat was treated with Curaclox LC in both udder halves in Trial 1.

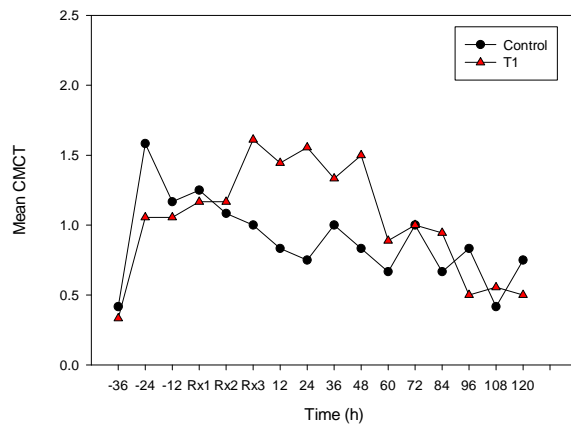
The SCC of the right udder half at the start was  $61\,367 \times 10^3$  cells/ml and the SCC of the left udder half at the start was  $16\,942 \times 10^3$  cells/ml.

The SCC of the left udder half remained relatively stable irrespective of treatment.

The peaks of SCC for the right udder half did not correspond to the treatment. This could have been due to the high irritation level present in the chronically infected damaged udder before treatment.

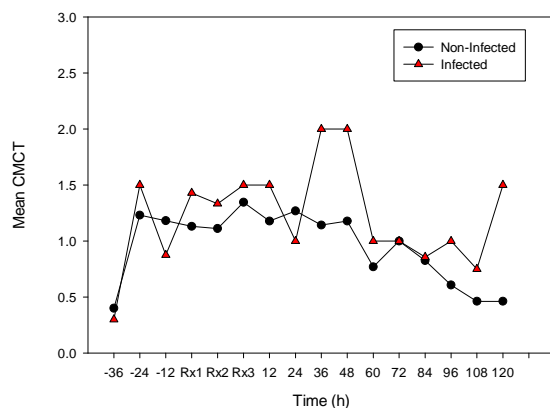
## 4.6 California Milk Cell Test (CMCT) Graphs and Chi-square Tests

### 4.6.1 Graphs of California Milk Cell Test (CMCT) versus Time (h)



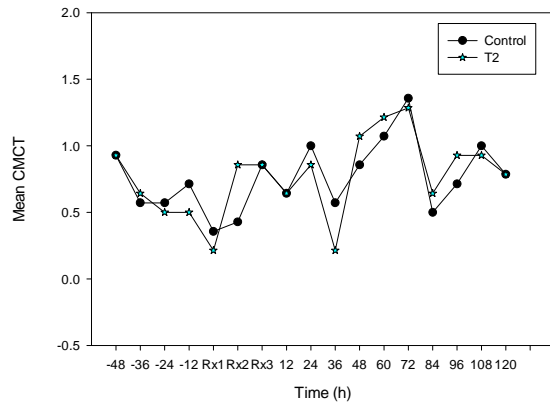
**FIGURE 4.21: MEAN CALIFORNIA MILK CELL TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 1.**

Mean CMCT of treatment group increased after treatment with Curaclox LC at Rx2 and returned to that of the control group at 72h. Mean CMCT of the control group was high at the start and then decreased and remained relatively unstable, although it was lower than that of the treatment group between treatment times Rx2 and 72h.

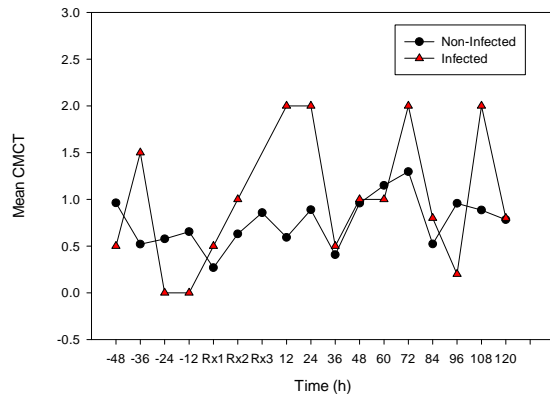


**FIGURE 4.22: MEAN CALIFORNIA MILK CELL TEST OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIAL 1.**

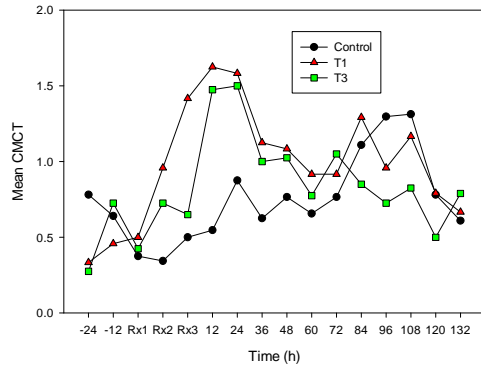
Mean CMCT of infected and non-infected udder halves was low at the start and then increased and remained unstable for the remainder of the trial. Mean CMCT of infected udder halves increased suddenly at 36h, 48h and 120h. In general the mean CMCT of infected udder halves was higher than that of the non-infected udder halves except at two treatment times (-36, -12h & 24h). The mean CMCT of infected and non-infected udder halves did not correspond to the antibiotic treatment times.



**FIGURE 4.23: MEAN CALIFORNIA MILK CELL TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 2.**  
Mean CMCT of treatment group increased after treatment with Spectrazol at Rx1, returned to baseline at 36h, then increased again at 48h and remained unstable for the remainder of the trial. Mean CMCT of the control group remained unstable throughout the trial.

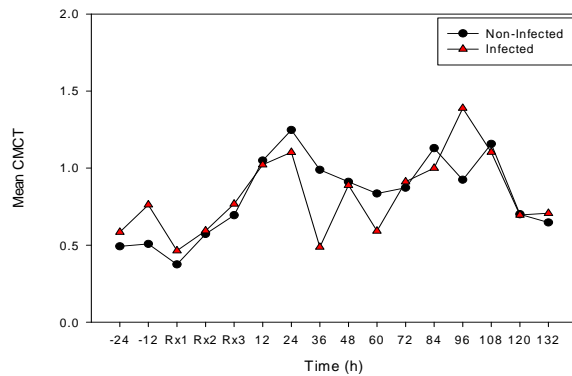


**FIGURE 4.24: MEAN CALIFORNIA MILK CELL TEST OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIAL 2.**  
Mean CMCT of non-infected udder halves was higher at the start than that of infected udder halves, remained unstable throughout the trial and did not correspond to the antibiotic treatment times. Mean CMCT of infected udder halves was unstable at the start, decreased to baseline at -12h, then increased after treatment at Rx1 and returned to baseline at 96h remaining unstable for the remainder of the trial.



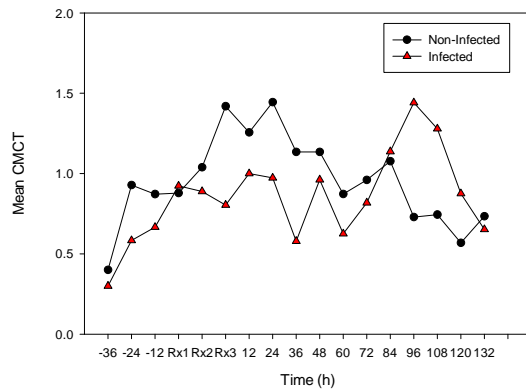
**FIGURE 4.25: MEAN CALIFORNIA MILK CELL TEST RESULTS OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIAL 3.**

Mean CMCT of T3 (Rilexine) increased after treatment at Rx1 and returned to baseline at 60h. Mean CMCT of T1 (Curaclox LC) increased after treatment at Rx1 and returned to baseline at 60h and then remained unstable for the remainder of the trial. Mean CMCT of the control group remained unstable for the duration of the trial, although it was lower than that of T1 and T3 between treatment times Rx1 and 96h.



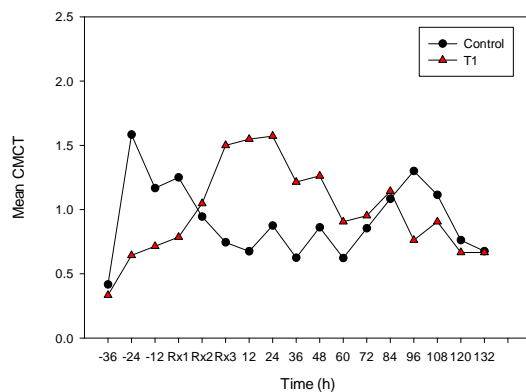
**FIGURE 4.26: MEAN CALIFORNIA MILK CELL TEST OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIAL 3.**

Mean CMCT of infected and non-infected udder halves was low at the start and increased after treatment at Rx1. Mean CMCT of infected and non-infected udder halves remained unstable for the remainder of the trial, although the mean CMCT of infected udder halves decreased at two treatment times (36h & 60h).



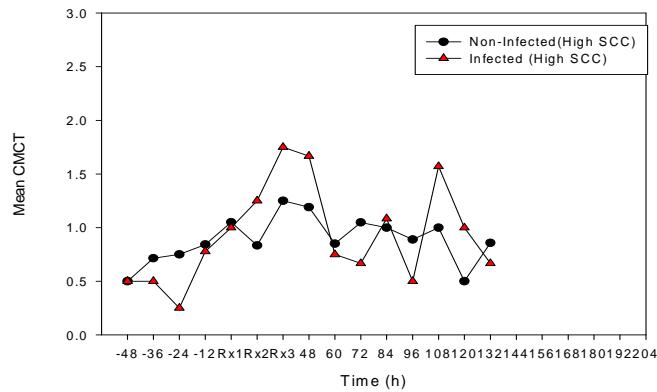
**FIGURE 4.27: MEAN CALIFORNIA MILK CELL TEST OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES: TRIALS 1&3 (CURACLOX LC).**

Mean CMCT of infected and non-infected udder halves was low at the start and steadily increased. Mean CMCT of infected udder halves remained unstable for the remainder of the trial and was lower than that of non-infected udder halves between treatment times Rx2 and 72h. Mean CMCT of non-infected udder halves increased after treatment at Rx1 and returned to baseline at 96h. This pattern differed to that of Curaclox LC in Trial 1 and Trial 3 separately, see (Figures 4.22 & 4.26).



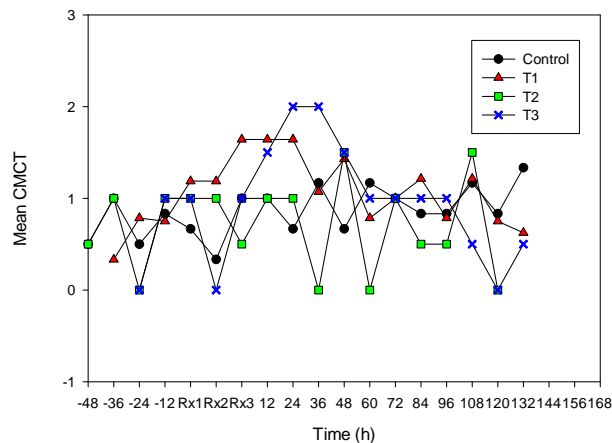
**FIGURE 4.28: MEAN CALIFORNIA MILK CELL TEST OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP: TRIALS 1&3 (CURACLOX LC).**

Mean CMCT of control group began at the same level as the treatment group then suddenly increased at -24h and then decreased and remained unstable for the duration of the trial. However the mean CMCT of the control group was lower than that of the treatment group between treatments times Rx2 and 84h. Mean CMCT of the treatment group was low at the start; it increased after treatment at Rx1 and returned to baseline at 96h, later than Curaclox LC in Trial 1 and Trial 3 separately. (See Figures 4.21 & 4.25.)



**FIGURE 4.29: MEAN CALIFORNIA MILK CELL TEST OF UDDER HALVES WITH CLINICAL MASTITIS WHERE BACTERIAL INFECTION WAS IDENTIFIED OR NOT.**

Mean CMCT of infected (high SCC) and non-infected (high SCC) udder halves was low at the start. Mean CMCT of infected udder halves increased after treatment at Rx1, returned to baseline at 72h and then remained unstable for the remainder of the trial. Mean CMCT of non-infected (high SCC) udder halves remained unstable throughout the trial, although it was lower than that of infected (high SCC) udder halves between treatment times Rx2 and 48h.



**FIGURE 4.30: MEAN CALIFORNIA MILK CELL TEST OF UDDER HALVES WITH CLINICAL MASTITIS IN TREATMENT GROUPS (T1=CURACLOX LC, T2= SPECTRAZOL MILKING COW, T3= RILEXINE 200 LC) VERSUS UDDER HALVES WITH CLINICAL MASTITIS IN THE CONTROL GROUP.**

Mean CMCT of the control group remained unstable throughout the trial. Mean CMCT of T2 (Spectrazol) remained unstable throughout the trial. Mean CMCT of T1 (Curaclox LC) increased after treatment at Rx1, returned to baseline at 60h and then remained unstable for the remainder of the trial. Mean CMCT of T3 (Rilexine) increased after treatment at Rx2 and returned to baseline at 120h and then increased again slightly at 132h.

#### 4.6.2 Chi-square Tests

**TABLE 4.49: THE ASSOCIATION BETWEEN TWO TREATMENTS AND CALIFORNIA MILK CELL TEST RATING (%) TRIAL 1.**

| Treatment        | CMCT=0     | CMCT=1      | CMCT=2 & 3 | Total Sample Numbers per Treatment |
|------------------|------------|-------------|------------|------------------------------------|
| T1 (Curaclon LC) | 67 (23.3%) | 155 (53.8%) | 66 (22.9%) | 288                                |
| Control (C1)     | 42 (21.9%) | 129 (67.2%) | 21 (10.9%) | 192                                |
| CMCT Total       | 109        | 284         | 87         | 480                                |

The Chi-square test performed on the data in the table above was highly significant ( $\chi^2 = 12.7$ ;  $P = 0.002$ ; degrees of freedom = 2). Thus the number of CMCT counts per category did depend on treatment.

The percentage udder halves with a CMCT score of 1 were highest for both treatments (> 50%), but for C1 (67.2%) it was significantly higher than that for T1 (53.8%).

The frequencies between CMCT = 0 and CMCT = 1 were not significantly different ( $\chi^2 = 1.254$  NS).

The frequencies between CMCT = 0 and CMCT = 2 & 3 were not significantly different ( $\chi^2 = 3.960$  NS).

The frequencies between CMCT = 1 and CMCT = 2 & 3 were significantly different ( $\chi^2 = 11.659$ ).

**TABLE 4.50: THE ASSOCIATION BETWEEN TWO TREATMENTS AND CALIFORNIA MILK CELL TEST RATING (%) TRIAL 2.**

| Treatment       | CMCT=0     | CMCT=1      | CMCT=2 & 3 | Total Sample Numbers per Treatment |
|-----------------|------------|-------------|------------|------------------------------------|
| T2 (Spectrazol) | 99 (41.6%) | 98 (41.2%)  | 41 (17.2%) | 238                                |
| Control (C2)    | 90 (37.8%) | 116 (48.7%) | 32 (13.4%) | 238                                |
| CMCT Total      | 189        | 214         | 73         | 476                                |

The Chi-square test performed on the data in the table above was not significant ( $\chi^2 = 3.052$ ;  $P = 0.216$ ; degrees of freedom = 2). Thus the number of CMCT counts per category did not depend on treatment.

The percentage udder halves with a CMCT score of 1 were highest for C2 (48.7%). For T2 the percentage udder halves with a CMCT score of 0 were slightly higher (41.6%), than those with a CMCT score of 1 (41.2%).

**TABLE 4.51: THE ASSOCIATION BETWEEN TWO TREATMENTS AND CALIFORNIA MILK CELL TEST RATING (%) TRIAL 3.**

| Treatment        | CMCT=0      | CMCT=1      | CMCT=2 & 3  | Total Sample Numbers per Treatment |
|------------------|-------------|-------------|-------------|------------------------------------|
| Control (C)      | 443 (43.3%) | 398 (38.9%) | 183 (17.9%) | 1024                               |
| T1 (Curaclon LC) | 116 (30.2%) | 161 (41.9%) | 107 (27.9%) | 384                                |
| T3 (Rilexine)    | 242 (38.1%) | 260 (40.9%) | 134 (21.1%) | 636                                |
| CMCT Total       | 801         | 819         | 424         | 2044                               |

The Chi-square test performed on the data in the table above was highly significant ( $\chi^2 = 26.745$ ;  $P < 0.0001$ ; degrees of freedom = 4). Thus the number of CMCT counts per category did depend on treatment.

The percentage udder halves with a CMCT score of 0 were highest for C (43.3%). However, the percentage udder halves with a CMCT score of 1 were highest for T1 (Curaclon LC) (41.9%) & T3 (Rilexine) (40.9%).

The frequencies between CMCT = 0 and CMCT = 1 were significantly different



( $\chi^2 = 10.165$ ).

The frequencies between CMCT = 0 and CMCT = 2 & 3 were significantly different

( $\chi^2 = 25.791$ ).

The frequencies between CMCT = 1 and CMCT = 2 & 3 were not significantly different

( $\chi^2 = 5.799$  NS).

**TABLE 4.52: THE ASSOCIATION BETWEEN TWO TREATMENTS AND CALIFORNIA MILK CELL TEST RATING (%), CURACLOX LC IN TRIALS 1 & 3.**

| Treatment        | CMCT=0      | CMCT=1       | CMCT=2 & 3  | Total Sample Numbers per Treatment |
|------------------|-------------|--------------|-------------|------------------------------------|
| Control (C)      | 485 (39.9%) | 527 (43.3%)  | 204 (16.8%) | 1216                               |
| T1 (Curaclor LC) | 183 (27.2%) | 316 (47.02%) | 173 (25.7%) | 672                                |
| CMCT Total       | 668         | 843          | 377         | 1888                               |

The Chi-square test performed on the data in the table above was highly significant ( $\chi^2 = 38.331$ ;  $P < 0.0001$ ; degrees of freedom = 2). Thus the number of CMCT counts per category did depend on treatment.

The percentage udder halves with a CMCT score of 0 were highest for the control group (C) (39.9%). However, the percentage udder halves with a CMCT score of 1 were highest for T1 (47.02%) and control (C) (43.3%). However, the percentage udder halves with a CMCT score of 1 were highest for T1 (Curaclor LC) (41.9%) & T3 (Rilexine) (40.9%), see (Table 4.51).

The frequencies between CMCT = 0 and CMCT = 1 were significantly different

( $\chi^2 = 16.701$ ).

The frequencies between CMCT = 0 and CMCT = 2 & 3 were significantly different

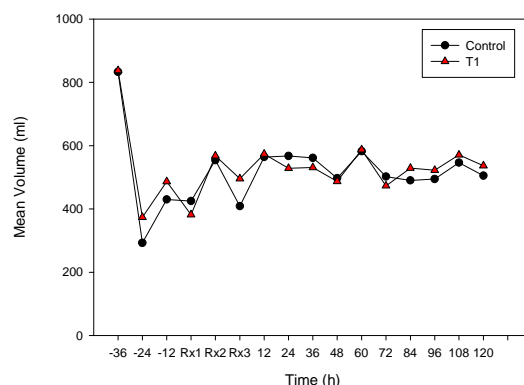
( $\chi^2 = 35.875$ ).

The frequencies between CMCT = 1 and CMCT = 2 & 3 were significantly different

( $\chi^2 = 7.314$ ).

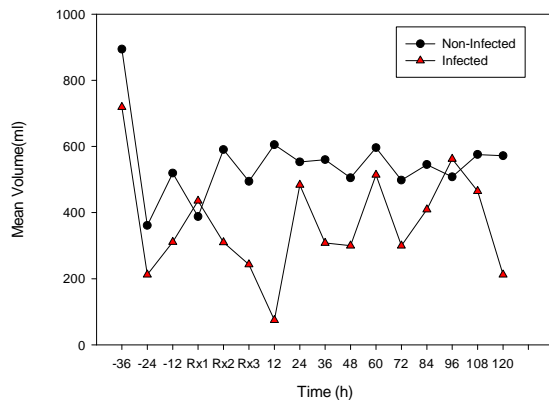
#### **4.7 Milk Production Volume: Graphs and Correlations between Volumes and Other Variables.**

##### **4.7.1 Curaclor LC from Trial 1 (low producers) Only**



**FIGURE 4.31: MEAN MILK VOLUME PRODUCTION OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP.**

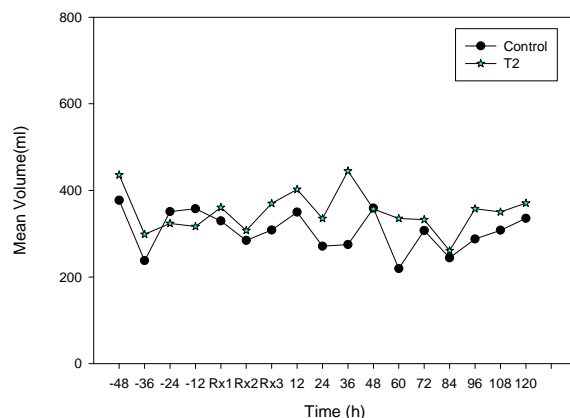
There were 8 goats (16 udder halves) in the treatment group and 6 goats (12 udder halves) in the control group of Trial 1. Mean milk volume for both treatment and control group at the start was over 800ml and then decreased to under 400ml by the second sampling. (These were low producers.) Mean milk volume of both treatment and control group remained unstable throughout the trial and did not correspond to the antibiotic treatment times.



**FIGURE 4.32: MEAN MILK PRODUCTION VOLUME OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES.**

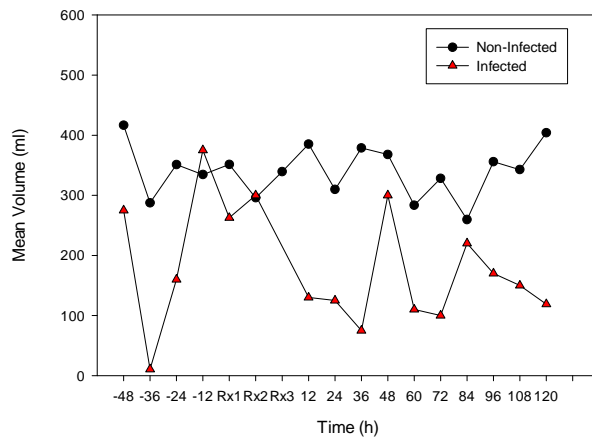
There were 15 goats (30 udder halves) used in Trial 1. Mean milk volume of infected and non-infected udder halves was high at the start, then decreased by the second milking, remained unstable throughout the trial and did not correspond to the antibiotic treatment times. Mean milk volumes of non-infected udder halves were higher at the start, than that of the infected and remained higher throughout the trial except at Rx1 and at 96h.

#### 4.7.2 Trial 2: Spectrazol (low producers)



**FIGURE 4.33: MEAN MILK VOLUME PRODUCTION OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP.**

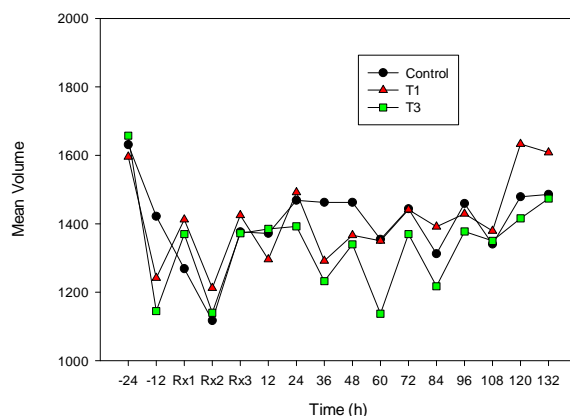
There were 7 goats (14 udder halves) in the treatment group and 7 goats (14 udder halves) in the control group of Trial 2. Mean milk volumes of treatment and control group were high at the start and then decreased at the second milking. (These were low producers.) Mean milk volume of the treatment group increased after treatment at Rx2 and reached a baseline at 84h and remained unstable for the remainder of the trial. Mean milk volume of the treatment group was higher than that of the control group except at two treatment times (-24h & -12h). Mean milk volume of the control group remained unstable throughout the trial and did not correspond to the antibiotic treatment times.



**FIGURE 4.34: MEAN MILK VOLUME PRODUCTION OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES.**

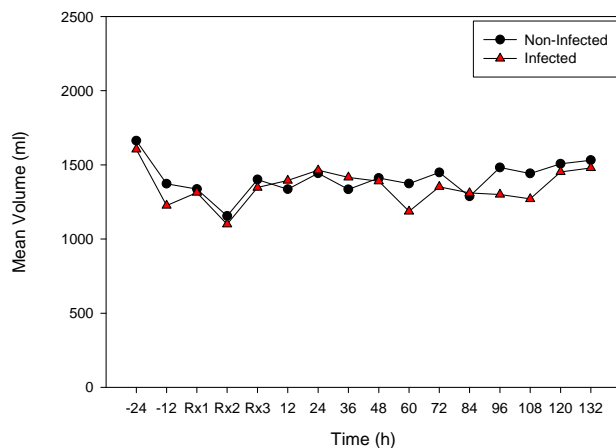
There were 14 goats (28 udder halves used in trial 2). Mean milk volume of non-infected udder halves was higher than that of infected udder halves except at -12h. Mean milk volume of non-infected udder halves remained unstable throughout the trial and did not correspond to the antibiotic treatment times. Mean milk volume of infected udder halves was high at the start, then decreased, then remained unstable throughout the trial and did not correspond to the antibiotic treatment times.

#### 4.7.3 Trial 3: Curaclox LC (T1) & Rilexine (T3) (mid & high producers)



**FIGURE 4.35: MEAN MILK VOLUME PRODUCTION OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP.**

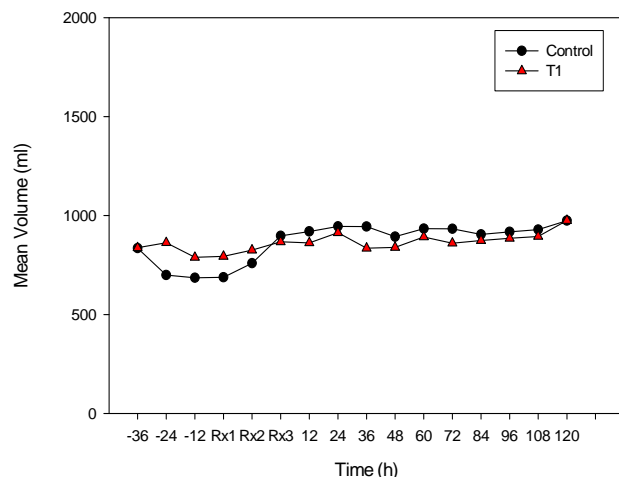
There were 12 goats (24 udder halves) treated with Curaclox LC (T1), 20 goats (40 udder halves) treated with Rilexine (T3) and 32 goats (64 udder halves) in the control group of Trial 3. Mean milk volume of goats in T1 (Curaclox LC), T3 (Rilexine) and the control group was high at the start at approximately 1600ml (mid & high producers). Mean milk volume of goats in T3 increased after treatment at Rx2, reached a baseline at 60h and then remained unstable for the remainder of the trial. Mean milk volume of goats in T1 and the control group remained unstable throughout the trial.



**FIGURE 4.36: MEAN MILK VOLUME PRODUCTION OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES.**

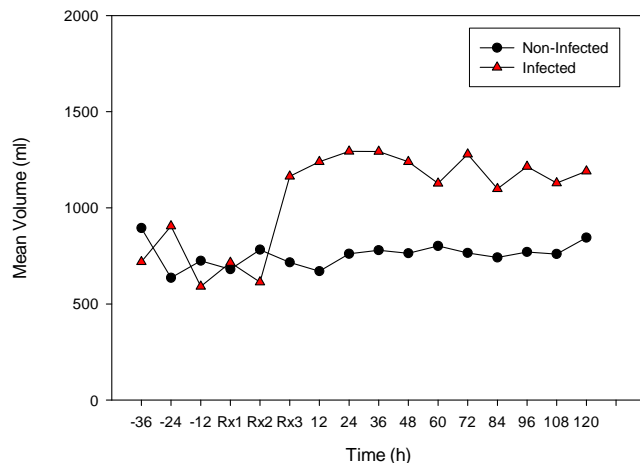
There were 64 goats (128 udder halves) in Trial 3. Mean milk volume of infected and non-infected udder halves was relatively high at the start but then decreased at the first treatment (Rx1), until a slight increase in volume was visible after Rx2. After this time mean milk volume of both infected and non-infected udder halves remained relatively steady.

#### 4.7.4 Curaclox LC from Trials 1 & 3 Combined (Trial 1, low producers; Trial 3, mid & high producers)



**FIGURE 4.37: MEAN MILK VOLUME PRODUCTION OF UDDER HALVES OF TREATMENT GROUP VERSUS CONTROL GROUP.**

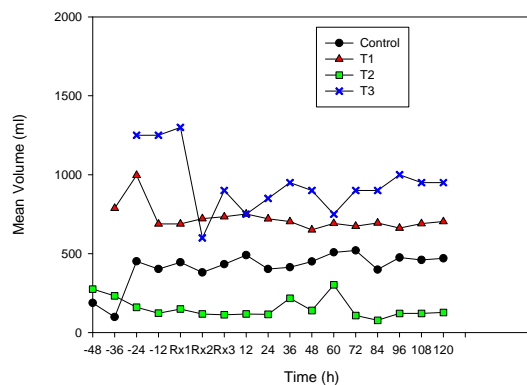
There were 20 goats (40 udder halves) treated with Curaclox LC and 18 goats (36 udder halves) in the control group of Trials 1 & 3 combined for Curaclox LC. Mean milk volume was the same for goats treated with Curaclox LC in Trial 1 & Trial 3 for both the treatment and control groups. (Trial 1: low producers & Trial 3: mid & high producers.) Mean milk volume of the control group then decreased sharply until Rx2 where it increased to a volume similar to that produced by the goats in the treatment group. Mean milk volume of the treatment group remained stable throughout the trial except for the sudden increase at the last sampling (132h). This was different from the pattern of treatment and control groups of Curaclox LC in Trials 1 & 3 analysed separately. (See Figures 4.31 & 4.35.)



**FIGURE 4.38: MEAN MILK VOLUME PRODUCTION OF INFECTED UDDER HALVES VERSUS NON-INFECTED UDDER HALVES.**

There were 38 goats (76 udder halves) in Trials 1 & 3 combined for Curaclox LC. Mean milk volume of non-infected udder halves varied but was similar to that of infected udder halves until the Rx2 treatment. The mean milk volume of non-infected udder halves was not co-ordinated with the antibiotic treatment times. Mean milk volume of infected udder halves increased after treatment at Rx2 and remained at a higher level for the remainder of the trial.

**4.7.5 All Data for Goats with Clinical Mastitis (T1=Curaclox LC, T2= Spectrazol Milking Cow, T3= Rilexine 200 LC) (Trial 1 & Trial 2: low producers, Herd C: low & mid producers, Trial 3: mid & high producers)**



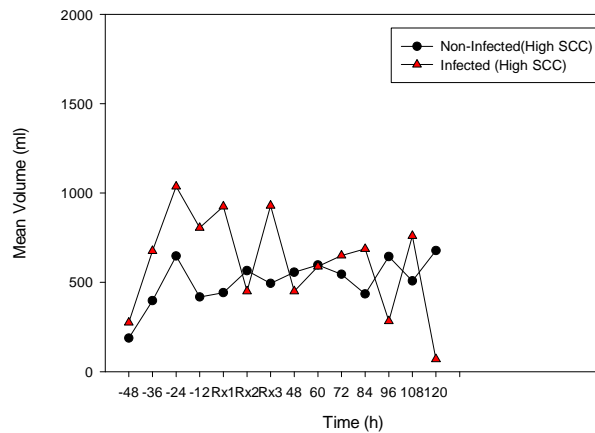
**FIGURE 4.39: MEAN MILK VOLUME PRODUCTION OF UDDER HALVES WITH CLINICAL MASTITIS OF TREATMENT GROUPS VERSUS UDDER HALVES WITH CLINICAL MASTITIS OF THE CONTROL GROUP.**

There were a total of 511 udder halves with clinical mastitis. The Control group, T1(Curaclox LC), T2 (Spectrazol) and T3 (Rilexine) had a sample size of 97, 308, 74 and 32 udder halves respectively.

Mean milk volume of goats treated with T3 (Rilexine) was the highest (Trial 3: high producers), although it started at a relatively high level, then decreased and remained relatively unstable throughout the trial and was not co-ordinated with the antibiotic treatment times. Mean milk volume of T1 (Curaclox LC) was second highest (Trial 1: low producers, Herd C: low & mid producers & Trial 3: mid & high producers), remained stable (see Figure 4.39) throughout the trial. Mean milk volume of T1 was not co-ordinated with the antibiotic treatment times in (Figure 4.39).

Mean milk volume of goats treated with T2 (Spectrazol) was the lowest (Trial 2: low producers & Herd C mid producers). Mean milk volume of T2 remained stable throughout the

trial and did not correspond to antibiotic treatment times. Mean milk volume of control group (Trial 1 & 2: low producers, Herd C: mid producers and Trial 3 high producers) was low at the start, then remained stable between treatment times –24h and 120h.



**FIGURE 4.40: MEAN MILK PRODUCTION VOLUME OF UDDER HALVES WITH CLINICAL MASTITIS WHERE BACTERIAL INFECTION WAS IDENTIFIED OR NOT.**

There were a total of 511 udder halves with clinical mastitis, of those bacteria was isolated in 134 udder halves and in the remaining 377 udder halves no bacteria was isolated.

Mean milk volume of non-infected and infected udder halves was low at the start and then increased and remained unstable throughout the trial. Mean volume of infected udder halves was higher than that of non-infected udder halves, except at treatment times Rx2, 48h, 96h, 120h and 180h. Mean volume of infected and non-infected animals was low from treatment time 144h to the end (204) because volumes at these times included only udder halves from Herd C: low & mid producers. Mean volumes of infected and non-infected udder halves did not correspond to the antibiotic treatment times.

#### 4.8 Tables of Statistical Linear Correlation Coefficients

**TABLE 4.53: CORRELATION MATRIX FOR TRIAL 1 (CURACLOX LC).**

|                        |   |        |        |        |        |       |
|------------------------|---|--------|--------|--------|--------|-------|
| Log SCC                | 1 | 1.000  |        |        |        |       |
| Volume                 | 2 | -0.614 | 1.000  |        |        |       |
| CMCT                   | 3 | 0.482  | -0.540 | 1.000  |        |       |
| Lactation Number       | 4 | 0.462  | 0.029  | -0.077 | 1.000  |       |
| Withdrawal Period TRIS | 5 | 0.378  | -0.701 | 0.557  | -0.229 | 1.000 |
|                        | 1 | 2      | 3      | 4      | 5      |       |

Degrees of freedom = 14

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as these were only weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationships were: the moderate negative correlation between Log SCC and volume ( $R^2 = -0.614$ ), the moderate negative correlation between volume and CMCT ( $R^2 = -0.540$ ). There was a moderate positive correlation between CMCT and withdrawal period according to TRIS ( $R^2 = 0.557$ ) and a weak positive correlation; between CMCT and Log SCC ( $R^2 = 0.482$ ), between lactation number and Log SCC ( $R^2 = 0.462$ ) and between withdrawal period as measured by TRIS and Log SCC ( $R^2 = 0.378$ ).

The correlation coefficient, also known as Pearson's coefficient of correlation or the product moment correlation coefficient, is a measure of the linear relationship between two random variates ( $-1 < r < 1$ ). Note that this only shows the extent to which two variates are linearly related and does not imply any causal relationship (Draper & Smith, 1981).

**TABLE 4.54: CORRELATION MATRIX FOR TRIAL 2 (SPECTRAZOL).**

|                        |   |        |        |        |        |       |
|------------------------|---|--------|--------|--------|--------|-------|
| Log SCC                | 1 | 1.000  |        |        |        |       |
| Volume                 | 2 | -0.120 | 1.000  |        |        |       |
| CMCT                   | 3 | -0.432 | -0.051 | 1.000  |        |       |
| Lactation Number       | 4 | -0.003 | -0.236 | -0.580 | 1.000  |       |
| Withdrawal Period TRIS | 5 | 0.181  | -0.493 | 0.323  | -0.218 | 1.000 |
|                        | 1 | 2      | 3      | 4      | 5      |       |

Degrees of freedom = 10

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as there were only weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationships were the moderate negative correlations between lactation number and CMCT ( $R^2 = -0.580$ ) and between withdrawal period according to TRIS and volume ( $R^2 = -0.493$ ). There was a weak negative correlation between Log SCC and CMCT ( $R^2 = -0.432$ ).

**TABLE 4.55: CORRELATION MATRIX FOR TRIAL 3 (CURACLOX LC & RILEXINE).**

|                        |   |        |       |       |       |       |
|------------------------|---|--------|-------|-------|-------|-------|
| Log SCC                | 1 | 1.000  |       |       |       |       |
| Volume                 | 2 | -0.120 | 1.000 |       |       |       |
| CMCT                   | 3 | 0.035  | 0.251 | 1.000 |       |       |
| Lactation Number       | 4 | -0.161 | 0.218 | 0.482 | 1.000 |       |
| Withdrawal Period TRIS | 5 | -0.100 | 0.006 | 0.085 | 0.236 | 1.000 |
|                        | 1 | 2      | 3     | 4     | 5     |       |

Degrees of freedom = 30

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as there were only weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationship, was the moderate positive correlation between lactation number and CMCT ( $R^2 = 0.482$ ).

**TABLE 4.56: CORRELATION MATRIX FOR CURACLOX LC IN TRIALS 1 & 3 COMBINED.**

|                   |   |        |        |        |        |        |        |        |       |
|-------------------|---|--------|--------|--------|--------|--------|--------|--------|-------|
| Log SCC           | 1 | 1.000  |        |        |        |        |        |        |       |
| Withdrawal Period | 2 | 0.459  | 1.000  |        |        |        |        |        |       |
| TRIS              |   |        |        |        |        |        |        |        |       |
| CMCT              | 3 | 0.026  | 0.082  | 1.000  |        |        |        |        |       |
| Lactation Number  | 4 | 0.467  | 0.621  | -0.058 | 1.000  |        |        |        |       |
| Lactation Stage   | 5 | -0.390 | -0.669 | -0.039 | -0.505 | 1.000  |        |        |       |
| Volume            | 6 | -0.347 | -0.070 | -0.043 | 0.146  | 0.267  | 1.000  |        |       |
| Floccules         | 7 | -0.004 | -0.159 | 0.245  | -0.107 | 0.086  | -0.106 | 1.000  |       |
| Udder Palpation   | 8 | -0.031 | 0.265  | -0.150 | 0.399  | -0.288 | 0.026  | -0.037 | 1.000 |
|                   |   | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8     |

Degrees of freedom = 38

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as there were only weak correlations between most of the variables. The correlations in the above matrix that showed any linear relationship, were: the moderate positive correlations between: lactation number and log SCC ( $R^2 = 0.467$ ); between withdrawal period as measured by TRIS and log SCC ( $R^2 = 0.459$ ); between lactation number and withdrawal period according to TRIS ( $R^2 = 0.621$ ). The moderate negative correlations were: between lactation stage and lactation number ( $R^2 = -0.505$ ); and between lactation stage and withdrawal period according to TRIS ( $R^2 = -0.669$ ).

There was a weak negative correlation between milk volume and log SCC ( $R^2 = -0.347$ ); between lactation stage and log SCC ( $R^2 = -0.390$ ); and between udder palpation score and lactation stage ( $R^2 = -0.288$ ). There was a weak positive correlation between volume and lactation stage ( $R^2 = 0.267$ ); between floccules in the milk and CMCT ( $R^2 = 0.245$ ); between udder palpation and lactation number ( $R^2 = 0.399$ ); and between udder palpation and withdrawal period as measured by TRIS ( $R^2 = 0.265$ ).

**TABLE 4.57: CORRELATION MATRIX FOR ALL DATA FROM GOATS WITH CLINICAL MASTITIS (CURACLOX LC, SPECTRAZOL & RILEXINE).**

|                  |   |        |        |        |
|------------------|---|--------|--------|--------|
| Log SCC          | 1 | 1.000  |        |        |
| Volume           | 2 | -0.283 | 1.000  |        |
| CMCT             | 3 | 0.408  | -0.002 | 1.000  |
| Lactation Number | 4 | 0.225  | -0.617 | -0.021 |
|                  |   | 1      | 2      | 3      |
|                  |   |        |        | 4      |

Degrees of freedom = 332 Without Withdrawal Period as measured by TRIS.

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as there were only weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationships were: the moderate negative correlation between lactation number and milk volume ( $R^2 = -0.617$ ); and the weak positive correlation between Log SCC and CMCT ( $R^2 = 0.408$ ).

**TABLE 4.58: CORRELATION MATRIX OF ALL DATA FROM GOATS WITH CLINICAL MASTITIS (CURACLOX LC, SPECTRAZOL & RILEXINE).**

|                        |   |        |        |       |        |
|------------------------|---|--------|--------|-------|--------|
| Withdrawal Period TRIS | 1 | 1.000  |        |       |        |
| Volume                 | 2 | -0.812 | 1.000  |       |        |
| Log SCC                | 3 | 0.839  | -0.833 | 1.000 |        |
| Lactation Number       | 4 | 0.167  | -0.438 | 0.228 | 1.000  |
| CMCT                   | 5 | 0.563  | -0.270 | 0.379 | -0.362 |
|                        |   | 1      | 2      | 3     | 4      |
|                        |   |        |        |       | 5      |

Degrees of freedom = 16 With Withdrawal Period as measured by to TRIS.

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship, as there were only weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationships; were the fairly



strong negative correlation between withdrawal period as measured by TRIS and milk volume ( $R^2 = -0.812$ ); the fairly strong positive correlation between withdrawal period as measured by TRIS and Log SCC ( $R^2 = 0.839$ ); the fairly strong negative correlation between Log SCC and volume ( $R^2 = -0.833$ ); and the positive correlation between withdrawal period as measured by TRIS and CMCT ( $R^2 = 0.563$ ). There were weak negative correlations between lactation number and volume ( $R^2 = -0.438$ ); and between withdrawal period as measured by TRIS and lactation number ( $R^2 = -0.362$ ). There was a weak positive correlation between withdrawal period as measured by TRIS and Log SCC ( $R^2 = 0.379$ ).

Table 4.57 shows a correlation matrix with 332 pairs of data excluding withdrawal period as measured by TRIS. Therefore the correlation coefficients of Table 4.57 are more significant than those of Table 4.58 with only 16 pairs of data.

**TABLE 4.59: CORRELATION MATRIX FOR ALL DATA COMBINED, TRIAL 1, TRIAL 2 & TRIAL 3 (CURACLOX LC, SPECTRAZOL & RILEXINE).**

| Log SCC                | 1 | 1.000  |        |       |       |       |
|------------------------|---|--------|--------|-------|-------|-------|
| Volume                 | 2 | -0.301 | 1.000  |       |       |       |
| CMCT                   | 3 | 0.144  | -0.220 | 1.000 |       |       |
| Lactation Number       | 4 | 0.240  | -0.373 | 0.240 | 1.000 |       |
| Withdrawal Period TRIS | 5 | 0.322  | -0.511 | 0.494 | 0.720 | 1.000 |
|                        | 1 | 2      | 3      | 4     | 5     |       |

Degrees of freedom = 78

The above table shows the linear correlation coefficients ( $R^2$ ), which indicated no significant linear relationship with weak correlations between most of the variables. The only correlations in the above matrix that showed any linear relationship was; the moderate positive correlation between withdrawal period as measured by TRIS and CMCT ( $R^2 = 0.494$ ); the moderate negative correlation between withdrawal period according to TRIS and volume ( $R^2 = -0.511$ ); and the fairly strong positive correlation between withdrawal period as measured by TRIS and lactation number ( $R^2 = 0.720$ ).

#### **4.9 Statistical Analysis of Butterfat, Protein and Lactose.**

##### **4.9.1 Statistical Analysis of Butterfat, Protein and Lactose of Curaclox LC (T1) from Trial 1 Only**

**TABLE 4.60: DIFFERENCES IN BUTTERFAT (%) BETWEEN TREATMENT (T1) AND CONTROL (C1) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Butterfat % at (-12h)         | Size (n) | Butterfat % at (12h)          | Size (n) | Butterfat % at (108h)         | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|----------------------------------|---|
|           | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |          | Mean $\pm$ Standard Deviation |          |                                  |   |
| T1        | 4.29 $\pm$ 1.52               | 18       | 4.16 $\pm$ 1.51               | 17       | 4.07 $\pm$ 1.41               | 17       | 0.984NS                          | 0.729 NS  |
| C1        | 4.56 $\pm$ 0.63               | 12       | 3.99 $\pm$ 0.79               | 12       | 3.99 $\pm$ 0.46               | 12       |                                  |   |

There was no significant difference of mean butterfat percentage between treatment (T1) and control (C1) groups ( $P = 0.984$ ) or between different treatment times ( $P = 0.729$ ).

**TABLE 4.61: DIFFERENCES IN PROTEIN (%) BETWEEN TREATMENT (T1) AND CONTROL (C1) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Protein % at (-12h)<br>Mean ± Standard Deviation | Size (n) | Protein % at (12h)<br>Mean ± Standard Deviation | Size (n) | Protein % at (108h)<br>Mean ± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|--|----------|---|----------|--|----------|----------------------------------|---|
| T1        | 3.35±0.59  | 18       | 3.21±0.44                                       | 17       | 3.17±0.40  | 17       | 0.992NS                          | 0.701 NS  |
| C1        | 3.24±0.54  | 12       | 3.33±0.27                                       | 12       | 3.18±0.38  | 12       |                                  |   |

There was no significant difference of mean protein percentage between treatment (T1) and control (C1) groups ( $P = 0.984$ ) or between different treatment times ( $P = 0.729$ ).

**TABLE 4.62: DIFFERENCES IN LACTOSE (%) BETWEEN TREATMENT (T1) AND CONTROL (C1) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Lactose % at (-12h)<br>Mean ± Standard Deviation | Size (n) | Lactose % at (12h)<br>Mean ± Standard Deviation | Size (n) | Lactose % at (108h)<br>Mean ± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|--|----------|---|----------|--|----------|----------------------------------|---|
| T1        | 4.32±0.94  | 18       | 4.32±0.41                                       | 17       | 4.52±0.27  | 17       | 0.460 NS                         | 0.583 NS  |
| C1        | 4.22±0.43  | 12       | 4.44±0.26                                       | 12       | 4.23±0.45  | 12       |                                  |   |

There was no significant difference of mean lactose percentage between treatment (T1) and control (C1) groups ( $P = 0.131$ ) or between different treatment times ( $P = 0.156$ ).

#### 4.9.2 Statistical Analysis of Butterfat, Protein and Lactose of Trial 2 (Spectrazol (T2))

**TABLE 4.63: DIFFERENCES IN BUTTERFAT (%) BETWEEN TREATMENT (T2) AND CONTROL (C2) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Butterfat % at (Rx1)<br>Mean ± Standard Deviation | Size (n) | Butterfat % at (12h)<br>Mean ± Standard Deviation | Size (n) | Butterfat % at (120h)<br>Mean ± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|---|----------|---|----------|--|----------|----------------------------------|---|
| T2        | 3.83±1.05   | 14       | 4.05±1.14   | 14       | 3.55±0.67  | 14       | 0.022*                           | 0.566 NS  |
| C2        | 3.48±0.62   | 14       | 3.39±0.53   | 14       | 3.28±0.78  | 14       |                                  |   |

There was a significant difference ( $P = 0.022$ ) of mean butterfat percentage between treatment (T2) and control group (C2). However this was a statistical difference, and probably not a meaningful biological difference in butterfat in practice. There was no significant difference ( $P = 0.566$ ) of mean butterfat between different treatment times.

**TABLE 4.64: DIFFERENCES IN PROTEIN (%) BETWEEN TREATMENT (T2) AND CONTROL (C2) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Protein % at (Rx1)<br>Mean ± Standard Deviation | Size (n) | Protein % at (12h)<br>Mean ± Standard Deviation | Size (n) | Protein % at (120h)<br>Mean ± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|---|----------|---|----------|--|----------|----------------------------------|---|
| T2        | 3.07±0.64                                       | 14       | 3.03±0.44                                       | 14       | 3.04±0.49  | 14       | 0.885 NS                         | 0.973 NS  |
| C2        | 3.11±0.41                                       | 14       | 3.09±0.37                                       | 14       | 3.00±0.45  | 14       |                                  |   |

There was no significant difference of mean protein percentage between treatment (T2) and control (C2) groups ( $P = 0.885$ ) or between different treatment times ( $P = 0.973$ ).

**TABLE 4.65: DIFFERENCES IN LACTOSE (%) BETWEEN TREATMENT (T2) AND CONTROL (C2) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treat-ment | Lactose % at (Rx1)<br>Mean $\pm$ Standard Deviation | Size (n) | Lactose % at (12h)<br>Mean $\pm$ Standard Deviation | Size (n) | Lactose % at (120h)<br>Mean $\pm$ Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|------------|---|----------|---|----------|--|----------|----------------------------------|---|
| T2         | 4.33 $\pm$ 0.71                                     | 14       | 4.18 $\pm$ 0.48                                     | 14       | 4.25 $\pm$ 0.43                                      | 14       | 0.426 NS                         | 0.780 NS  |
| C2         | 4.19 $\pm$ 0.64                                     | 14       | 4.25 $\pm$ 0.49                                     | 14       | 4.03 $\pm$ 0.6                                       | 14       |                                  |   |

There was no significant difference of mean lactose percentage between treatment (T2) and control (C2) groups ( $P = 0.426$ ) or between different treatment times ( $P = 0.780$ ).

#### 4.9.3 Statistical Analysis of Butterfat, Protein and Lactose of Trial 3 (Curaclox LC (T1) & Rilexine (T3))

**TABLE 4.66 DIFFERENCES IN BUTTERFAT (%) BETWEEN TREATMENT AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treat-ment | Butterfat % at (Rx1)<br>Mean $\pm$ Standard Deviation | Size (n) | Butterfat % at (12h)<br>Mean $\pm$ Standard Deviation | Size (n) | Butterfat % at (132h)<br>Mean $\pm$ Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|------------|---|----------|---|----------|--|----------|----------------------------------|---|
| C          | 3.53 $\pm$ 0.63                                       | 32       | 3.39 $\pm$ 0.61                                       | 15       | 3.72 $\pm$ 1.10  | 27       | 0.320 NS                         | 0.199 NS  |
| T1         | 3.19 $\pm$ 0.54                                       | 12       | 3.55 $\pm$ 0.31                                       | 12       | 3.48 $\pm$ 0.51  | 10       |                                  |   |
| T3         | 3.39 $\pm$ 0.60                                       | 20       | 3.85 $\pm$ 0.52                                       | 18       | 3.66 $\pm$ 0.58  | 19       |                                  |   |

There was no significant difference of mean butterfat percentage between treatments (T1), (T3) and control (C) groups ( $P = 0.320$ ) or between different treatment times ( $P = 0.199$ ).

**TABLE 4.67: DIFFERENCES IN PROTEIN (%) BETWEEN TREATMENT AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treat-ment | Protein % at (Rx1)<br>Mean $\pm$ Standard Deviation | Size (n) | Protein % at (12h)<br>Mean $\pm$ Standard Deviation | Size (n) | Protein % at (132h)<br>Mean $\pm$ Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|------------|---|----------|---|----------|--|----------|----------------------------------|---|
| C          | 3.13 $\pm$ 0.21                                     | 32       | 3.02 $\pm$ 0.22                                     | 15       | 3.09 $\pm$ 0.23                                      | 27       | 0.054*                           | <0.001***   |
| T1         | 3.24 $\pm$ 0.29                                     | 12       | 3.29 $\pm$ 0.23                                     | 12       | 3.15 $\pm$ 0.22                                      | 10       |                                  |   |
| T3         | 3.30 $\pm$ 0.25                                     | 20       | 2.77 $\pm$ 0.44                                     | 18       | 3.34 $\pm$ 0.98                                      | 19       |                                  |   |

There was a significant difference ( $P = 0.054$ ) of mean protein percentage between treatments (T1), (T3) and control (C) groups and a highly significant difference ( $P < 0.001$ ) between different treatment times.

**TABLE 4.68: DIFFERENCES IN LACTOSE (%) BETWEEN TREATMENT AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Lactose % at (Rx1)<br>Mean ± Standard Deviation | Size (n) | Lactose % at (12h)<br>Mean ± Standard Deviation | Size (n) | Lactose % at (120h)<br>Mean ± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|---|----------|---|----------|--|----------|----------------------------------|---|
| C         | 4.58±0.17                                       | 32       | 4.57±0.22                                       | 15       | 4.59±0.16  | 27       | 0.755 NS                         | 0.001***  |
| T1        | 4.69±0.22                                       | 12       | 4.42±0.20                                       | 12       | 4.60±0.28  | 10       |                                  |   |
| T3        | 4.69±0.16                                       | 20       | 4.48±0.18                                       | 18       | 4.61±0.19  | 19       |                                  |   |

There was no significant difference ( $P = 0.755$ ) of mean lactose percentage between treatments (T1), (T3) and control (C) groups. There is a highly significant difference ( $P = 0.001$ ) between different treatment times.

#### 4.9.4 Statistical Analysis of Butterfat, Protein and Lactose of Curaclox LC (T1) in Trials 1 & 3 Combined

**TABLE 4.69: DIFFERENCES IN BUTTERFAT (%) BETWEEN TREATMENT (T1) AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Mean Butterfat % (Before Treatment)<br>± Standard Deviation | Size (n) | Mean Butterfat % (During Treatment)<br>± Standard Deviation | Size (n) | Mean Butterfat % (After Treatment)<br>± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|---|----------|---|----------|--|----------|----------------------------------|---|
| C         | 3.81±0.78   | 44       | 3.66±0.75   | 27       | 3.80±0.95  | 39       | 0.359 NS                         | 0.963 NS  |
| T1        | 3.91±1.35   | 28       | 3.97±1.25   | 26       | 3.85±1.19  | 27       |                                  |   |

There was no significant difference of mean butterfat percentage between treatments (T1) and control (C) groups ( $P = 0.359$ ) or between different treatment times ( $P = 0.963$ ).

**TABLE 4.70: DIFFERENCES IN PROTEIN (%) FOR TREATMENT AND (T1) AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Mean Protein % (Before Treatment)<br>± Standard Deviation | Size (n) | Mean Protein % (During Treatment)<br>± Standard Deviation | Size (n) | Mean Protein % (After Treatment)<br>± Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|---|----------|---|----------|--|----------|----------------------------------|---|
| C         | 3.16±0.33   | 44       | 3.16±0.29   | 27       | 3.11±0.29  | 39       | 0.069 NS                         | 0.611 NS  |
| T1        | 3.31±0.50   | 28       | 3.25±0.38   | 26       | 3.16±0.34  | 27       |                                  |   |

There was no significant difference of mean protein percentage between treatments (T1) and control (C) groups ( $P = 0.069$ ) at the 5% level of significance or between different treatment times ( $P = 0.611$ ).

**TABLE 4.71: DIFFERENCES IN LACTOSE (%) BETWEEN TREATMENT (T1) AND CONTROL (C) GROUPS: ANALYSIS OF AN UNBALANCED DESIGN.**

| Treatment | Mean Lactose % (Before Treatment) $\pm$ Standard Deviation | Size (n) | Mean Lactose % (During Treatment) $\pm$ Standard Deviation | Size (n) | Mean Lactose % (After Treatment) $\pm$ Standard Deviation | Size (n) | F Probability between treatments | F Probability interaction between treatments at different times |
|-----------|--|----------|--|----------|---|----------|----------------------------------|---|
| C         | 4.48 $\pm$ 0.31  | 44       | 4.51 $\pm$ 0.24  | 27       | 4.48 $\pm$ 0.32   | 39       | 0.494 NS                         | 0.474 NS  |
| T1        | 4.45 $\pm$ 0.78  | 28       | 4.34 $\pm$ 0.34  | 26       | 4.55 $\pm$ 0.27   | 27       |                                  |   |

There was no significant difference of mean protein percentage between treatments (T1) and control (C) groups ( $P = 0.494$ ) or between different treatment times ( $P = 0.474$ ).

**4.10 Statistical Analysis of Withdrawal Periods for Goats Compared to Withdrawal Periods Recommended for Use in Cattle with or without the 24h Safety Margin**

**TABLE 4.72: WITHDRAWAL PERIODS (WP) OF INTRAMAMMARY ANTIBIOTICS FOR GOATS COMPARED TO WITHDRAWAL PERIODS (WP) RECOMMENDED FOR USE IN CATTLE WITH OR WITHOUT THE 24H SAFETY MARGIN (ONE SAMPLE T-TESTS).**

| Original test (Table Number)        | Withdrawal Periods Measured by Different Methods | F Probability (Original test) | F Probability Withdrawal Period Recommended for use in Cattle -24h |
|-------------------------------------|--|-------------------------------|--|
| Table 4.9<br>Trial 2;<br>Spectrazol | WP TRIS  | <0.001**8                     | <0.001***  |
| Table 4.11<br>Trial 3;Rilexine      | WP TRIS  | <0.001***                     | <0.001***  |
| Table 4.14<br>Trials 1 & 3;         | WP TRIS  | <0.0018**                     | 0.006**  |
| Curaclox LC                         | WP Colour Dye                                    | 0.160NS                       | <0.001***  |
|                                     | WP Cloxacillin Parallax                          | 0.002**                       | <0.001***  |
| Table 4.18<br>Trial 1;              | WP Ampicillin Parallax                           | 0.060NS                       | <0.001***  |
|                                     | WP TRIS  | 0.646NS                       | <0.001***  |
|                                     | WP Colour Dye                                    | <0.001***                     | <0.001***  |
| Curaclox LC                         | WP Cloxacillin Parallax                          | <0.001***                     | <0.001***  |
|                                     | WP Ampicillin Parallax                           | 0.003**                       | <0.001***  |
| Table 4.21<br>Trial 3;              | WP TRIS  | <0.001***                     | <0.001***  |
|                                     | WP Colour Dye                                    | <0.001***                     | <0.001***  |
|                                     | WP Cloxacillin Parallax                          | 0.272NS                       | <0.001***  |
| Curaclox LC                         | WP Ampicillin Parallax                           | 0.760NS                       | <0.001***  |

There were significant differences in all the one sample t-tests between withdrawal periods for goats compared to withdrawal periods recommended for use in cattle without the 24h (safety margin). Not all of these tests were significant when the comparison was with withdrawal periods as recommended for use in cattle.

## CHAPTER 5: DISCUSSION

### 5.1 Withdrawal Periods and Correlations with Other Variables

Withdrawal periods as recommended for use in cattle have a 24h safety margin added. The withdrawal periods determined within this research have not had this 24h safety margin added. A study on Alpine does showed that withdrawal period according to the Penzyme Test, the Delvotest P and the TRIS test were 72h for Penicillin G and 120h for Cephapirin (Zeng *et al.*, 1996).

The raw data used in the trials on cattle to determine the recommended withdrawal period for use in cattle were not available for the antibiotics used in this research. Therefore comparisons between maximum and minimum withdrawal periods as well as the range of withdrawal periods at the 95% confidence level could not be made for each product used in this research.

The results discussed below show that the Parallax test was the most sensitive for testing goat milk for antibiotic residues. However, the Parallax test was also much more expensive to run than the TRIS test. In cattle the TRIS test is routinely used for the detection of antibiotic residues. A negative TRIS test indicates that the cow milk is safe for human consumption i.e., it is an indication of the Maximum Residue Limits (MRLs) and safe tolerance levels for antibiotic residues in milk ( $\mu\text{g}/\text{kg}$ ) fixed by Codex Alimentarius, European Community (EC) and United States Food and Drug Administration (USFDA).

**TABLE 5.1: THE MAXIMUM RESIDUE LIMITS (MRLs) AND SAFE/TOLERANCE LEVELS FOR RESIDUES OF ANTI-MICROBIALS IN MILK ( $\mu\text{g}/\text{kg}$ ) FIXED BY CODEX ALIMENTARIUS, EUROPEAN COMMUNITY (EC) AND FOOD AND DRUG ADMINISTRATION (FDA) (1996) (Honkanen-Buzalski & Reybroeck, 1997).**

| Antibiotics    | MRL Codex | MRL EC | Safe/Tolerance FDA                    |
|----------------|-----------|--------|---------------------------------------|
| Ampicillin     | 4         | 4      | 10/10                                 |
| Cloxacillin    | 4         | 30     | 10/10                                 |
| Cephalosporins | 100       | 100*   | 50 <sup>(a)</sup> /100 <sup>(b)</sup> |
| Neomycin       | 500*      | 500*   | 150/150                               |

\* preliminary or compounds on agenda, (a) parent drug, (b) total of parent and metabolite

Further research is required on MRLs and safe/tolerance levels of antibiotic residues specifically for goat milk and the most reliable test for achieving these results needs to be determined.

Research is also required on antibiotic withdrawal periods after treatment during the dry period.

In this research it was practical to insert a whole tube of intramammary antibiotic into each udder half. However, this could lead to an increased antibiotic concentration in the udder, since the goat udder is smaller than a cow udder and goats produce a smaller volume of milk than cows. Further research is necessary to determine if the longer withdrawal period in goat milk is because of the higher antibiotic concentration and if it will be viable to design smaller tubes especially for intramammary preparations in goats. Research is also required to design intramammary syringes with thinner and shorter tips, to minimize teat end damage in goats.

#### **5.1.1 Withdrawal Periods: Trial 2; Spectrazol**

In Trial 2 the mean withdrawal period as measured by TRIS for Spectrazol was significantly ( $P < 0.001$ ) higher (95h) than that recommended for use in cattle (60h) (Table 4.9). Therefore the withdrawal period of Spectrazol approved for cattle was not applicable for goats.

There is no significant difference ( $P = 0.815$ ) between mean withdrawal period as measured by TRIS of left and right udder halves (Table 4.9). Almost all inter-half variance comparisons in this research were not significant.

The 95% confidence interval showed that if this trial were to be repeated on the same number of animals under the same conditions a mean withdrawal period between 86h and 104h would be expected.

The presence of bacteria did not affect the mean withdrawal period as measured by TRIS (Table 4.8).

The difference in withdrawal period as measured by TRIS between udder halves with and without clinical mastitis was not significant ( $P = 0.087$ ) at the 5% level. However, further research with a larger number of goats, is necessary to confirm this, as other aspects showed that there was an influence. Due to the small numbers of clinical udder halves, the mean was unreliable (Table 4.7).

### **5.1.2 Withdrawal Periods: Trial 3; Rilexine**

In Trial 3 the mean withdrawal period as measured by TRIS for Rilexine was significantly ( $P = 0.034$ ) lower (38h) than that recommended for use in cattle (96h), (Table 4.10). Therefore, the withdrawal period of Rilexine approved for cattle was acceptable for goats because goats have a shorter withdrawal period for Rilexine than cows. However, this would result in unnecessary discarding of milk.

There was no significant difference ( $P = 0.135$ ) of withdrawal period as measured by TRIS between left and right udder halves.

The 95% confidence interval was between 33h & 40h.

The presence of bacteria did affect the withdrawal period. Infected udder halves had a significantly higher withdrawal period as measured by TRIS than non-infected udder halves. There were not enough data of udder halves with clinical mastitis treated with Rilexine to perform valid statistical analysis.

### **5.1.3 Withdrawal Periods: Curaclox LC from Trials 1 & 3 Combined**

The withdrawal period as measured by TRIS was significantly ( $P < 0.001$ ) lower than that recommended for use in cattle. (72h). The difference in withdrawal periods as measured by TRIS between left and right udder halves (inter-half variance) was not significant at the 5% level. However, further research with larger numbers is necessary to confirm this. The 95% confidence interval was between 51h & 66h.

There was a moderate positive correlation ( $R^2 = 0.459$ ) between withdrawal period as measured by TRIS and log SCC, and between withdrawal period as measured by TRIS and lactation number ( $R^2 = 0.621$ ) for Curaclox LC in Trials 1 & 3 (Table 4.56). Therefore log SCC and lactation number increased as withdrawal period as measured by TRIS increased. There was a strong negative ( $R^2 = -0.669$ ) correlation between withdrawal period as measured by TRIS and lactation stage (Table 4.56). Therefore, withdrawal period as measured by TRIS decreased with each progressive increasing stage of lactation. However, as withdrawal period as measured by TRIS decreased so did log SCC; thus log SCC decreased with each progressively increasing stage of lactation (Table 4.56).

The withdrawal period as measured by the Parallax testing for cloxacillin residues was significantly ( $P = 0.002$ ) higher at the 5% level than that recommended for use in cattle (72h).

The withdrawal period as measured by the Parallax testing for ampicillin residues was not significantly ( $P = 0.06$ ) different at the 5% level from that recommended for use in cattle (72h). However, this high F probability value could have been due to few samples tested because of the high expense of the test. Therefore, further research is necessary with a greater number of samples. The 95% confidence interval for the withdrawal period as measured by the Parallax testing for cloxacillin, was between 78h & 96h. The 95% confidence interval as measured by the Parallax testing for ampicillin, was between 72h & 89h.

Therefore, according to the Parallax test cloxacillin residues remained longer in the milk than ampicillin residue (Table 4.14).

The withdrawal period as measured by TRIS was significantly shorter than that recommended for use in cattle, thus proving that withdrawal times approved for use in cattle for Curaclox LC applied for goats. However, the withdrawal period as measured by the Parallax test showed that withdrawal times approved for use in cattle for Curaclox LC, did not apply for

goats. This showed that the Parallax test was more sensitive than the TRIS test (Table 4.14). There was a highly significant ( $P < 0.001$ ) difference, between withdrawal period as measured by TRIS and withdrawal period as measured by the Parallax testing for both ampicillin and cloxacillin residues separately (Table 4.15).

The standard acceptable test used for the testing of antibiotic residues in milk is the TRIS test. Therefore, perhaps further research is necessary on more sensitive tests like for example the Parallax, or High Performance Liquid Chromatography (HPLC).

The 95% confidence for the withdrawal period as measured by colour dye, was between 70h & 81h.

There was no significant difference ( $P = 0.160$ ) between the withdrawal period as measured by the blue dye in the milk and the withdrawal period recommended for use in cattle. Therefore, the withdrawal period as measured by the blue dye in the milk showed that the withdrawal times approved for use in cattle for Curaclox LC, did apply for goats, when using TRIS as a measure of antibiotic residues in milk. However, the withdrawal times approved for use in cattle for Curaclox LC, did not apply for goats, when using the Parallax as a measure of antibiotic residues in milk. There was a highly significant ( $P < 0.001$ ) difference between the withdrawal period according to TRIS and the withdrawal period as measured by the colour dye, at the 1% level (Table 4.15). There was also no significant difference ( $P = 0.605$ ) between withdrawal period as measured by colour dye between left and right udder halves (Table 4.14). Therefore for the combined data of Curaclox LC in Trials 1 & 3, when the colour dye had been excreted there were still antibiotic residues present in the milk according to the Parallax test. Thus the excretion of the colour dye was not a good indicator of the absence of antibiotic residues in the milk according to the Parallax test. Although, it was an acceptable indicator of antibiotic residues in the milk as measured by the TRIS test, which, is widely used, more sensitive antibiotic residue tests like the Parallax and HPLC are not widely used because they are so costly. The colour dye did not substantiate the hypothesis that the withdrawal period of goats was longer than that of cows for the combined data of Curaclox in Trials 1 & 3, although it did substantiate the hypothesis for the Curaclox LC data in Trial 1. This could have been due to the fact that Trial 3 was carried out with moderate to high yielding goats, while Trial 1 was carried out with only low producing animals. Therefore, the residues were “washed out” more quickly in the higher producers than in the lower producers due to the dilution factor.

The presence of bacteria did not significantly affect the any of the withdrawal periods (as measured by TRIS, as measured by the Parallax testing for Cloxacillin and Ampicillin and as measured by the colour dye) (Table 4.13).

There was no significant difference in withdrawal periods (as measured by TRIS and as measured by the Parallax testing for Cloxacillin and Ampicillin) between udder halves with and without clinical mastitis. There was no significant difference in withdrawal period as measured by the colour dye between udder halves with and without clinical mastitis, at the 5% level. However, further research with greater numbers of goats is necessary to confirm this.

#### **5.1.4 Withdrawal Periods: Curaclox LC from Trial 1 Only**

In Trial 1 the withdrawal period as measured by TRIS was not significantly different from that recommended for use in cattle (72h) (Table 4.18). The withdrawal period as measured by TRIS was longer for Curaclox LC in Trial 1 (74h) than for the combined data of Curaclox LC from Trials 1 & 3 (59h). The 95% confidence interval of the withdrawal period according to TRIS was between 64h & 85h. The withdrawal period of Curaclox LC in Trial 1 as measured by TRIS showed that the withdrawal time approved for cattle was applicable for goats. Therefore, although the withdrawal periods for Curaclox LC differed between Trial 1 and the combined data from Trial 1 & 3, they both did not significantly exceed the withdrawal period recommended for use in cattle. However, a safety margin of 24h has been added to the withdrawal periods, recommended for use in cattle. If a safety margin were added to the withdrawal period as measured by TRIS for Curaclox in Trial 1, then the withdrawal period approved for cattle would no longer be applicable for goats.



The withdrawal periods as measured by colour dye (90h) and as measured by the Parallax testing for cloxacillin (99h) and ampicillin (93h) were significantly higher than that recommended for use in cattle (72h) (Table 4.18). Therefore, the withdrawal time approved for use in cattle was not applicable for use in goats according to withdrawal periods measured by the colour dye and the Parallax testing for both cloxacillin and ampicillin residues. The 95% confidence interval of the withdrawal period as measured by the Parallax testing for cloxacillin was between 91h & 108h. The 95% confidence interval of the withdrawal period as measured by the Parallax testing for ampicillin, was between 82h & 103h.

The withdrawal period as measured by TRIS was lower than the withdrawal periods as measured by the colour dye and the Parallax testing for both cloxacillin and ampicillin residues. This showed again that the TRIS test was not as sensitive as the colour dye indicator and the Parallax test. It took longer to excrete the blue dye than it did to excrete the antibiotic residues. Therefore, the colour dye indicator was too sensitive.

The colour dye excretion of Curaclox LC in Trial 1 was an indicator of the antibiotic residues in the milk as measured by the Parallax test, although the colour dye excretion also substantiated the hypothesis that goats have a longer antibiotic withdrawal period than cows. The 95% confidence interval of the withdrawal period as measured by colour dye was between 81h & 99h.

The presence of bacteria did not affect the withdrawal periods as measured by TRIS and colour dye (Table 4.17). There were too few samples to test if the presence of bacteria affected the withdrawal period as measured by the Parallax testing for both cloxacillin and ampicillin. There were only two non-infected udder halves, but at least five samples are necessary for a meaningful two-sample t-test.

The withdrawal period for udder halves without clinical mastitis (81h), as measured by TRIS was significantly ( $P = 0.009$ ) longer than that for udder halves with clinical mastitis (54h) (Table 4.16). However, the withdrawal period as measured by the colour dye was significantly ( $P = 0.036$ ) shorter for goats without clinical mastitis (85h) than for goats with clinical mastitis (105h). It was shown that clinical mastitis has an affect on the withdrawal period (Table 4.16). However there were only four udder halves with clinical mastitis, but a two-sample t-test strictly needs a minimum of five samples. Therefore the mean withdrawal period of the udder halves without clinical mastitis was more reliable than that of the udder halves with clinical mastitis. The shorter withdrawal period for udder halves with clinical mastitis as measured by TRIS could have been due to the small sample number of udder halves with clinical mastitis.

Udder halves with clinical mastitis had high SCC, floccules in the milk and bacteria present, with or without udder damage. Udder halves with intramammary infection (IMI) were defined as those that had bacteria present.

A study on Alpine does showed that withdrawal period as measured by the Penzyme test, the Delvotest P and the TRIS test were 72h for Penicillin G and 120h for Cephapirin (Zeng *et al.*, 1996).

### 5.1.5 Withdrawal periods: Curaclox LC from Trial 3 Only

There was a highly significant difference at the 1% level between withdrawal periods as measured by TRIS and colour dye and withdrawal period recommended for use in cattle (72h) (Table 4.21). The 95% confidence of the withdrawal period as measured by TRIS was between 39h & 45h. The withdrawal period as measured by TRIS and colour dye was significantly shorter than that recommended for use in cattle, although the 24h safety margin added to the withdrawal period approved for cattle has not been added to the withdrawal period as measured by TRIS and colour dye in goats.

The 95% confidence interval of the withdrawal period as measured by the Parallax testing for cloxacillin, was between 67h & 88h. The 95% confidence interval of the withdrawal period as measured by the Parallax testing for ampicillin, was between 61h & 80h. The 95% confidence interval of the withdrawal period as measured by colour dye, was between 61h & 68h.

There was no significant difference between the withdrawal periods as measured by the Parallax testing for cloxacillin and ampicillin residues and the withdrawal period recommended for use in cattle (72h) (Table 4.21).

Therefore, the withdrawal time approved for use in cattle (TRIS) was applicable for use in goats according to withdrawal periods measured by the colour dye and the Parallax testing for both cloxacillin and ampicillin residues. The withdrawal period as measured by TRIS was lower than the withdrawal periods as measured by the colour dye and the Parallax testing for both cloxacillin and ampicillin residues. This showed again that the TRIS test was not as sensitive as the colour dye indicator and the Parallax test.

The colour dye excretion of Curaclox LC in Trial 3, was an indicator of the antibiotic residues in the milk as measured by the TRIS test but not as measured by the Parallax test, although the colour dye excretion did not substantiate the hypothesis that goats have a longer antibiotic withdrawal period than cows for Curaclox LC in Trial 3, (Table 4.21) unlike the results for Curaclox LC in Trial 1 and in Trials 1 & 3 (Table 4.14).

The goats in Trial 3 were mainly high producing animals, with a few mid producers, whereas the goats in Trial 1 were all low producers. Thus the shorter withdrawal periods in the combined data of Trials 1 & 3 and the even shorter withdrawal periods in Trial 3 could be due to a “washing out” effect, because of a higher dilution factor than that of the low producing animals of Trial 1.

The presence of bacteria did not affect the withdrawal periods (as measured by colour dye and as measured by the Parallax testing for cloxacillin and ampicillin residues). However, the presence of bacteria did affect the withdrawal period of Curaclox LC for Trial 3 as measured by TRIS (Table 4.20). In Trial 3 the mean withdrawal period as measured by TRIS for infected animals (40h) was significantly lower ( $P = 0.024$ ) than that for non-infected (47h) animals. This was not the case for Trial 1 (Table 4.17) and for the combined Curaclox LC data of Trial 1 & 3 (Table 4.13).

There were no significant differences between withdrawal periods (as measured by TRIS, colour dye and Parallax testing for ampicillin residues) for udder halves with and without clinical mastitis, of Trial 3. However, there was a significant difference between withdrawal periods as measured by TRIS and colour dye for udder halves with and without clinical mastitis, of Trial 1 (Table 4.16). However, for the combined Curaclox LC data of Trials 1 & 3 there was no significant difference in withdrawal periods (as measured by TRIS, colour dye and Parallax testing for cloxacillin and ampicillin) of udder halves with and without clinical mastitis (Table 4.12). There was a significant ( $P = 0.046$ ) difference at the 5% level between withdrawal periods as measured by the Parallax testing for cloxacillin of udder halves with and without clinical mastitis for Trial 3. This could have been due to the small sample number of only four udder halves tested by the Parallax for cloxacillin and ampicillin residues, although a two-sample t-test strictly required a minimum of five samples for a reliable result. Only a few samples were tested using the Parallax due to the high cost involved. Therefore, the mean withdrawal periods as measured by TRIS and colour dye were more reliable than that of the Parallax testing for cloxacillin and ampicillin (Table 4.19). In Trial 3 the mean withdrawal period as measured by the Parallax testing for cloxacillin (70h) for udder halves with clinical mastitis and was shorter than that for udder halves without clinical mastitis (87h) (Table 4.19).

#### **5.1.6 Withdrawal Periods: Trial 3; Curaclox LC & Rilexine**

There was no significant difference ( $P = 0.07$ ) between withdrawal period as measured by TRIS between left and right udder halves at the 5% level (Table 4.22). However, further research with a greater number of goats is necessary to confirm this. There was no significant ( $P = 0.586$ ) difference between withdrawal period as measured by colour dye between left and right udder halves (Table 4.22).

The presence of bacteria did not affect the withdrawal period as measured by TRIS or by colour dye at the 5% level (Table 4.23). However, further research with a greater number of goats is necessary to confirm this.

### **5.1.7 Withdrawal Periods: All Clinical Data Combined; Trial 1 (Curaclox LC), Trial 2 (Spectrazol), Trial 3 (Curaclox LC & Rilexine) and Herd C (Curaclox LC & Spectrazol).**

There was no significant difference between withdrawal periods as measured by TRIS and colour dye between left and right udder halves infected with clinical mastitis (Table 4.24). The presence of bacteria did not significantly affect the withdrawal periods of goats with clinical mastitis, as measured by TRIS and colour dye between infected and non-infected udder halves (Table 4.25).

There was a moderate positive correlation ( $R^2 = 0.563$ ) between withdrawal period as measured by TRIS and log SCC (Table 4.58). Therefore, as withdrawal period increased, log SCC increased. There was a weak negative correlation ( $R^2 = -0.362$ ) between withdrawal period as measured by TRIS and lactation number (Table 4.58).

The withdrawal period as measured by TRIS was excluded, in the analysis shown in Table 4.57, and there were 332 degrees of freedom compared with 16 degrees of freedom when the withdrawal period as measured by TRIS was included (Table 4.58). Therefore the information shown in (Table 4.57) was more reliable than the information shown in (Table 4.58), but there were no correlations with withdrawal period as measured by TRIS in Table 4.57.

### **5.1.8 Graphs of Mean TRIS Results**

All the graphs of TRIS versus time showed that the TRIS test result became positive after the start of treatment at Rx2. The withdrawal periods indicated in these graphs (Figures 4.1, 4.2, 4.3, 4.4 & 4.5) were in agreement with the statistical data shown in the tables (Tables 4.7, 4.11, 4.14, 4.18 & 4.21).

Withdrawal periods were longer for milk from udder halves of goats with clinical mastitis (Figure 4.4), compared to those without clinical mastitis for Spectrazol and Rilexine (Figures 4.2 & 4.3). However, the withdrawal periods as measured by TRIS for Curaclox LC were shorter for udder halves with clinical mastitis (Figure 4.4) than for udder halves without clinical mastitis (Figures 4.1, 4.3 & 4.5). The results obtained above for Curaclox LC, were contrary to those obtained for Spectrazol and Rilexine. It is difficult to explain this apparent contradiction.

The above results were illustrated by the regression model, which was formulated using the data from all goats with clinical mastitis. (See section 4.3.1). This model indicated that udder damage determined by palpation was a factor that increased withdrawal time. It also showed that the presence of floccules was associated with a reduction in withdrawal time. Perhaps there were effects of the degree of udder damage in goats in the different trials and the appearance of floccules in the milk. However, it was not possible to test these factors statistically because of the low numbers of goats with clinical mastitis. Goats were classified as having clinical mastitis if there were floccules in the milk and high SCC, with or without udder damage.

### **5.2 Regression Analysis of All Data from Goats with Clinical Mastitis**

There was very little previous research on withdrawal periods of goats with clinical mastitis. The regression model of (Table 4.26) was valid because the final  $R^2$  was 95.7%. (The closer the final  $R^2$  value to 100%, the better the model of regression.)

According to the linear model of regression, the withdrawal period as measured by TRIS increased at the evening milking by 4.7h compared to the measurements from the morning milking. Although this was the apparent trend shown by the linear model, it was not tested statistically because of the small numbers of goats with clinical mastitis. This was probably due to the extra stress placed on the goats from the change of regular milking times to 12hourly intervals and milking after sunset.

When udder damage was present as indicated by udder palpation the withdrawal period increased by 22h. This increase of withdrawal period was due to the presence of chronic udder damage. A possible explanation could be that, the antibiotic residues took longer to be excreted from udders with atrophy or fibrosis, due to the anatomical changes in the damaged udder.

The presence of floccules in the milk indicated clinical mastitis in goats. The linear model of regression (Table 4.26) showed that the presence of floccules in the milk was associated with a decrease in the withdrawal time of 13.6h. This was not tested statistically because of the small numbers of goats with clinical mastitis.

The regression model (Table 4.26) indicated that increasing volume of milk was not significantly associated with withdrawal period.

### **5.3 Somatic Cell Counts (SCC) and Correlations with Other Variables**

In this research all SCC were done using the Fossomatic, which was the most reliable and practical method.

Zeng & Escobar (1996) showed that there was no significant effect of breed or milking method on SCC.

According to Timms & Schultz, (1985), there was a moderate positive linear correlation ( $R^2 = 0.54$ ) between SCC and N-Acetyl-B-D-glucosaminidase activity (NAGase). Therefore further research is necessary to compare SCC and NAGase sensitivity for the diagnosis of intramammary infection and mastitis.

In a study done on sheep, the storage method had a significant effect on the SCC variation.

The average fresh, refrigerated and frozen sample counts were 125 000, 110 000 and 82 000 cells/mL for foremilk and 201 000, 192 000 and 145 000 cells/mL for strippings respectively, measured by the Fossomatic (Gonzalo *et al.*, 1993). In cattle foremilk samples are mostly used for mastitis diagnosis and frozen milk samples can be used for reliable microbiological tests but not for SCC (Sandholm *et al.*, 1995). In this research fresh foremilk samples were used, with the exception of the samples used for the Parallax test, which were frozen. Further research is required to determine the effects of storage methods on SCC for goat milk. Research is also necessary to determine which type of milk sample is most effective for determining accurate SCC and withdrawal periods: foremilk, composite samples or strippings (last milk).

In this study most of the bacteria present were coagulase negative staphylococci. This was in agreement with previous research by (Dulin *et al.*, 1982; Lerondelle & Poutrel, 1984; Pettersen, 1981; Sheldrake *et al.*, 1981;). In this research a few udder halves were infected with *Staphylococcus aureus* and one goat with *Klebsiella*. Therefore in this research there were not sufficient numbers of different types of bacteria present to assess SCC of goats with different bacteria. Mean SCC was only assessed between infected and non-infected goats. In another study commercial goat dairy herds 8.6 % of the producers had  $SCC < 750 \times 10^3$  cells/mL and 34.5 % were  $< 1000 \times 10^3$  cells/mL, and higher SCC were observed in goat milk than in cow milk (Droke *et al.*, 1993). In a study by Zeng & Escobar (1996) the mean SCC was  $930 \times 10^3$  cells/mL, but during the entire lactation period, 51 % of the milk samples had a SCC of above  $1000 \times 10^3$  cells/mL.

**TABLE 5.2: COMPARISON OF SCC (FOSSOMATIC) FOR INFECTED AND NON-INFECTED GOATS IN DIFFERENT STUDIES.**

| Author                                      | Breed                             | SCC of Non-infected Goats          | SCC of Infected Goats               |                                     |
|---|-----------------------------------|------------------------------------|-------------------------------------|-------------------------------------|
|   |                                   |                                    | Major Pathogens                     | Minor Pathogens                     |
| Lerondelle <i>et al.</i> , 1992             | Alpine                            | 520 X 10 <sup>3</sup> <sup>a</sup> | 7890 X 10 <sup>3</sup> <sup>a</sup> | 1040 X 10 <sup>3</sup> <sup>a</sup> |
| Lerondelle & Poutrel 1984                   |                                   | 614 X 10 <sup>3</sup> <sup>a</sup> | 4804 X 10 <sup>3</sup> <sup>a</sup> | 1293 X 10 <sup>3</sup> <sup>a</sup> |
| Luengo <i>et al.</i> , 2004                 | Murciano-Granadina                | 645 X 10 <sup>3</sup> <sup>b</sup> | 4073 X 10 <sup>3</sup> <sup>b</sup> | 1023 X 10 <sup>3</sup> <sup>b</sup> |
| Poutrel <i>et al.</i> , 1997                | Alpine, Saanen & Crossbreed goats | 687 X 10 <sup>3</sup> <sup>a</sup> | 4213 X 10 <sup>3</sup> <sup>a</sup> | 1462 X 10 <sup>3</sup> <sup>a</sup> |
| Kalogridou-Vassiliadou <i>et al.</i> , 1992 |                                   | 270 X 10 <sup>3</sup> <sup>b</sup> |                                     |                                     |
| De Cremoux <i>et al.</i> , 1996             |                                   | 272 X 10 <sup>3</sup> <sup>b</sup> |                                     |                                     |
| Contreras <i>et al.</i> , 1996              |                                   | 396 X 10 <sup>3</sup> <sup>b</sup> |                                     |                                     |
| Zeng & Escobar 1996                         |                                   | 930 X 10 <sup>3</sup> <sup>a</sup> |                                     |                                     |

<sup>a</sup> arithmetic mean; <sup>b</sup> geometric mean

**TABLE 5.3: COMPARISON OF SCC (FOSSOMATIC) FOR INFECTED AND NON-INFECTED GOATS IN THIS STUDY.**

| Trials                             | Breed                                  | SCC of Non-infected Goats            | SCC of Infected Goats               |
|------------------------------------|--|--------------------------------------|-------------------------------------|
| 07:00 Trial 1                      | Saanen                                 | 3639 X 10 <sup>3</sup> <sup>a</sup>  | 9274 X 10 <sup>3</sup> <sup>a</sup> |
| 19:00 Trial 1                      |  | 2288 X 10 <sup>3</sup> <sup>a</sup>  | 6415 X 10 <sup>3</sup> <sup>a</sup> |
| 07:00 Trial 2                      | Saanen                                 | 3279 X 10 <sup>3</sup> <sup>a</sup>  | 2845 X 10 <sup>3</sup> <sup>a</sup> |
| 19:00 Trial2                       |  | 3304 X 10 <sup>3</sup> <sup>a</sup>  | 5456 X 10 <sup>3</sup> <sup>a</sup> |
| 06:00 Trial3                       | Saanen & Saanen-Toggenburg Crossbreeds | 1817 X 10 <sup>3</sup> <sup>a</sup>  | 1928 X 10 <sup>3</sup> <sup>a</sup> |
| 18:00 Trial3                       |  | 2103 X 10 <sup>3</sup> <sup>a</sup>  | 1927 X 10 <sup>3</sup> <sup>a</sup> |
| 06:00 Curaclox LC (Trials 1 & 3)   | Saanen & Saanen-Toggenburg Crossbreeds | 2300 X 10 <sup>3</sup> <sup>a</sup>  | 2870 X 10 <sup>3</sup> <sup>a</sup> |
| 18:00 Curaclox LC (Trials 1 & 3)   |  | 2103 X 10 <sup>3</sup> <sup>a</sup>  | 1927 X 10 <sup>3</sup> <sup>a</sup> |
| 06:00 Goats with clinical mastitis | Saanen & Saanen-Toggenburg Crossbreeds | *7053 X 10 <sup>3</sup> <sup>a</sup> | 8479 X 10 <sup>3</sup> <sup>a</sup> |
| 18:00 Goats with clinical mastitis |  | *7948 X 10 <sup>3</sup> <sup>a</sup> | 6476 X 10 <sup>3</sup> <sup>a</sup> |

<sup>a</sup> arithmetic mean; <sup>b</sup> geometric mean, \* micro organisms not isolated

The result obtained by Luengo *et al.* (2004) for non-infected udder halves of goats, was considerably higher than that obtained for other dairy ruminants such as those reported by Gonzalo *et al.* (2002) for sheep (77 X 10<sup>3</sup> cells/mL) and Schepers *et al.* (1997) for cattle (14 X 10<sup>3</sup> cells/mL). This result confirmed the report of Paape *et al.* (2001) who showed that SCC of goat milk from goats without mastitis (ranged from 270 to 2000 X 10<sup>3</sup> cells/ml). This illustrated that it was impossible to use SCC as a diagnostic tool for mastitis in goats.

### 5.3.1 SCC: Curaclox LC from Trial 1 Only

SCC had to be transformed for the statistical tests to be valid ( $\log_{10}$ ).

In Trial 1 (Curaclox LC), there was a highly significant difference ( $P < 0.001$ ) between mean log SCC of the treatment (T1) and control (C1) groups at both treatment times (Table 4.27). This showed that treatment with Curaclox LC significantly increased the log SCC. This was illustrated by the data shown in (Figure 4.6), which showed an increase of mean SCC of the treatment group after treatment at Rx1. The increase in SCC indicated a degree of tissue irritation in the udder caused by infusion of the intramammary antibiotic (Curaclox LC). The presence of bacteria did not significantly affect the mean log SCC at both treatment times (Table 4.28). Mean SCC of non-infected udder halves remained stable throughout the trial, and was not affected by treatment (Figure 4.7). Mean SCC of infected udder halves remained unstable throughout the trial, although it did increase after the second treatment at Rx3 (Figure 4.7). This substantiated the fact that the mean SCC was not affected by the presence of bacteria. This is in agreement with another study on goats, where the use of SCC for predicting intramammary infection was found to be difficult (Lerondelle *et al.*, 1992). However, this does not agree with work done in cows where infected cows show a higher SCC than non-infected cows (Sandholm *et al.*, 1995).

Both mean SCC and mean log SCC were significantly different between early and late lactation for treatment times 07:00 ( $P = 0.035$ ) and 19:00 ( $P = 0.015$ ), (Table 4.29). This was not consistent with results in other studies, in which SCC increased with increasing stage of lactation (De Cremoux *et al.*, 1996; Paape *et al.*, 2001; Poutrel *et al.*, 1997; Wilson *et al.*, 1995; Zeng & Escobar, 1995; Zeng & Escobar 1996). A study by Luengo *et al.* (2004) on Murciano-Granadina goats also showed that SCC increased with increasing lactation stage for goats with a lactation of 150days. Goats with a shorter lactation (<150days) had a significantly ( $P < 0.05$ ) higher SCC than goats with a longer lactation (>250days) (Luengo *et al.*, 2004). The findings of this research did not agree with similar measurements in cows where SCC increased with increasing stage of lactation (Sandholm *et al.*, 1984). Milk samples from the goats had higher SCC in early stages of lactation than in mid lactation. This could be related to increased stress due to the adaptation to the milking routine after kidding. In the above study by (Sandholm *et al.*, 1984) milk trypsin inhibitor was used as an indicator of mastitis in cattle and compared to SCC. Perhaps this could be done in goats in further research, to evaluate if milk trypsin inhibitor is a better indicator of mastitis in goats than SCC is. However, a study by Poutrel *et al.* (1997), as in this research, showed that geometric means of SCC were below 800 x 10<sup>3</sup> cells/mL for uninfected halves of goats more than 200 days in milk, having a parity more than three. Another study (De Cremoux *et al.*, 1996) showed that stage of lactation and parity had no effect on SCC for milk from udder halves infected by major pathogens.

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC between udder halves of lactation numbers 2, 3, 4, 5 & 7, at both treatment times (Table 4.30). Insufficient data were available for lactation numbers 1 & 6. Mean SCC was high in the second lactation, and then decreased in the third and fourth lactation and increased again in the fifth and seventh lactation, with the highest mean SCC in the fifth lactation. This was not consistent with findings that goats of the first and second lactation group had the lowest SCC (Luengos *et al.*, 2004). This pattern of mean SCC for different lactation numbers does not agree with that of cows, which has been shown to be very low in the first and second lactations and then to increase with each subsequent lactation. However, several authors have described a progressive increase in SCC for higher parities, but only for healthy udder halves (Dulin *et al.*, 1983; Sinapis & Vlachos, 1999; Wilson *et al.*, 1995). No evidence of this effect was

recorded for halves infected by minor and major pathogens where alterations of SCC caused by bacterial infection masked the effect of parity (De Cremoux *et al.*, 1996; Luengo *et al.*, 2004). However, this was not consistent with the research of other authors, who did not detect any effect of parity on goat milk SCC (Randy *et al.*, 1988; Zeng & Escobar, 1995).

In another study least square means of mean SCC were low in the first lactation, increased significantly ( $P < 0.05$ ) in the second lactation and then decreased significantly in the third lactation and in later lactations for infected goats. For uninfected goats in the same study mean SCC decreased significantly from the first to the second lactation and then increased again for the third lactation and later lactations (Dulin *et al.*, 1983). Therefore according to Dulin *et al.*, (1983) the pattern of SCC changes in relation to lactation number was different for infected and non-infected Saanen, Toggenburg and Nubian goats. In this study the effect of parity on SCC was not studied separately for infected and non-infected animals. Therefore the irregular patterns of SCC between respective lactation numbers in this research could be because the data of infected and non-infected goats were not separated.

### 5.3.2 SCC: Trial 2: Spectrazol

In Trial 2 (Spectrazol) there was no significant difference ( $P = 0.227$ ) between mean log SCC of treatment (T2) and control (C2) group at treatment time 07:00 (Table 4.31). For treatment time 19:00 there was no significant difference between T2 and C2 at the 5% level of significance. However, further research with a greater number of goats is necessary to confirm this. However the information in (Figure 4.8) showed that mean SCC of both treatment and control groups remained unstable throughout the trial, mean SCC was higher at the start for T2 than for C2 and the increase in mean SCC did not correspond to the treatment times. This substantiated the fact that treatment with Spectrazol did not cause a significant increase in SCC. Therefore, it was clear that Spectrazol caused the least degree of tissue irritation in the udder.

In another study factors such as nutritional disorders and vaccination have caused an erratic increase in SCC (Lerondelle *et al.*, 1992).

In Trial 2 the presence of bacteria had an affect on mean log SCC at treatment time 19:00 but not at treatment time 07:00. There was a significant difference ( $P = 0.01$ ) of mean log SCC between infected and non-infected udder-halves at 19:00 (Table 4.32). Infected udder halves had a significantly higher mean log SCC than non-infected udder halves at 19:00. However at 07:00 there was no significant difference ( $P = 0.320$ ) between mean log SCC (Table 4.32). This showed that the presence of bacteria significantly increased the mean log SCC at the evening milking. Generally SCC was higher at evening milkings than at morning milkings and this might have been due to the increased stress on the goats when they were milked after sunset.

In Trial 2 there was a highly significant difference ( $P < 0.001$ ) between udder halves of mid and late lactation for both treatment times (Table 4.33). As in Trial 1 (Table 4.29), mean SCC was significantly lower for goats in late lactation than in early or mid lactation. This trend of a decrease in mean SCC with each subsequent stage of lactation again does not agree with findings in cattle, where SCC was found to increase with each subsequent lactation stage. However there was a very weak negative linear correlation between SCC and volume for Trial 2 (Table 4.54).

In a similar way to Trial 1 there was a highly significant difference ( $P < 0.001$ ) of mean log SCC between udder halves of lactation numbers 2, 3, 4, 5 & 7, at both treatment times (Table 4.34). But in this trial mean SCC was high in the second lactation, increased in the third lactation, decreased in the fourth lactation and increased again in the fifth and seventh lactations, with the highest SCC in the third lactation from samples measured at 19:00 and in the seventh lactation from samples measured at 07:00. This was different from the pattern SCC showed through the different lactation numbers in Trial 1 (Curaclox LC), although in both Trials 1 & 2 there was a decrease in SCC in the fourth lactation.

### 5.3.3 SCC: Trial 3 (Curaclor LC & Rilexine)

In Trial 3 there was a highly significant difference ( $P < 0.001$ ) of mean log SCC between Treatment groups T1 (Curaclor LC), T3 (Rilexine) and control group (C), at both treatment times (Table 4.35). SCC of both treatment groups T1 and T3 was higher than that of the control group, although SCC increased more for T1 (Curaclor LC) than for T3 (Rilexine). This was substantiated by the data shown in Figure 4.10, which also showed an increase in SCC for both treatment groups compared to the control group, although T1 increased more in mean SCC than T3. This showed that although both Curaclor and Rilexine caused a degree of tissue irritation in the udder, Curaclor LC caused a higher degree of tissue irritation than Rilexine. Therefore the product that caused the most tissue irritation in the udder as indicated by SCC was Curaclor LC; Rilexine caused less tissue irritation and Spectrazol caused the least tissue irritation in the udder.

In Trial 3 the presence of bacteria had an affect on mean log SCC at treatment time 06:00 but not at treatment time 18:00. There was a significant difference ( $P = 0.041$ ) at the 5% level of significance of mean log SCC between infected and non-infected udder-halves at 06:00 (Table 4.36). Infected udder halves had a significantly higher mean log SCC than non-infected udder halves at 06:00. However at 18:00 there was no significant difference ( $P = 0.993$ ) between mean log SCC of infected and non-infected goats (Table 4.36). These results were the opposite of those measured in Trial 2 where bacteria had an effect on SCC of the evening rather than the morning milking (Table 4.32). Trial 3 commenced on a commercial dairy farm where a 12 hourly milking interval and milking in the dark were part of the goats' routine. In another study (Wilson *et al.*, 1995) SCC of goats increased with intramammary infection. However non-infected goats frequently had SCC greater than  $100 \times 10^3$  cells/mL. Most of the differences in SCC were not due to intramammary infection as 77% of the variation in SCC was unexplained.

In Trial 3 there was no significant difference in mean log SCC between early and mid lactation stages (Table 4.37). This was different from the results in Trial 1 (Table 4.29), which showed that SCC of early lactation were significantly higher than SCC of late lactation and in Trial 2 (Table 4.33), which showed that SCC of mid lactation was significantly higher than that of late lactation. There was a significant difference between mid and late lactation in Trial 2 and between early and late lactation in Trial 1. Trial 2 did not have goats in early lactation and Trial 1 did not have goats in late lactation. These results differ from other work done on different breeds of goats where SCC increases with subsequent lactation stage and is the highest in late lactation. Most goats in Trial 3 were in the first lactation and there were too few goats in the second lactation to be able to compare mean log SCC between lactation numbers statistically as in Trials 1 & 2. In another study (Wilson *et al.*, 1995) increasing stage of lactation was associated with increased SCC in Alpine goats with or without diagnosis of intramammary infection. Goats lactating during months with highest mean days in milk had increased SCC.

### 5.3.4 SCC: Curaclor LC from Trials 1 & 3 Combined

Goats in Trial 1 at Onderstepoort (Herd A) had lower milk yields than goats in Trial 3 on a commercial goat farm, near Louis Trichardt (Makhado) in the Limpopo province (Herd B), although in both Trials 1 & 3 all goats were treated with Curaclor LC. Therefore, Curaclor LC data from Trials 1 & 3 were analysed separately (5.3.1 & 5.3.3) and combined (5.3.4). There was a highly significant difference of mean SCC ( $P < 0.001$ ) between treatment (T1) and control (C) groups (Table 4.38). Mean SCC was significantly higher for udder halves treated with Curaclor LC in Trials 1 & 3, than for udder halves of the control group. This was also the case with Curaclor LC in Trial 1 (Table 4.27) and Trial 3 (Table 4.35) separately. This showed that Curaclor LC caused a high degree of tissue irritation in goats' udders, when mean SCC was used as the indicator. This is substantiated by the data shown in Figure 4.12. Mean SCC of Treatment group (T1), increased after treatment with Curaclor LC at Rx1 and returned to baseline at 108h. The same pattern was seen in the graphs of Curaclor LC in Trial 1 (Figure 4.6) and Trial 3 (Figure 4.10) separately.



The presence of bacteria did not affect the mean log SCC of goat udder halves treated with Curaclox LC in Trials 1 & 3 combined (Table 4.39). This was also the case for Curaclox LC in trial 1 (Table 4.28) and in Trial 3 at 18:00 (Table 4.36). In Trial 3, mean log SCC was significantly higher for infected than for non-infected udder halves. After treatment SCC might be expected to increase because of udder irritation. This was not shown in (Figure 4.13), where the mean SCC of infected udder halves remained stable throughout the trial. The mean SCC of non-infected udder increased after treatment at Rx2 and returned to baseline at 72h. The presence of bacteria did not affect the mean SCC (Figure 4.12) and (Table 4.39). This confirmed that SCC was not an effective method for diagnosing intramammary infection in goats.

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC between early, mid and late lactation of Curaclox LC in Trials 1 & 3 combined (Table 4.40). Mean SCC began high in early lactation, decreased in mid lactation and increased again in late lactation. The high SCC in early lactation was different to that from cows, which has been shown to be low in early lactation and increased with each subsequent lactation stage (Sandholm *et al.*, 1995). This pattern (in Table 4.40) was also different to that in Trial 1 (Table 4.29), where SCC was high in early lactation and decreased in late lactation. In Trial 3 (Table 4.37), SCC was high in mid lactation and decreased in late lactation. However, the increase in SCC in late lactation was in agreement with other studies on goats with healthy udder halves (Dulin *et al.*, 1983; Sinapis & Vlachos, 1999; Wilson *et al.*, 1995).

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC between lactation numbers 1, 2, 3, 4, 5 & 7 (Table 4.41). The mean log SCC was low in the first lactation increased in the second, third, fourth and fifth lactation and decreased at the seventh lactation at 06:00. From the samples taken at 18:00 the mean log SCC was low in the first lactation, increased in the second and third lactation, decreased in the fourth lactation, increased in the fifth lactation and decreased at the seventh lactation. Mean log SCC were the highest at the fifth lactation for both treatment times. In Trial 1 (Table 4.30) there were no first lactating animals and the mean SCC of the second lactation was high, and the SCC decreased in the seventh lactation. Therefore, as shown in (Tables 4.30 & 4.41) perhaps the mean SCC of goats was low in the first lactation as for cattle and then increased in the second lactation. There was a moderate positive correlation ( $R^2 = 0.459$ ) between log SCC and withdrawal period as measured by TRIS and between log SCC and lactation number ( $R^2 = 0.467$ ) for Curaclox LC in Trials 1 & 3, (Table 4.56). Therefore withdrawal period as measured by TRIS increased with increasing lactation number. There was a weak negative correlation ( $R^2 = -0.390$ ) between log SCC and lactation stage and between log SCC and volume ( $R^2 = -0.347$ ) (Table 4.56). Therefore as lactation stage and volume increased, log SCC decreased for Trials 1 & 3 with Curaclox LC.

### 5.3.5 SCC: Combined Data for All Products for Trials 1, 2 & 3

There was a weak positive linear correlation ( $R^2 = 0.322$ ) between log SCC and withdrawal period as measured by TRIS (Table 4.59). Therefore as log SCC increased, withdrawal period as measured by TRIS increased.

### 5.3.6 SCC: All Data for Goats with Clinical Mastitis

There was a highly significant difference ( $P < 0.001$ ) of mean log SCC between treatment groups, T1 (Curaclox LC), T2 (Spectrazol), T3 (Rilexine) and control group for goats with clinical mastitis (Table 4.42). Treatment groups in order of SCC from highest to lowest were: T1, T2, C & T3 at 06:00 and T2, T1, C & T3 at 18:00. Curaclox LC resulted in the highest SCC in clinically infected goats at 06:00. As for goats without clinical mastitis, Curaclox LC also caused the most tissue irritation in the udder of goats with clinical mastitis. In goats with clinical mastitis treated with Spectrazol there was an increased SCC, especially at 18:00. This could be because Spectrazol caused a higher degree of tissue irritation in clinical than in non-clinical animals (Table 4.31). Goats treated with Spectrazol had a higher mean SCC at night than in the morning. This might have been because, in Trial 2 (Spectrazol), goats were stressed more in the evening than in the other trials, because of power failures, resulting in

stopping and starting of machine milking; and milking had to be completed by hand at some evening samplings throughout Trial 2. In goats with clinical mastitis treatment with Rilexine caused the lowest degree of tissue irritation in the udder, as demonstrated by the lowest increase in SCC. Mean SCC of the clinical group was higher than that of the goats treated with Rilexine, because these were goats with clinical mastitis that did not receive treatment. Therefore if the mean SCC of the control group was taken as a baseline, treatment with Curaclox LC and Spectrazol increased mean SCC, which indicated increased tissue irritation in the udders of goats with clinical mastitis. However, treatment with Rilexine decreased mean SCC, which indicated decreased tissue irritation in the udders of goats with clinical mastitis. This could have been because Rilexine contained prednisolone, a corticosteroid that helps in reducing inflammation in goats with clinical mastitis and inflamed udders, thus reducing the mean SCC.

The mean SCC of goats treated with Rilexine increased after treatment at Rx2, but returned to baseline at 60h (Figure 4.10). Mean SCC of the control group was higher than that of Rilexine at all times except at treatment time 12h. Mean SCC of goats treated with Curaclox LC remained unstable throughout the trial, although mean SCC did increase with treatment. Mean SCC of goats treated with Spectrazol had a higher mean SCC than the control group, according to the data in (Figure 4.8) for both morning and evening sampling times. This could have been due to the influence of the increased mean SCC of the evening milking for goats treated with Spectrazol in Trial 2.

The presence of bacteria did not affect the mean log SCC of clinical udder halves significantly at 06:00 (Table 4.43). However, the presence of bacteria affected the mean log SCC significantly ( $P = 0.001$ ) at 18:00. This showed that the mean log SCC of non-infected udder halves with clinical mastitis increased at night. This could have been due to the increase in the withdrawal period as measured by TRIS in the evening milking as shown in the regression model (Table 4.26).

The mean SCC of both infected and non-infected udder halves with clinical mastitis remained unstable throughout this trial (Figure 4.15). The mean SCC of infected udder halves was only higher than that of that of the non-infected udder halves, for only some of the treatment times, -48h, -36h, 48h, 60h, 72h, 96h & 120h. Mean SCC of infected and non-infected udder halves with clinical mastitis did not show the expected rise at times of antibiotic treatment (Figure 4.15).

For goats with clinical mastitis udder halves there was a highly significant ( $P < 0.001$ ) difference of mean log SCC between early, mid and late lactation (Table 4.44). The SCC was high in early, lactation, decreased significantly in mid lactation and then increased significantly in late lactation. The decrease of SCC in mid lactation was in agreement with the decrease in SCC in Trial 3. The increase in SCC in late lactation was in agreement with previous research done on goats and cattle, showing that SCC increases in late lactation (Dulin *et al.*, 1983; Sandholm *et al.*, 1995; Sinapis & Vlachos, 1999; Wilson *et al.*, 1995). However, this increase in SCC in late lactation was found only in the case of the goats with clinical mastitis and not in the other goats in Trials 1, 2 & 3. In Trials 1 & 2, SCC decreased in late lactation, which was the opposite of what was found in goats with clinical mastitis. For these goats with clinical mastitis there was a highly significant difference ( $P < 0.001$ ) of mean log SCC between lactation numbers 1, 2, 3, 4, 5 & 7 (Table 4.45). Mean SCC was high in the first lactation, decreased in the second lactation, increased in the third, fourth and fifth lactation and decreased again in the seventh lactation at 6:00 & 18:00. This pattern of increase and decrease of mean SCC at different lactation numbers was different from that of Trial 1 & 2 which all showed an increase in SCC at the seventh lactation, (Table 4.30; Table 4.34). However, the pattern of increasing and decreasing mean SCC in (Table 4.45) was similar to that of Trials 1 & 2 and different to that of cows, which had been found to be low in first lactations and increases with each subsequent lactation number.

### 5.3.7 Peak in Somatic Cell Counts (SCC)

There was a significant difference between the log SCC Peak of T1 (Curaclox LC), T2 (Spectrazol) and T3 (Rilexine), (Table 4.46). This showed that each product used peaked at a

unique log SCC value. The goats treated with Curaclox LC showed the highest peak log SCC, and the peaks for goats treated with Rilexine and Spectrazol were lower. This substantiates the fact that Spectrazol caused the least tissue irritation and Curaclox caused the most tissue irritation in the udder, as indicated by the log SCC.

There was no significant difference in the time in hours from start of the trial to peak in SCC between treatment groups, T1 (Curaclox LC), T2 (Spectrazol), T3 (Rilexine), (Table 4.47). There was no significant difference in the time in hours from the peak in SCC to the end of the trial between treatment groups, T1 (Curaclox LC), T2 (Spectrazol) and T3 (Rilexine) (Table 4.48). Therefore all three products used peaked at similar times, although the SCC values at which they peaked were different. This showed that treatment with all three products caused a rise in SCC at the same time, therefore all three products caused a degree of tissue irritation in the udder, although the degree of tissue irritation caused by each product was different.

The mean SCC over time for both right and left udder halves is shown for one goat from the control group in Trial 3 (Figure 4.16). There was no peak in SCC since both these udder halves were untreated. The same lack of response was shown for Trial 1 (Figure 4.17), and for Trial 2 (Figure 4.18).

In Trial 2 (Spectrazol) only nine udder halves were used in the analysis (Table 4.46; Table 4.47 & Table 4.48). This was because only nine udder halves from the group treated with Spectrazol had peaks in SCC. The remainder of the udder halves treated with Spectrazol in Trial 2 showed no peak in SCC (Figure 4.19) as for the untreated controls of the same trial (Figure 4.18).

The mean SCC of one goat with clinical mastitis in Trial 1 treated with Curaclox LC in the right udder half is shown in (Figure 4.20). Although mean SCC of both udder halves was high, mean SCC of the left udder half remained stable, while mean SCC of the left clinically infected udder half remained unstable. In both udder halves of this clinical goat there was no peak in SCC corresponding to treatment with Curaclox LC. This showed that in clinically infected goats, there was already a high degree of tissue irritation present in the udder, and this was not significantly altered by the administration of intramammary antibiotics.

#### **5.4 California Milk Cell Test (CMCT)**

Tissue irritation of udder tissue was measured by CMCT & SCC. A negative CMCT score and a low SCC should then indicate tissue tolerance.

This research confirmed that the CMCT was more useful for ruling out than for diagnosing mastitis (Smith & Sherman, 1994).

##### **5.4.1 CMCT: Curaclox LC from Trial 1 Only**

The Chi-squared test was highly significant; therefore the number of CMCT counts per category was associated with treatment (Table 4.49). There was a significantly higher percentage of samples with a CMCT score of 1 for the control (C1) compared with the treatment group (T2). This showed that more of the untreated goats had a positive CMCT score of 1 in Trial 1, than in the other trials. This agrees with the statement that a negative result is a good indicator of absence of infection, but a positive test does not always indicate infection (Contreras *et al.*, 1996 & Lewter *et al.*, 1984). There was no significant difference between the number of samples with CMCT scores of 0 and the number of samples with CMCT scores of 1. There was also no significant difference between samples with CMCT scores of 0 and samples with CMCT scores of 2&3. There were too few udder halves with CMCT score of 3 to be analysed separately; therefore the CMCT scores of 2 & 3 were combined for the Chi-squared test. There were significantly more CMCT scores of 1 than CMCT scores of 2 & 3.

According to the data shown in (Figure 4.21) the mean CMCT of the treatment group (T1) increased after treatment and returned to baseline at 72h. In Trial 1 the mean SCC increased after treatment at Rx1, decreased at 72h but only returned to baseline at 108h (Figure 4.6). Therefore antibiotic treatment with Curaclox LC in Trial 1 caused an increase in both the

CMCT and the SCC. This showed that treatment with Curaclox LC caused tissue irritation in goats.

There was a positive moderate correlation between SCC and CMCT for Trial 1 ( $R^2 = 0.482$ ) (Table 4.53). This was in accordance with findings on cow milk, that CMCT is an indication of the SCC in the milk. Therefore the higher (more positive) the CMCT score, the higher the SCC.

Mean CMCT of infected udder halves was higher than that of non-infected udder halves at all treatment times except at -36h, -12h & 24h (Figure 4.22). This showed that CMCT was not a reliable method for determining an intramammary infection in goats. As for cows, CMCT should only be used in conjunction with other methods of diagnosing mastitis for example, SCC, udder palpation, and microbiological tests. Another study (Poutrel & Lerondelle, 1983) also showed that CMCT was not an accurate indicator of infection in goats and did not correspond with SCC of infected and uninfected goats.

Mean SCC of infected and non-infected udder halves in Trial 1, increased after treatment at Rx3, decreased at 24h & 36h and then increased again at 48h, returning to baseline at 96h. SCC only increased after the second treatment and then remained unstable between treatment times 24h and 36h, otherwise SCC of infected udder halves was generally higher than that of non-infected udder halves (Figure 4.7). As with CMCT, this showed that SCC was also not a reliable method for determining intramammary infection in goats and should be used in conjunction with udder palpation and microbiological tests for mastitis diagnosis.

#### **5.4.2 CMCT: Trial 2; Spectrazol**

The Chi-squared test for Trial 2 data was not significant (Table 4.50). Therefore the numbers of CMCT counts per category were not significantly associated with treatment with Spectrazol. Treatment with Spectrazol also did not cause a significant difference in mean SCC (Table 4.32).

In Trial 2 there was a negative linear correlation ( $R^2 = -0.432$ ), between SCC and CMCT (Table 4.54). This suggested that as SCC increased, CMCT would decrease and vice versa. However, this was neither a weak ( $R^2 = -0.3$  to  $+0.3$ ), nor a moderate ( $R^2 = \pm 0.5$ ) linear correlation.

Although mean CMCT of treatment group (T2) increased after treatment with Spectrazol after Rx1, it then remained unstable for the remainder of the trial. Therefore, mean CMCT of Trial 2 did not show the expected increase associated with antibiotic treatment times (Figure 4.23). The mean CMCT of the control (C2) group also remained unstable throughout the Trial and did not show a significant difference in mean CMCT between T2 and C2 (Figure 4.23) as was shown for Trial 1 (Figure 4.21).

There was no significant difference of mean SCC between treatment (T2) and control (C2) groups at both treatment times (Table 4.31).

The mean CMCT of infected and non-infected udder halves was unstable throughout Trial 2. Although mean CMCT of infected udder halves was higher than that of non-infected udder halves at treatment times -36h, Rx1 to 36h, 72h & 108h (Figure 4.23). This showed that CMCT was not a reliable method for determining an intramammary infection in goats. As for cows, CMCT should only be used in conjunction with other methods of diagnosing mastitis, such as SCC, udder palpation, and microbiological tests.

The mean SCC of non-infected udder halves remained stable, while the mean SCC of infected udder halves was unstable and increased between treatment times Rx2 and 84h. This showed that infected udder halves had an increase SCC after treatment while non-infected udder halves did not and infected udder halves had lower mean SCC values at the start than non-infected udder halves (Figure 4.9). Therefore, with CMCT, this showed that SCC was also not a reliable method for determining intramammary infection in goats and should be used in conjunction with udder palpation and microbiological tests for mastitis diagnosis.

#### **5.4.3 CMCT: Trial 3; Curaclox LC & Rilexine**

The Chi-squared test was highly significant therefore the number of CMCT counts per category were associated with treatment (Table 4.51). The control group had the highest

percentage (43.3%) udder-halves with a CMCT score of 0. The samples with a CMCT score of 1 had the highest percentage of udder halves for T1 (Curaclon LC) (41.9%) and T3 (Rilexine) (40.9%). This showed that more of the treated goats had a positive CMCT score of 1 in Trial 3. There was a significant difference between the number of samples with CMCT scores of 0 and the number of samples with CMCT scores of 1 and between samples with CMCT scores of 0 and samples with CMCT scores of 2&3 (Table 4.51). There were too few udder halves with CMCT score of 3 to be analysed separately and therefore the CMCT scores of 2 & 3 were combined for the Chi-squared test. There was no significant difference between samples with CMCT scores of 1 and those with CMCT scores of 2 & 3. This differed from that of Trial 1 and Trial 2 as explained above.

Mean CMCT of Treatment groups T1 (Curaclon LC) and T3 (Rilexine), increased after treatment at Rx1, returned to baseline at 72h and then remained unstable for the remainder of the trial (Figure 4.25). The mean CMCT of the control group was unstable throughout the trial, it was higher than that of both treatment groups at the start, then decreased and remained lower than that of both treatment groups only to increase again at 96h & 108h and then decreased again. Although mean CMCT did increase with treatment, there was also an increase in mean CMCT of the control group. Mean SCC of T1 and T3 increased with treatment after Rx1 and returned to baseline at 72h, although mean SCC of T1 increased more than that of T3 (Figure 4.10). The SCC of the control group remained relatively low and stable throughout the trial. This showed that both SCC and CMCT increased with antibiotic treatment for both Curaclon LC and Rilexine.

There was a weak positive linear correlation ( $R^2 = 0.035$ ) between SCC and CMCT in Trial 3 (Table 4.55). Although this was a weak linear correlation it still showed that as SCC increased, CMCT also increased.

Mean CMCT of infected and non-infected udder halves remained unstable throughout Trial 3. Mean CMCT of infected udder halves was not higher than that of non-infected udder halves (Figure 4.26). This showed that CMCT was not a reliable method for determining an intramammary infection in goats. As for cows, CMCT should only be used in conjunction with other methods of diagnosing mastitis, such as SCC, udder palpation, and microbiological tests.

Mean SCC of infected and non-infected udder halves also remained unstable throughout Trial 1 but at different times compared to those of Trial 3. In Trial 3, mean SCC of infected udder-halves was only higher than that of non-infected udder halves at sampling times -12h, -24h, 12h, 72h, 84h, 96h & 132h (Figure 4.26). Therefore, as with CMCT this showed that SCC was also not a reliable method for determining intramammary infection in goats and should be used in conjunction with udder palpation and microbiological tests for mastitis diagnosis.

#### 5.4.4 CMCT: Curaclon LC from Trials 1 & 3 Combined

The Chi-squared test was highly significant therefore the counts of CMCT per category were associated with treatment (Table 4.52). In this trial the control group had the highest percentage (39.9%) udder-halves with a CMCT score of 0. However, the CMCT score of 1 had the highest percentage of udder halves for T1 (Curaclon LC) (47.0%) and for the control group (43.3%). This showed that more treated and control goats had a positive CMCT score of 1 in Trials 1 & 3. There was a significant difference between the percentage udder halves from samples with a CMCT score of 0 and samples with a CMCT score of 1, between the percentage udder halves with samples with a CMCT score of 0 and 2 & 3 and between the percentage udder halves with samples with a CMCT score of 1 & 2 & 3 (Table 4.52). Percentage udder halves with CMCT score of 1 and 2 & 3 increased in the treatment group (T1) compared to the control group.

There was a weak positive linear correlation between CMCT and log SCC, (Table 4.56). The mean CMCT score of the control group was shown to be unstable and the mean CMCT score of the treatment group (T1) increased after treatment at Rx2 and returned to baseline at 96h (Figure 4.28). This showed that the CMCT score increased with treatment as in Trial2 (Spectrazol) (Figure 4.24). This was an indication of the increased tissue irritation caused by the infusion of the intramammary antibiotic. Mean SCC also increased after treatment at Rx2

and returned to baseline at 108h (Figure 4.12). Therefore mean SCC and mean CMCT score both increase with antibiotic treatment, although there is a weak correlation between CMCT and log SCC ( $R^2 = 0.026$ ) (Table 4.56).

The mean CMCT scores of infected and non-infected udder halves remained unstable (Figure 4.27). The CMCT scores of non-infected udder halves increased after treatment, this could have been due to more non-infected than infected goats in the treatment group. A similar pattern was observed for mean SCC (Figure 4.13). Showing that both CMCT and SCC were not affected by intramammary infection but rather by treatment. However, CMCT was a poor indicator of intramammary infection (Figure 4.27). Therefore, like with CMCT this showed that SCC was also not a reliable method for determining intramammary infection in goats and should be used in conjunction with udder palpation and microbiological tests for effective mastitis diagnosis.

There was a weak positive correlation ( $R^2 = 0.245$ ) between CMCT and floccules present in the milk (Table 4.56). Therefore although this was a weak linear correlation as CMCT increased, the floccules present in the milk increased. This showed that CMCT was to some extent an indicator of the presence of mastitis in the milk. However, CMCT was not a reliable method for mastitis diagnosis and should only be used for diagnosis together with other methods like SCC and microbiological tests.

#### **5.4.5 CMCT: Combined Data for All Products for Trials 1, 2 & 3**

There was a moderate positive ( $R^2 = 0.494$ ) linear correlation between CMCT and withdrawal period according to TRIS (Table 4.59). Therefore as withdrawal period according to TRIS increased the CMCT score increased. There was a weak positive ( $R^2 = 0.240$ ) linear correlation between CMCT and lactation number (Table 4.59). Therefore CMCT score increased with each increasing lactation number. In a study by Boscos *et al.*, (1996), parity, breed and stage of lactation differences were not found to have any effect on mean CMCT scores in goat milk.

#### **5.4.6 CMCT: All Data for Goats with Clinical Mastitis**

There were not enough data to perform Chi-squared tests on the data from goats with clinical mastitis.

The mean CMCT scores of the control group (C) and of T2 (Spectrazol) were unstable throughout the trial (Figure 4.30). However, mean CMCT score of T1 (Curaclox LC) and T3 (Rilexine) increased with antibiotic treatment.

The mean SCC increased the most for Spectrazol, then for Curaclox LC and the least for Rilexine (Figure 4.14).

However there was a strong positive correlation between CMCT and log SCC (Table 4.57) and a moderate positive correlation between CMCT and log SCC (Table 4.56). This positive correlation did not explain why SCC increased the most after treatment with Spectrazol (T2), while CMCT increased the most after treatment with Rilexine.

This showed that CMCT and SCC were both inaccurate indicators of tissue irritation in goats, which is different to the findings in cows.

The mean CMCT scores were unstable for both infected and non-infected udder halves (Figure 4.29). Subsequently the mean SCC scores were unstable for infected and non-infected udder halves (Figure 4.15). Therefore CMCT and SCC were both not reliable methods for determining intramammary infection in goats and should be used in conjunction with udder palpation and microbiological tests for effective mastitis diagnosis.

### **5.5 Milk Production Volume and Correlations with Other Variables**

All animals in this research were milked twice daily at 12 hourly intervals. A study on dairy goats in the Canary Islands showed that goats milked twice daily had a higher milk yield than goats milked once daily, in both the first and the second lactation (Capote *et al.*, 2000).

### 5.5.1 Milk Volume: Trial 2 (Spectrazol)

These were low producing animals (less than 1.3 L) in Trial 2. There was a moderate negative correlation ( $R^2 = -0.493$ ) between withdrawal period as measured by TRIS and milk volume in Trial 2 (Table 4.54). Therefore withdrawal period increased as milk production volume decreased. This showed that the increased withdrawal period as measured by TRIS on low producing goats could also have been partly attributed to the low milk production volumes of these goats. Milk production volumes of treatment (T2) and control (C) groups were unstable during the trial and did not correspond to treatment times (Figure 4.33). This showed that treatment with Spectrazol did not affect the milk production volume of goats in Trial 2. Milk production volumes of infected udder halves were unstable and lower than those of non-infected udder halves (Figure 4.34). This showed that the presence of bacteria did affect the milk production of goats in Trial 2. Therefore, infected goats had a lower milk production and can be expected to have a longer withdrawal period as measured by TRIS, than non-infected goats (Table 4.54; Figure 4.34).

### 5.5.2 Milk Volume: Curaclox LC from Trials 1 & 3 Combined

These were mostly high producing goats with a few mid-level producing goats in Trial 3 and there were low producing goats in Trial 1.

The mean milk production volumes of the treatment group (T1) remained relatively stable throughout the trial and did not correspond to the antibiotic treatment times (Figure 4.37). The mean milk production of the control group began at the same level as that of the treatment group, then decreased at -24h, then steadily increased, until passing the level of the treatment group at Rx3, then remained relatively stable for the remainder of the trial, only to increase at the last sampling, like that of the treatment group. The high volume at 132h was because only high producing goats from Trial 3 were sampled at this time, Trial 1 with low producing animals ended at 120h (Figures 4.37 & 4.38). This showed that treatment with Curaclox LC in Trials 1 & 3 did not affect the milk production volumes of goats.

The mean milk production volume of non-infected goats remained relatively stable throughout the trial, although it was higher at the start than that of the infected goats (Figure 4.38). The mean volume of the infected goats, was lower at the start than that of non-infected goats, and then was unstable until treatment time Rx2, after which volume increased, and remained increased for the remainder of the trial. This did not show that the presence of bacteria negatively affected the milk production volume of the low, mid-level & high producing goats of Trials 1 & 3 as it did for the low producers of Trial 1. This pattern differed from that of Trial 3 (Figure 4.35). However, the presence of bacteria did not affect the milk production volume, when the majority of the animals were high producers (Figures 4.38 & 4.35).

There was a weak negative linear correlation ( $R^2 = -0.347$ ) between volume and log SCC for Curaclox LC in Trials 1 & 3 (Table 4.56). Although this was a weak linear correlation, it still showed that as volume increased log SCC decreased. Withdrawal period as measured by TRIS had a moderate positive correlation with log SCC ( $R^2 = 0.459$ ) (Table 4.56). Therefore as log SCC increased, withdrawal period as measured by TRIS increased. Therefore as volume increased withdrawal period as measured by TRIS and log SCC both would decrease. This showed that the dilution factor, with a higher or lower milk volume might decrease or increase the withdrawal period respectively. However, there was no linear correlation between withdrawal period as measured by TRIS and milk volume (Table 4.56).

### 5.5.3 Milk Volume: Curaclox LC from Trial 1 Only

These were low producing animals (less than 1.3 L) in Trial 1. There was a strong negative correlation ( $R^2 = -0.701$ ) between withdrawal period as measured by TRIS and milk volume (Table 4.53). Therefore withdrawal period as measured by TRIS increased as milk production volume decreased, because of the dilution factor as found in research on cows. Milk production volumes of treated (T1) and control goats in Trial 1 were unstable

(Figure 4.31). This showed that treatment with Curaclox LC in Trial 1 did not affect milk production volume. According to another study (Wilson *et al.*, 1995) elevated SCC has not always been associated with reduced milk production in goats. Lower milk production in high SCC does may have been caused more by the effects of advanced lactation than mastitis or elevated SCC.

Milk production volumes of infected and non-infected udder halves were unstable throughout the trial and did not correspond to antibiotic treatment times (Figure 4.32). Milk production volumes of non-infected goats were higher at the start and remained higher than those of infected goats throughout Trial 1. This showed that the presence of bacteria affected the milk production volume negatively on low producing goats treated with Curaclox LC in Trial 1. This was in accordance with the milk production volumes on low producing goats in Trial 2 treated with Spectrazol. However, this was not the case in the high and mid-level producing goats in Trial 3 treated with Curaclox LC and Rilexine. Therefore, the presence of bacteria decreased the milk production volume in low producing goats but not in mid-level and high producing goats.

#### 5.5.4 Milk Volume: Trial 3 (Curaclox LC & Rilexine)

These were mostly high producing goats (greater than 1.5 L) with a few mid-level producing goats in Trial 3. There was no linear correlation between volume and withdrawal period as measured by TRIS for Trial 3 (Table 4.55). Therefore, the shorter withdrawal period as measured by TRIS for goats was not necessarily because this trial was done on high and mid-level producing goats compared to low producers. Therefore for Rilexine goats have a significantly shorter withdrawal period than that recommended for use in cattle.

The withdrawal period of Curaclox LC in Trial 1 was significantly ( $P < 0.001$ ) shorter than that recommended for use in cattle as measured by TRIS and the colour dye. However withdrawal period of Curaclox LC in Trial 1 as measured by the Parallax testing for ampicillin and cloxacillin was not significantly different from that recommended for use in cattle (Table 4.21). This showed again that the Parallax test was more sensitive than the TRIS test and the colour dye. Withdrawal periods for Curaclox LC were shorter in Trial 3, than in Trial 1 or in Trial 1 & 3 combined. Trial 3 had mostly high producing animals with a few mid producers, whereas trial 1 had low producing animals. Therefore although there was no linear correlation between withdrawal period as measured by TRIS and volume, it was noted that the lower producers in trial 1 had a longer withdrawal period than that of the higher producers in Trial 3 for Curaclox LC. The withdrawal periods for the Curaclox LC combined for Trials 1 & 3 were higher than those in Trial 1 but lower than those in Trial 3 and included low (less than 1.3 L), mid-level (1.3 L to 1.5 L) and high producing animals (greater than 1.5 L). This substantiates the finding that as milk production volume increased, withdrawal periods decreased.

Mean milk production volume of T3 (Rilexine), T1 (Curaclox) and control groups were unstable throughout Trial 3 and did not correspond to antibiotic treatment times (Figure 4.35). Therefore treatment with T1 (Curaclox LC) & T3 (Rilexine), did not affect the milk production volume in Trial 3.

The milk production volume of infected and non-infected udder halves remained unstable throughout the trial and did not correspond to antibiotic treatment times. Therefore the presence of bacteria did not affect the milk production volume of mid and high producers in Trial 3 as it did for the low producers in Trial 2.

#### 5.5.5 Milk Volume: Combined Data for All Products for Trials 1, 2 & 3

There was a weak negative linear correlation ( $R^2 = -0.373$ ) between volume and lactation number and between volume and log SCC ( $R^2 = -0.301$ ) in the combined data of Trials 1, 2 & 3 (Table 4.59). Therefore volume increased with decreasing log SCC and lactation number. This was not the case in a study by (Dulin *et al.*, 1983) where milk yield increased with increasing lactation number. There was a moderate negative ( $R^2 = -0.511$ ) correlation between volume and withdrawal period as measured by TRIS (Table 4.59). Therefore as volume increased withdrawal period as measured by TRIS decreased.



### 5.5.6 Milk Volume: Data for Goats with Clinical Mastitis

There were, low, mid and high producing goats in the clinical group. There was a weak negative correlation ( $R^2 = -0.270$ ) between withdrawal period as measured by TRIS and milk volume (Table 4.58). This showed that although there was a weak linear correlation, as withdrawal period as measured by TRIS increased, volume decreased for goats with clinical mastitis. There was a moderate negative correlation ( $R^2 = -0.438$ ) between volume and lactation number (Table 4.58). This showed that volume decreased with increasing lactation number. There was a strong negative correlation ( $R^2 = -0.812$ ) between volume and log SCC for goats with clinical mastitis (Table 4.58). This showed that as log SCC increased, volume decreased.

Goats treated with Rilexine (T3) had the highest milk production volumes; these were mostly high producing goats from Trial 3 In (Figure 4.39). Goats treated with Curaclox LC (T1) had the next highest milk production volume, these were low, high and mid-level producing animals, except for the low milk production at treatment times 144h, 156h & 168h. At these last three treatment times there were only low and mid-level producers from Herd C, as Trial 1 ended at 120h and Trial 3 ended at 132h.

Goats treated with Spectrazol in Trial 2, had the lowest milk production volumes (Figure 4.39). This could have been because most of the goats with clinical mastitis treated with Spectrazol were low producers from Trial 2 and a few were low and mid-level producers from Herd C.

The milk production volume of the untreated control group of the goats with clinical mastitis, was low at the start, increased at -24h, remained relatively stable until 108h and then increased at 120h.

Goats with clinical mastitis treated with Spectrazol (T2), caused the greatest increase in mean SCC (Figure 4.14). This was in agreement with the strong negative ( $R^2 = -0.812$ ) linear correlation (Table 4.58). This showed that as SCC increased, milk production volume decreased. The same was true for Rilexine, which showed the lowest increase in SCC (Figure 4.14) and the highest milk production volume (Figure 4.39). Curaclox LC also showed a higher milk production volume (Figure 4.39) and a moderate increase in SCC (Figure 4.14). The mean milk production volume of infected and non-infected goats with clinical mastitis remained unstable throughout the Trial (Figure 4.40). The low production volumes of infected and non-infected goats between treatment times 144h and 204h are from the low and mid-level producing animals of Herd C. Trial 1 & 2 ended at 120h and Trial 3 ended at 132h. This showed that the presence of bacteria did not affect the mean milk production volume of goats with clinical mastitis.

Mean SCC of infected and non-infected goats with clinical mastitis remained unstable throughout the trial (Figure 4.15). Although mean SCC of infected udder halves had an increased SCC after treatment at Rx3 and returned to baseline at 108h. This showed that more infected animals were affected by antibiotic treatment than in the control group. This could have been due to the greater number of infected animals in the treatment group and more non-infected animals in the control group. Mean SCC of infected and non-infected udder halves with clinical mastitis remained unstable throughout the trial (Figure 4.15). Mean milk volume of non-infected and infected udder halves with clinical mastitis, was low at the start and then increased and remained unstable throughout the trial (Figure 4.40). Therefore, the strong negative correlation between log SCC and milk volume (Table 4.58) was not shown in (Figures 4.15 & 4.40).

When the withdrawal period as measured by TRIS was excluded, there were 332 degrees of freedom (Table 4.57) compared to 16 degrees of freedom with the withdrawal period as measured by TRIS included (Table 4.58). Therefore correlations indicated in (Table 4.57) were more reliable than those indicated in (Table 4.58).

Moderate negative correlations between milk volume and lactation number were shown (Tables 4.57 & 4.58). Therefore, milk volume decreased with increasing lactation number for Saanen goats with clinical mastitis. In another study on Saanen, Toggenburg and Nubian goats milk volume increased with increasing lactation number (Dulin *et al.*, 1983).

## 5.6 Analysis of Butterfat, Protein and Lactose

Butterfat, protein and lactose concentration were measured only at three treatment times during each trial, once before treatment, once during treatment, and once when antibiotic residues were no longer present in the milk.

### 5.6.1 Butterfat

Significant differences of butterfat, protein and lactose percentages in this trial may have been statistically different, but these were not necessarily biologically meaningfully different.

**TABLE 5.4: BUTTERFAT PERCENTAGES IN DIFFERENT STUDIES.**

| Authors                       | Breed                                   | Butterfat %<br>Mean $\pm$ Standard Error (se) or<br>Standard Deviation (sd) |
|-------------------------------|---|---|
| Park, 1991                    | French Alpine and Anglo<br>Nubian goats | 3.94 $\pm$ 1.21 (sd)  |
| Park & Humphrey, 1986         | Alpine and Nubian goats                 | 4.47 $\pm$ 0.13 (se)  |
| Calderon <i>et al.</i> , 1984 | French Alpine goats                     | 3.40 $\pm$ 0.09 (se)  |
| Donkin <i>et al.</i> , 2000   | Saanen goats                            | 3.29 $\pm$ 0.58 (se)  |
| Donkin <i>et al.</i> , 2000   | Saanen-Indigenous crossbred<br>goats    | 5.22 $\pm$ 0.61 (se)  |

**TABLE 5.5: BUTTERFAT PERCENTAGES IN THIS STUDY** (Table 4.60;  
Table 4.63 & Table 4.66).

| Trials and Products Used                     | Breed  | Butterfat %<br>Mean $\pm$ Standard Deviation |
|--|--|--|
| Trial 1: Curaclox LC<br>Treatment Group (T1) | Saanen                                       | 4.29 $\pm$ 1.52                              |
| Trial 1: Control Group<br>(C1)               |  | 4.56 $\pm$ 0.63                              |
| Trial 2: Spectrazol<br>Treatment Group (T2)  |  | 3.83 $\pm$ 1.05                              |
| Trial 2: Control Group<br>(C2)               |  | 3.48 $\pm$ 0.62                              |
| Trial 3: Control Group (C)                   | Saanen and Saanen-<br>Toggenburg Crossbreeds | 3.53 $\pm$ 0.63                              |
| Trial 3: Curaclox LC<br>Treatment Group (T1) |  | 3.19 $\pm$ 0.54                              |
| Trial 3: Rilexine Treatment<br>Group (T3)    |  | 3.39 $\pm$ 0.60                              |

According to Donkin *et al.*, (2000), there were differences in percentage butterfat between Saanen and Saanen-Indigenous Crossbreds in first and second lactation. Trials 1 & 2 have Saanen goats in second, third, fourth, fifth and seventh lactations. Trial 3 has mostly Saanen goats with a few Saanen-Toggenburg Crossbreeds, which were mostly in the first lactation, with a few goats in the second lactations.

In research done by Calderon *et al.*, (1984), butterfat percentage increased with each subsequent stage of lactation and butterfat percentage decreased when goats were fed a high concentrate diet. Trial 1 had goats in early and late lactation. Trial 2 had goats in mid and late lactation and Trial 3 had goats in mid lactation.

In a previous study (Park & Humphrey, 1986) there was a moderate strong correlation between percentage butterfat and percentage protein in goat milk, therefore as percentage butterfat increased so did percentage protein. In another study (Park, 1991) there were also moderate and strong positive correlations between percentage protein and percentage butterfat

and moderate negative correlations between percentage butterfat and electrical conductivity. Therefore as percentage butterfat increased electrical conductivity decreased. In this research, electrical conductivity measurements were taken, but due to the unreliability of the conductivity meter there were not enough data to try correlation between electrical conductivity, butterfat, and protein and lactose percentages. In another study (Zeng & Escobar, 1996) overall butterfat percentage for Alpine and Nubian goats was 4.08 %. Nubian does produced a significantly higher ( $P < 0.05$ ) percentage butterfat than Alpine does.

#### 5.6.1.1 Curaclox LC from Trial 1 Only

In Trial 1 there was no significant difference ( $P = 0.984$ ) of percentage butterfat between treatment (T1) and control (C1) groups (Table 4.60). There was also no significant difference ( $P = 0.729$ ) between percentage butterfat of treatment and control groups between the three respective treatment times (Table 4.60). Therefore in Trial 1, treatment with Curaclox LC did not affect the butterfat percentage.

#### 5.6.1.2 Trial 2: (Spectrazol)

In Trial 2 there was no significant difference ( $P = 0.566$ ) between percentage butterfat between the three respective treatment times for both treatment and control groups, (Table 4.63). Therefore there was no significant difference of the percentage butterfat, before treatment, during treatment and after antibiotic residues were no longer present in the milk. There was a significant difference ( $P = 0.022$ ) in percentage butterfat between treatment (T2) and control (C2) groups (Table 4.63). The treatment (T2) group had a significantly higher percentage butterfat than the control (C) group. However, there was a significant difference in butterfat percentage between the treatment and control groups, at all three treatment times. This showed that the butterfat percentage of the goats in the treatment (T2) group was higher than those of the control (C) group to begin with. Although this was a statistically significant difference, this difference in butterfat between treatment groups was not biologically meaningful. This statistically significant difference in percentage butterfat between treatment (T2) and control (C2) group could have been due to the goats in Trial 2 being low producers, and/or due to the high fat content of the carrier substance of Spectrazol (a succinylated fatty acid and triglycerides). Therefore treatment with Spectrazol did not have a meaningful effect on the butterfat percentage in Trial 2.

#### 5.6.1.3 Trial 3: (Curaclox LC & Rilexine)

In Trial 3 there was no significant difference ( $P = 0.320$ ) of percentage butterfat between treatments (T1) Curaclox LC, (T3) Rilexine and control (C) (Table 4.66). There was also no significant difference ( $P = 0.199$ ) between percentage butterfat of treatments (T1) Curaclox LC, (T3) Rilexine and control (C), between the three respective treatment times (Table 4.66). Therefore in Trial 3, treatment with Curaclox LC (T1) and Rilexine (T3) did not affect the butterfat percentage. Times, before, during and after treatment (Table 4.66) were equivalent to treatment times Rx1, 12h & 132h respectively in Trial 3 and -12h, 12h & 108h respectively in Trial 1.

#### 5.6.1.4 Curaclox LC from Trials 1 & 3 Combined

There was no significant difference ( $P = 0.359$ ) in mean butterfat percentage between treatment (T1) and control (C) groups for Curaclox LC between treatments for Trials 1 & 3 (Table 4.69). There was also no significant difference ( $P = 0.963$ ) in mean butterfat percentage of treatment and control groups at different treatment times, before, during and after treatment (Table 4.69). This was also the case for Curaclox LC in Trial 1 and Trial 3 separately (Tables 4.60 & 4.66).

### **5.6.2 Protein**

According to Donkin *et al.*, (2000), there were differences in percentage protein between Saanen and Saanen-Indigenous crossbred goats in first and second lactation. Trials 1 & 2 were carried out with Saanen goats in second, third, fourth, fifth and seventh lactations. Trial

3 included mostly Saanen goats with a few Toggenburg-Saanen Crossbreeds, which were mostly in the first lactation, with a few goats in the second lactation.

**TABLE 5.6: PROTEIN PERCENTAGES IN DIFFERENT STUDIES.**

| Authors                       | Breed                                   | Protein %<br>Mean $\pm$ Standard Error (se) or<br>Standard Deviation (sd) |
|-------------------------------|---|---|
| Park, 1991                    | French Alpine and Anglo<br>Nubian goats | 3.51 $\pm$ 0.77 (sd)  |
| Park & Humphrey, 1986         | Alpine and Nubian goats                 | 3.42 $\pm$ 0.05 (se)  |
| Calderon <i>et al.</i> , 1984 | French Alpine goats                     | 3.30 $\pm$ 0.06 (se)  |
| Donkin <i>et al.</i> , 2000   | Saanen goats                            | 2.85 $\pm$ 0.37 (se)  |
| Donkin <i>et al.</i> , 2000   | Saanen-Indigenous crossbred<br>goats    | 3.77 $\pm$ 0.27 (se)  |

Percentage protein remained the same during treatment with a high concentrate diet, but increased after treatment (Calderon *et al.*, 1984). There were moderate and strong negative correlations between percentage protein and electrical conductivity (Park, 1991). According to (Donkin *et al.*, 2000), percentage protein between Saanen and Saanen-Indigenous crossbred goats remained relatively stable in first and second lactations. Trials 1 & 2 involved Saanen goats in second, third, fourth, fifth and seventh lactations. Trial 3 consisted mostly of Saanen goats with a few Toggenburg-Saanen crosses, which were mostly in the first lactation, with a few goats in the second lactation. In another study overall protein percentage for Alpine and Nubian goats was 3.20 %. Nubian does produced a significantly ( $P < 0.05$ ) higher percentage protein than Alpine does (Zeng & Escobar, 1996).

**TABLE 5.7: PROTEIN PERCENTAGES IN THIS STUDY** (Table 4.61; Table 4.64 & Table 4.67).

| Trials and Products Used                     | Breed  | Protein %<br>Mean $\pm$ Standard Deviation |
|--|--|--|
| Trial 1: Curaclox LC<br>Treatment Group (T1) | Saanen                                       | 3.35 $\pm$ 0.59                            |
| Trial 1: Control Group<br>(C1)               |  | 3.24 $\pm$ 0.54                            |
| Trial 2: Spectrazol<br>Treatment Group (T2)  | Saanen and Saanen-<br>Toggenburg Crossbreeds | 3.07 $\pm$ 0.64                            |
| Trial 2: Control Group<br>(C2)               |  | 3.11 $\pm$ 0.41                            |
| Trial 3: Control Group (C)                   |  | 3.13 $\pm$ 0.21                            |
| Trial 3: Curaclox LC<br>Treatment Group (T1) |  | 3.24 $\pm$ 0.29                            |
| Trial 3: Rilexine Treatment<br>Group (T3)    |  | 3.30 $\pm$ 0.25                            |

#### 5.6.2.1 Curaclox LC from Trial 1 Only

In Trial 1 there was no significant difference ( $P = 0.992$ ) of percentage protein between treatment (T1) and control (C1) groups (Table 4.61). There was also no significant difference ( $P = 0.701$ ) between percentage protein of treatment and control groups between the three respective treatment times (Table 4.61). Therefore in Trial 1, treatment with Curaclox LC (T1) did not affect the percentage protein in goat milk.

#### 5.6.2.2 Trial 2: (Spectrazol)

In Trial 2 there was no significant difference ( $P = 0.885$ ) of percentage protein between treatment (T2) and control (C2) groups, (Table 4.64). There was also no significant difference ( $P = 0.973$ ) between percentage protein of treatment and control groups between the three respective treatment times (Table 4.64). Therefore in Trial 2, treatment with Spectrazol (T2) did not affect the percentage protein in goat milk.

#### 5.6.2.3 Trial 3: (Curaclox LC & Rilexine)

In Trial 3 there was a significant difference ( $P = 0.054$ ) of percentage protein between treatments, (T1) Curaclox LC, (T3) Rilexine and control (C) at the 5% level of significance (Table 4.67). There was also a significant difference ( $P < 0.001$ ) between the percentage protein of treatments (T1) Curaclox LC, (T3) Rilexine and control (C), between the three respective treatment times (Table 4.67). Therefore in Trial 3 percentage protein was significantly different between treatment groups to begin with. However, for the control (C) group percentage protein decreased significantly during treatment and then increased significantly when antibiotic residues were no longer present in the milk. In the group treated with Curaclox LC (T1), there was a significant increase in percentage protein during treatment and a significant decrease when antibiotic residues were no longer present in the milk. In the group treated with Rilexine (T3), there was a significant decrease in percentage protein during treatment and a significant increase when antibiotic residues were no longer present in the milk. The untreated control group also showed significant differences in percentage protein between the three respective treatment times. This could have shown that the difference in percentage protein for T1 (Curaclox LC) and T3 (Rilexine) were not caused by the antibiotic treatment, but perhaps by nutritional changes on the commercial dairy farm. In Trial 1 treatment with Curaclox LC did not affect the percentage protein significantly between the three respective treatment times. However, the differences in percentage protein between the treatments, (T1) Curaclox LC, (T3) Rilexine and control (C), and between treatments (T1) Curaclox LC, (T3) Rilexine and control (C), between the three respective treatment times were statistically significant differences. These differences in percentage protein were probably not biologically meaningful.

#### 5.6.2.4 Curaclox LC from Trials 1 & 3 Combined

There was no significant difference ( $P = 0.069$ ) at the 5% level of significance in mean protein percentage between treatment (T1) and control (C) groups for Curaclox LC between treatments for Trials 1 & 3 (Table 4.70). However, if a larger number of samples had been used perhaps there would be a significant difference at the 5 % level of significance. There was no significant difference ( $P = 0.611$ ) in mean protein percentage of treatment and control groups at different treatment times, before, during and after treatment (Table 4.70). This was the case for Curaclox LC in Trial 1 (Table 4.61). However, in Trial 3 there was a significant difference in percentage protein between treatment and control groups ( $P = 0.054$ ) at the 5 % level of significance and between treatment and control groups between different treatment times ( $P < 0.001$ ) at the 0.1 % level of significance (Table 4.67). Times, before, during and after treatment (Table 4.70) were equivalent to treatment times Rx1, 12h & 132h respectively in Trial 3 and -12h, 12h & 108h respectively in Trial 1.

#### **5.6.3 Lactose**

In another study overall lactose percentage for Alpine and Nubian goats was 4.41 % (Zeng & Escobar, 1996).

**TABLE 5.8: LACTOSE PERCENTAGES IN DIFFERENT STUDIES.**

| Authors                     | Breed                             | Lactose %<br>Mean $\pm$ Standard Error |
|-----------------------------|-----------------------------------|--|
| Donkin <i>et al.</i> , 2000 | Saanen goats                      | 4.55 $\pm$ 0.19                        |
| Donkin <i>et al.</i> , 2000 | Saanen-Indigenous crossbred goats | 4.82 $\pm$ 0.12                        |

Percentage lactose remained the same between goats of first and second lactations, but differed between goats in first lactation in 1988 and goats in first lactation in 1989 (Donkin, *et al.*, 2000).

**TABLE 5.9: LACTOSE PERCENTAGES IN THIS STUDY** (Table 4.62; Table 4.65 & Table 4.68).

| Trials and Products Used                  | Breed                                    | Lactose %<br>Mean $\pm$ Standard Deviation |
|---|--|--|
| Trial 1: Curaclox LC Treatment Group (T1) | Saanen                                   | 4.32 $\pm$ 0.94                            |
| Trial 1: Control Group (C1)               |  | 4.22 $\pm$ 0.43                            |
| Trial 2: Spectrazol Treatment Group (T2)  |  | 4.33 $\pm$ 0.71                            |
| Trial 2: Control Group (C2)               |  | 4.19 $\pm$ 0.64                            |
| Trial 3: Control Group (C)                | Saanen and Saanen-Toggenburg Crossbreeds | 4.58 $\pm$ 0.17                            |
| Trial 3: Curaclox LC Treatment Group (T1) |  | 4.69 $\pm$ 0.22                            |
| Trial 3: Rilexine Treatment Group (T3)    |  | 4.69 $\pm$ 0.16                            |

#### 5.6.3.1 Curaclox LC from Trial 1 Only

In Trial 1 there was no significant difference ( $P = 0.131$ ) of percentage lactose between treatment (T1) and control (C1) groups (Table 4.62). There was also no significant difference ( $P = 0.156$ ) between percentage lactose of treatment and control groups between the three respective treatment times (Table 4.62). Therefore in Trial 1, treatment with Curaclox LC (T1) did not affect the percentage lactose in goat milk.

#### 5.6.3.2 Trial 2: (Spectrazol)

In Trial 2 there was no significant difference ( $P = 0.426$ ) of percentage lactose between treatment (T1) and control (C1) groups (Table 4.65). There was also no significant difference ( $P = 0.780$ ) between percentage lactose of treatment and control groups between the three respective treatment times (Table 4.65). Therefore in Trial 2, treatment with Spectrazol (T2) did not affect the percentage lactose in goat milk.

#### 5.6.3.3 Trial 3: (Curaclox LC & Rilexine)

In Trial 3 there was no significant difference ( $P = 0.755$ ) of percentage lactose between treatments (T1) Curaclox LC, (T3) Rilexine and control (C) (Table 4.68). However, there was a significant difference ( $P = 0.001$ ) between the percentage lactose of treatments (T1) Curaclox LC, (T3) Rilexine and control (C), between the three respective treatment times, at the 0.1% level of significance (Table 4.68). In the treatment groups (T1) Curaclox LC, (T3) Rilexine and control (C) group the percentage lactose decreased significantly during treatment and increased when antibiotic residues were no longer present in the milk. However, similar differences in percentage lactose were shown in the treatment groups (T1) and (T3) and the untreated control groups between the three respective times.

Therefore, the difference in percentage lactose between the three respective treatment times was not due to treatment with Curaclox LC (T1) or due to treatment with Rilexine (T3). In Trial 1 treatment with Curaclox LC did not affect the percentage lactose in goat milk. These differences between percentage lactose of treatment groups between the three respective treatment times were statistically significant differences. However, these differences were probably not biologically meaningful.

#### 5.6.3.4 Curaclox LC from Trials 1 & 3 Combined

There was no significant difference ( $P = 0.494$ ) in mean protein percentage between treatment (T1) and control (C) groups for Curaclox LC between treatments for Trials 1 & 3 (Table 4.71). There was no significant difference ( $P = 0.474$ ) in mean protein percentage of treatment and control groups at different treatment times, before, during and after treatment (Table 4.71). This was the case for Curaclox LC in Trial 1 (Table 4.62). However, in Trial 3 there was a no significant difference in percentage protein between treatment and control groups ( $P = 0.755$ ), but there was a significant difference ( $P < 0.001$ ) between treatment and control groups between different treatment times (Table 4.68). Times, before, during and after treatment in (Table 4.71) were equivalent to treatment times Rx1, 12h & 132h respectively in Trial 3 and -12h, 12h & 108h respectively in Trial 1.

### **5.7 Analysis of Withdrawal Periods for Goats Compared to Withdrawal Periods Recommended for Use in Cattle with or without the 24h Safety Margin**

The additional statistical tests shown in Table 4.72 were necessary to check if the withdrawal period recommended for use in cattle (with or without the 24h safety margin) had an impact on the comparisons with withdrawal periods for goats without the 24h safety margin, as determined in this research.

The raw data from the trials done on cattle was not available for this research. The withdrawal periods recommended for use in cattle have a 24h safety margin added to the longest withdrawal period in the trial. Therefore, by subtracting the 24h safety margin from the withdrawal period recommended for use in cattle an estimated withdrawal period was obtained for cows. This estimated withdrawal period for cows was compared to the withdrawal periods of intramammary antibiotics for goats as determined by different methods.

There were significant differences in all the one sample t-tests between withdrawal periods for goats compared to withdrawal periods recommended for use in cattle without the 24h (safety margin). Not all of these tests were significant when the comparison was with withdrawal periods as recommended for use in cattle.

## CONCLUSION

Antibiotic withdrawal periods on goat milk were different from those recommended for use in cattle for each of the products used and for the different intramammary antibiotics used. The withdrawal periods recommended for use in cattle have a 24h safety margin added to the longest withdrawal period in the trial. However, in this research, 24h safety margins were not added to withdrawal periods. Therefore, in practice a 24h safety margin should be added to all withdrawal periods determined in this research. However additional significant tests were carried out comparing withdrawal periods of intramammary antibiotics for goats in this research with withdrawal periods recommended for use in cattle with or without the 24h safety margin. There were significant differences in all the one sample t-tests between withdrawal periods for goats compared to withdrawal periods recommended for use in cattle without the 24h (safety margin). Not all of these tests were significant when the comparison was with withdrawal periods as recommended for use in cattle. Withdrawal periods for use in cattle were estimated by subtracting the 24h safety margin because the raw data for cattle was not available for this research.

Withdrawal periods were affected by volume of milk produced. High producers had a shorter withdrawal period than low producers treated with the same intramammary antibiotic. However, treatment with intramammary antibiotics did not significantly affect the volume of milk produced.

This research has shown the importance of measuring the volume of milk secreted, and any further research must take this into account. Further research is required to assess the effect of milk production volume on withdrawal periods when comparing withdrawal periods of different products.

There was a significant difference in withdrawal period as measured by TRIS ( $P = 0.009$ ) and colour dye ( $P = 0.036$ ) for mostly low producing Saanen goats in Trial 1. In the case of goats with clinical mastitis, the withdrawal period as measured by TRIS was associated positively with sampling time. For example, a sample taken at the evening milking was associated with a longer withdrawal period than a sample taken at the morning milking. There was no obvious reason for this relationship. In the case of goats with clinical mastitis, the withdrawal period as measured by TRIS was associated with damage to the udder secretory tissue as indicated by palpation. Where tissue damage was apparent, the withdrawal period was longer. In the case of goats with clinical mastitis, the withdrawal period as measured by TRIS was associated negatively with the presence of floccules in the milk. If floccules were present, the withdrawal period was shorter, according to the regression model. The regression model also indicated a negative association between milk volume and withdrawal period as measured by TRIS. This might have been because of a dilution effect. Further research is necessary to determine withdrawal periods of different intramammary antibiotics on goats with clinical mastitis.

The blue dye of Curaclox LC indicated the withdrawal of antibiotic residues for goats was similar to that for cows and confirmed that Curaclox LC had a longer withdrawal period in goat milk than in cow milk, for low producing goats.

Withdrawal periods were not affected by stage of lactation or parity.

However, Somatic Cell Counts (SCC), were affected by stage of lactation, parity and by the absence or presence of bacteria, indicating an intramammary infection.

The most effective methods for diagnosing intramammary infection, before treatment, were microbiological tests, udder palpation and examining the milk for floccules. Using a strip cup, SCC, CMCT and conductivity were unreliable methods of mastitis diagnosis.

However, CMCT and SCC were indicators of udder irritation (tissue tolerance). The degrees of tissue tolerance differed for different intramammary antibiotics and for healthy goats and goats with clinical mastitis.

The variability in SCC was largely unexplained, and an increased SCC did not necessarily indicate an intramammary infection in goats, as it does in cows.

Therefore further, research is required to assess SCC and all possible factors affecting it, including breed, stage of lactation, parity, nutrition, vaccination and farm management.





Further research is also required to find a more reliable method for mastitis diagnosis apart from SCC, for example, NAGase.

Treatment with intramammary antibiotics did not affect the composition of the goat milk (percentage butterfat, protein and lactose). Although some statistically significant differences were shown, these statistical differences were not biologically meaningful.

In this research foremilk samples were used for practical reasons. Further research should determine whether the use of foremilk samples is the most appropriate for mastitis diagnosis, or whether whole milk, or strippings should rather be used.

Further research is also required to determine withdrawal periods of goat milk in the dry period, compared to those recommended for use in cattle for different dry period intramammary antibiotics.

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