A COMPARATIVE STUDY ON THE EFFECT OF *CHINA OFFICINALIS* 12CH AND *CHINA OFFICINALIS* 12CH–*PARATHYROID* 4CH COMPLEX ON BLOOD CALCIUM LEVELS OF MULTIPAROUS DAIRY COWS

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by

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DECLARATION

I, Jurgens Staats, declare that this dissertation is my own, unaided work. It is being submitted for a Master's Degree in Technology: Homoeopathy at the Technikon Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other Technikon or University.

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ABSTRACT

Hypocalcemia in dairy cows is one of the most common metabolic diseases in the dairy industry. The economical impact it has is substantial and includes losses from deaths, premature culling, treatment costs and decreased milk production. Furthermore, incidences of clinical and subclinical hypocalcemia increase the incidence of related pathologies such as mastitis, dystocia, retained foetal membrane, displaced abomasum, ketosis and decreased fertility later in lactation. This study therefore addresses an area of great economical importance.

Homoeopathic medicines are relatively inexpensive and leave no residues or poisonous substances in the milk or tissues of dairy cows.

The aim of this study was to determine the effect of the respective remedies, *China officinalis* 12CH and *China officinalis* 12CH – *Parathyroid* 4CH, on the calcium metabolism of multiparous dairy cows as well as factors associated with hypocalcemia. Serum calcium, phosphate and magnesium levels as well as milk production and incidence of mastitis were recorded.

Eighteen multiparous dairy cows (Holstein breed) were used for the purposes of the research study. The study was carried out on the farm Welkom, District Christiana, from 17 November 2003 to 31 January 2004.

The eighteen subjects were randomly divided into three groups of six. Cows in group A received a simplex remedy (*China officinalis* 12CH), cows in group B received a complex remedy (*China officinalis* 12CH – *Parathyroid* 4CH), and cows in group C received a medically inactive substance (group C served as the control group). Medication was administered orally on a twice-weekly basis.

Results obtained from mastitis and mortality rates were recorded as a yes or no value and directly analysed. Results regarding milk production were compared

and analysed by using the ANOVA method. Results regarding serum calcium, magnesium and phosphate levels were compared and analysed by using both the ANOVA method as well as the Kruskal-Wallis test.

Results obtained from this study provided no statistically significant differences between the three groups used in the study. Although results regarding mastitis (group A) and mortality (group B) rates reflected negatively on the experimental groups, the number of subjects used in the study prevents one from drawing premature conclusions.

Recommendations regarding further studies related to hypocalcemia focuses on aspects regarding methodology as well as remedy and potency selection.

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NOMENCLATURE

Abomasum:

The fourth compartment and the glandular portion of the stomach of a ruminant.

Bovine Ketosis:

A common metabolic disease of cows which appears as a rule within a few weeks after parturition; characterized by hypoglycemia, ketonuria, loss of appetite, lethargy, loss of milk production, and rapid emaciation

Dystocia:

Difficult calving

Fat Cow Syndrome:

Excessively fat cows (condition score greater than four) are particularly susceptible to post-calving disorders. These cows typically have a reduced appetite before and after parturition. Decreased feed intake results in severe negative energy balance which may trigger metabolic events leading to clinical disorders.

Inanition:

Severe weakness and wasting as occurs from lack of food, defect in assimilation, or neoplastic disease.

Meteorism:

Swelling of the abdomen from gas in the intestinal or peritoneal cavity.

Multipara:

A female who has given birth to at least two offspring.

1 INTRODUCTION

Shortly before parturition in dairy cows, large amounts of calcium are removed from the blood stream and utilized in the mammary gland in the production of colostrum. Calcium demands in dairy cattle are at their nadir just prior to parturition. Thus, bone calcium resorption mechanisms become quiescent and intestinal calcium absorption mechanisms are passive just before parturition. Sudden calcium outflow occurs most commonly at the time of the initiation of lactation (Aiello ed., 1998; Roginski *et al.*, 2003;).

Calcium in the colostrum may be eight to ten times greater than in the blood supply. The rapid drop in calcium prior to parturition, and the failure of calcium absorption to increase fast enough after the onset of lactation, can predispose animals to hypocalcemia (Adams *et al.*, 1996; Van Horn *et al.*, 1992).

The average annual incidence of hypocalcemia in cows is about five to ten percent (Van Horn *et al.*, 1992; Whitaker, 2002), and two different studies have shown that two thirds of multiparous and one half of all adult dairy cows suffer from subclinical hypocalcemia at parturition each year (Beede *et al.*, 1992; Roginski *et al.*, 2003). The costs of this disease are very high due to clinical complications and milk production losses. Clinical hypocalcemia may bring about financial losses due to deaths, premature culling, treatment costs, and decreased milk production (about 14%) in the subsequent lactation. In addition, each episode of clinical hypocalcemia increases the risk for other parturient diseases, such as ketosis, displaced abomasum and coliform mastitis. As subclinical hypocalcemia is so widespread, the economical impact it has on the dairy industry may be far greater than that of clinical hypocalcemia (Roginski *et al.*, 2003). Therefore, any intervention that can beneficially impact this will be of great importance.

The term, Homoeopathy, is made up of the Greek words homoios, meaning 'like', and pathos, meaning 'illness'. Thus it is a method of medical treatment that uses substances which cause similar symptoms (in healthy individuals) to that of the symptoms treated (Salva, 2001), hence the law of similars (Hahnemann, 1997).

All individuals react differently to pain and illness. Homoeopathy takes this into account and therefore the totality of the individual's symptoms are evaluated (Salva 2001). The totality refers to the presenting symptoms as well as other individualising aspects like modalities, behaviour and general appearance.

By relating the law of similars (Hahnemann, 1997) as well as organotherapeutic principles (Anon. A, 2004) to hypocalcemia, homoeopathy may prove to be beneficial in the prevention of this metabolic disease. The use of *China officinalis* 12CH and *China officinalis* 12CH – *Parathyroid* 4CH complex comply with abovementioned laws.

1.1 Problem Statement

Hypocalcemia in dairy cows result in economical losses due to increased mortality rates and occurrence of related disorders, as well as decreased milk production (Roginski *et al.*, 2003). Current preventative measures may lack affordability, consistency, palatability, safety or simplicity (Aiello ed., 1998).

1.2 Hypothesis

China officinalis 12CH and *China officinalis* 12CH-*Parathyroid* 4CH support the physiological mechanisms for regulating serum calcium levels in multiparous dairy cows.

1.3 Purpose of the Study

The purpose of this research study is to determine and compare the effects of a simplex homoeopathic remedy (*China officinalis* 12CH) and a complex remedy (*China officinalis* 12CH-*Parathyroid* 4CH) respectively on the calcium levels of multiparous dairy cows. The comparison focuses mainly on blood calcium levels as well as other parameters associated with hypocalcemia in dairy cows.

2 LITERATURE REVIEW

2.1 Hypocalcemia in Perspective

Hypocalcemia (parturient paresis, paresis puerperalis, parturient apoplexy) is a non-febrile disease of adult dairy cows caused by an acute drop in blood calcium levels (Roginski *et al.*, 2003). Hypocalcemia is colloquially known as Milk Fever. This term is a misnomer because loss of body temperature control by the nervous system is one effect so cows are commonly cold rather than feverish (although they can get very hot if recumbent in the sun.) (Aiello ed., 1998; Roginski *et al.*, 2003; Whitaker; 2002).

Several factors are known to affect the incidence of hypocalcemia in dairy cattle. Older animals (second lactation or later) are more susceptible than are young cows (Kirkpatrick, 1998). Hypocalcemia occurs more frequently in Jerseys than in the other dairy breeds. High producing animals are more prone to hypocalcemia than low producers (Andrews *et al.*, 1992, Radostits, 2001, Roginski *et al.*, 2003). Cows with a previous history tend to have repeat cases of hypocalcemia (Schmidt *et al.*, 1988).

2.2 Signs Associated with Hypocalcemia

Hypocalcemia is divided into three stages based on clinical signs:

- Stage one has a short duration (less than one hour). Signs include loss of appetite, excitability, nervousness, hypersensitivity, weakness, weight shifting, and shuffling of the hind feet. Cows may be slightly ataxic, have fine tremors over the flank and loins, tetany without recumbency, and display ear twitching and head bobbing. Many cows at this stage will protrude their tongue when stimulated around the head (Rebhum, 1995).

- Stage two can last from one to twelve hours. The affected animal may turn its head into its flank or, if the head is extended, an S-shaped curve to the neck may be noted (Aiello ed., 1998; Andrews *et al.*, 1992). The animal appears dull and listless, has cold ears and a dry nose, exhibits incoordination when walking and muscular trembling and quivering are evident. Smooth muscle paralysis leads to gastrointestinal stasis, which can manifest as bloat, failure to defecate, and loss of anal sphincter tone. A decrease in body temperature is common. The heart rate will be rapid exceeding one hundred beats per minute. Pupils may be dilated and unresponsive to light due to atony of the pupillae muscle (Andrews *et al.,* 1992; Roginski *et al.*, 2003).

- Stage three is characterised by the animal's inability to stand and a progressive loss of consciousness leading to coma. Heart sounds become nearly inaudible and the heart rate increases to one hundred and twenty beats per minute or more. Cows in stage three will not survive for more than three hours without treatment (Adams *et al.*, 1996; Aiello ed., 1998). Death can be due to respiratory muscle paralysis, but suffocation is more often caused by bloat (Andrews *et al.,* 1992; Rebhum, 1995).

- Decreased serum calcium and phosphate levels, and increased serum magnesium levels are the most notable chemical changes that occur during hypocalcemia (Adams *et al.,* 1996).

2.2.1 Differential Diagnosis

Although hypocalcemia is a fairly common cause of recumbency in dairy cows there are other conditions that may cause similar symptoms. These conditions are: Toxaemia from mastitis or metritis, calving paralysis, physical injury (such as pelvic fracture, obturator paralysis, leg bone fracture, and ruptured gastrocnemius tendon), hypomagnesaemia, downer cow syndrome due to

pressure necrosis, inanition, pregnancy toxaemia, acidosis, hypothermia, bovine spongiform encephalopathy, and fat cow syndrome (Andrews *et al.*, 1992; Roginski *et al.*, 2003).

2.3 Treatment Protocol for Clinical Hypocalcemia

To avoid permanent muscular and nervous damage, treatment is commenced as soon as possible. Cows in stage one are usually given an oral or subcutaneous dose of calcium. Subcutaneous administration may however be inefficient if the cow is dehydrated, and peripheral circulation is insufficient. (Roginski *et al.*, 2003).

Cows in stages two and three require immediate intravenous calcium administration. Because calcium is cardiotoxic, the administration of calcium solution is given slowly while auscultating the heart. Should any dysrhythmias or bradycardia develop, the administration is halted until the heartbeat has returned to normal (Aiello ed., 1998). In addition to calcium administration, dairy cows in a lateral recumbent position are positioned in sternal recumbency to prevent regurgitation and aspiration (Van Horn *et al.*, 1992).

Intravenous administration of calcium raises the level of blood calcium well beyond the physiological threshold. The effect is a shutdown of Parathyroid Hormone (PTH) production and an increase in calcitonin release (Roginski *et al.*, 2003). This leaves the animal susceptible to a relapse once the exogenous calcium is eliminated from her system. Twenty five to thirty percent of cows that respond to treatment are prone to relapse within twenty four to forty eight hours afterwards (Aiello ed., 1998; Roginski *et al.*, 2003).

2.4 Subclinical Hypocalcemia

Subclinical hypocalcemia refers to metabolic calcium deficits without paresis (Van Horn *et al.*, 1992). The incidence of subclinical hypocalcemia is estimated by Roginski *et al.* (2003:824) to affect about fifty percent of all adult dairy cows. Subclinical hypocalcemia may predispose cows to present with retained placenta, mastitis, ketosis, dystocia, prolapsed uterus, metritis and displaced abomasa. In addition to abovementioned pathologies, dairy cows are also at risk of decreased dry matter intake after calving, decreased milk production and decreased fertility later in lactation (Radostits, 2001; Roginski *et al.*, 2003; Sanches, 1996).

2.5 The Physiology of Calcium Metabolism

2.5.1 Negative Feedback System

The physiology involved in calcium metabolism works according to a negative feedback system (Figure 2.5.2.1). Any decrease in ionized blood calcium concentration will cause the parathyroid glands to secrete PTH and inhibit the C-cells in the thyroid gland (Nielsen and Duke, 1991).

2.5.2 PTH

The chief cells of the parathyroid glands monitor the circulating calcium ion concentrations. When the calcium concentration of the blood falls below normal, the chief cells secrete PTH (Figure 2.5.2.1).

The net result of PTH secretion is an increase in calcium concentration in body fluids (Martini *et al.*, 1998). PTH has four major effects:

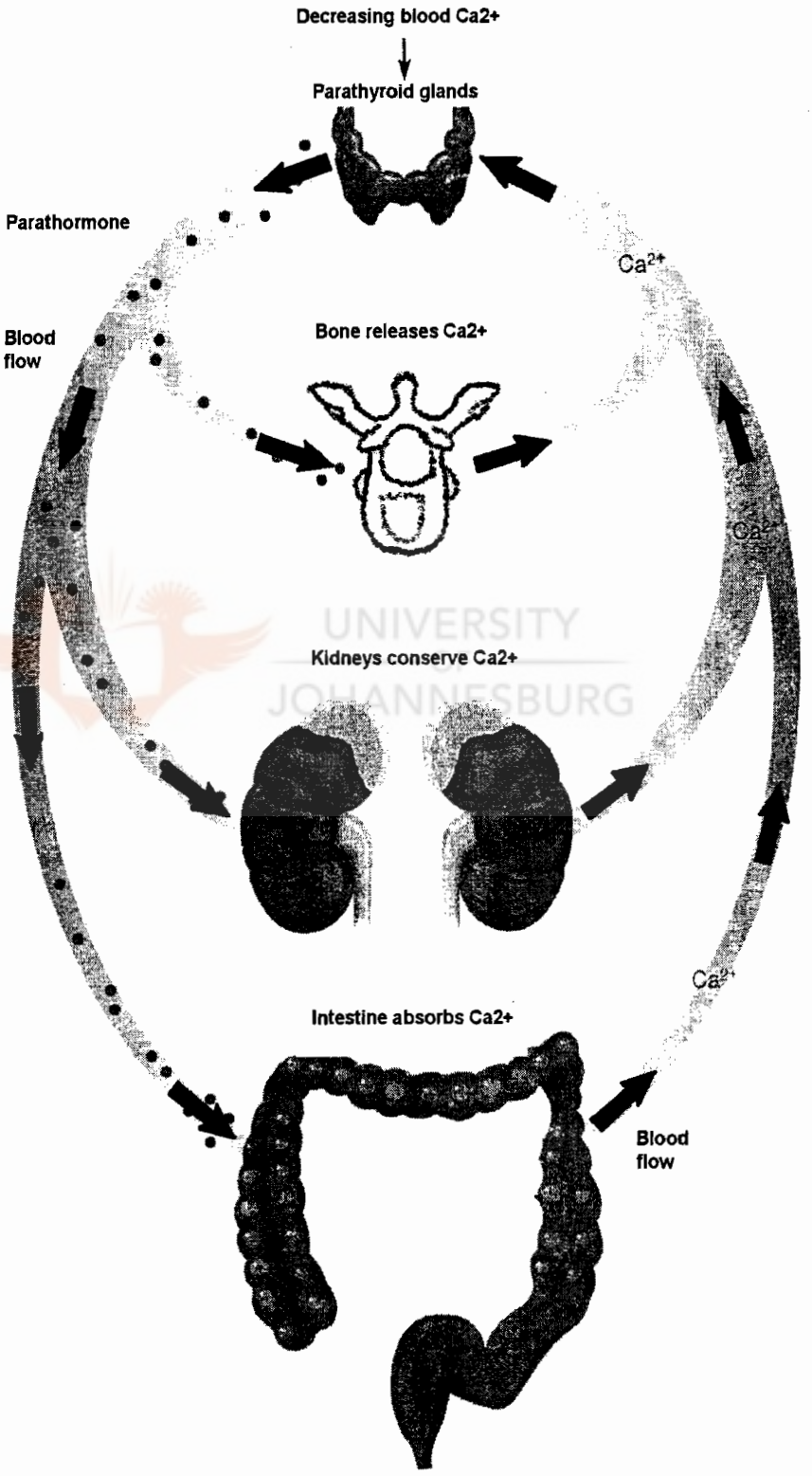

Figure 2.5.2.1 Hormonal calcium regulation (Miller and Harley, 1996)

2.5.2.1 Osteoclast Stimulation

PTH stimulates osteoclasts, accelerating mineral turnover and the release of calcium from bone (Martini *et al.*, 1998; Miller and Harley, 1996).

2.5.2.2 Osteoblast Inhibition

PTH inhibits osteoblasts, reducing the rate of calcium deposition in bone (Martini *et al.*, 1998; Miller and Harley, 1996).

2.5.2.3 Renal Calcium Retention

PTH reduces urinary excretion by causing renal tubular reabsorption of calcium (Martini *et al.*, 1998; Miller and Harley, 1996).

2.5.2.4 Calcitriol Formation

PTH stimulates the formation and secretion of calcitriol at the kidneys. Calcitriol is the activated form of Vitamin D3 (1,25-dihydroxyvitamin D) and in general compliments the action of PTH.

One major effect of calcitriol is the enhancement of calcium and phosphate absorption by the digestive tract (Martini *et al.*, 1998; Miller and Harley, 1996).

2.6 Additional Clinical Pathology Related to Calcium Metabolism

2.6.1 Magnesium

Low blood magnesium can reduce PTH secretion from the parathyroid glands. A magnesium deficit also reduces tissue responsiveness to PTH by inducing conformational changes in the PTH receptor. High levels of dietary potassium in turn reduce ruminal magnesium absorption by increasing the transruminal electrical potential. The rate of magnesium absorption is inversely related to the transruminal potential (Rebhum, 1995; Roginski *et al.*, 2003).

2.6.2 Phosphate

Excessive dietary phosphate intake during late gestation can induce milk fever by raising blood phosphorus concentrations to the point that phosphorus directly inhibits renal synthesis of calcitriol (Roginski *et al.*, 2003).

The amount of phosphate absorbed in the gastrointestinal tract is directly related to the amount of phosphate ingested. Phosphate is absorbed in the presence of calcitriol and plays a role in the acid-base buffer system (Clark, 2004).

Besides causing renal tubular calcium reabsorption, PTH also results in rapid loss of phosphate in the urine by diminishing renal tubular reabsorption of phosphate ions (Guyton and Hall, 1997).

2.6.3 Oestrogen

Oestrogen is a potent inhibitor of osteoclast activity. Blood oestrogen concentrations rise dramatically at the end of gestation and may blunt the effects of PTH on bone resorption (Roginski *et al.*, 2003)

2.6.4 Cortisol

Animals in a hypocalcemic state typically present with elevated cortisol concentrations (Roginski *et al.*, 2003).

Prior to parturition cortisol levels rise as a mechanism to help the fetus cope with physical and mental stress associated with the birthing process and entry into a new world (Guyton and Hall, 1997).

Excessive cortisol levels cause immunosuppression in periparturient animals and partially explains the increased susceptibility of hypocalcemic cows to infectious diseases like mastitis (Roginski *et al.*, 2003).

2.6.5 Glucose

The inability of pancreatic beta cells to secrete insulin when extracellular calcium levels are low combined with elevated cortisol levels explains why hyperglycaemia is also evident in hypocalcemia (Roginski *et al.*, 2003).

2.6.6 Fatty Liver

The liver is occasionally enlarged and infiltrated with fat resulting in a yellow discolouration. Although fatty liver is not pathognornic, cows presenting with it are more susceptible to hypocalcemia (Andrews *et al.*, 1992).

2.7 Current Preventative Measures in Hypocalcemia

2.7.1 The Dietary Cation-Anion Balance

The prevention of hypocalcemia relies mainly on the correct feeding ratios. Hypocalcemia can occur in cows as a result of excessive dietary cations. These cations, primarily potassium and sodium, induce metabolic alkalosis in the cow that impairs the physiological activity of PTH so that bone resorption and production of calcitriol is impaired (Bushinsky and Nilsson, 1996; Roginski *et al.*, 2003).

Acidic diets promote bone mobilization of calcium (a strong cation) because bone acts as a buffer against systemic acidity (Van Horn *et al.*, 1992:196). Therefore a method of preventing and controlling hypocalcemia is by balancing dry cow ratios for anions and cations. The dietary cation-anion balance equation is most often used to determine the ratio of feed. The most commonly fed salts are ammonium sulphate, calcium sulphate, magnesium sulphate, ammonium chloride, calcium chloride and magnesium chloride (Adams *et al.*, 1996).

After discontinuation of anionic salt feeding, there is delayed rebound hypocalcemia. In most circumstances no clinical effects are seen because the rebound occurs several days after calving when the dry matter intake of the cow is sufficient to supply the calcium needed for lactation (Rebhum, 1995)

One major drawback to the inclusion of anionic salts into the diets of dairy cows is that most are very unpalatable and therefore have a tendency to reduce dry matter intake. As negative energy balance is already a major problem for the cow following parturition, reduced dry matter intake may worsen the situation (Aiello ed., 1998; Oetzel and Barmore, 1993; Radostits, 2001).

Anions like hydrochloric acid have been proven to be very successful in the prevention of hypocalcemia. In its liquid form, however, hydrochloric acid remains dangerous, and problems with handling it will have to be overcome to allow widespread use of hydrochloric acid to prevent hypocalcemia (Goff and Horst, 1998).

2.7.2 Dietary Calcium Restriction

Feeding ratios low in calcium during late pregnancy has been helpful in stimulating parathyroid activity and reducing the incidence of hypocalcemia.

Such rations, however, are difficult to devise and, if continued for long periods in heavy-milking cows, may result in dangerous depletion of skeletal mineral reserves (Aiello ed., 1998; Radostits, 2001).

2.7.3 Calcitriol

Injection of calcitriol and its metabolites from eight to two days before parturition can also be used. If administration is stopped more than four days before calving, the cow is more susceptible to becoming hypocalcemic.

The milk produced by injected cows, however, cannot be used for human consumption for twenty-eight days after treatment; consequently the milk has to be discarded.

Furthermore, this method cannot be relied on to guarantee hypocalcemia prevention as it is not always possible to accurately predict the time of calving (Aiello ed., 1998; Andrews *et al.*, 1992; Whitaker, 2002).

2.7.4 Synthetic Bovine PTH

It is thought that intravenous administration of synthetic bovine PTH 60 hours before parturition or intramuscularly six days before parturition may prove to be more effective than vitamin D3 and its metabolites in preventing hypocalcemia (Aiello ed., 1998).

2.7.5 Suppression of Milk Production

Reducing milk production in order to prevent loss of calcium is another approach to prevent hypocalcemia. Air is blown into the udder of the cow to prevent formation and removal of milk.

This practice may aggravate latent mammary infections and increase the incidence of mastitis by introducing bacteria into the udder and promoting stasis of milk (Aiello ed., 1998; Whitaker, 2002).

2.8 Homoeopathy

2.8.1 Principles of Homoeopathy

Similia Similibus Curentur, translated as 'let likes be cured by likes', the Law of Similars, was created by Hippocrates and is a fixed and fundamental law from which Homoeopathy derives its name (Razlog, 2000; Salva, 2001).

According to this law, any therapeutic substance may be homoeopathic if it is given on the understanding that it may create cure because in other circumstances it may cause the same symptoms i.e. any medicine that is capable of provoking certain symptoms in a healthy person is capable of curing similar symptoms which occur spontaneously in a sick person (Razlog, 2000).

This law was rediscovered by Samuel Hahnemann in the late eighteenth century after translating an article written by an English doctor, William Cullen, on the subject of cinchona bark (Salva, 2001).

Following this discovery, Hahnemann tested more than a hundred remedies over a fifty-year period. The toxicity of some substances however remained a problem. The solution was to reduce the doses of the remedies until toxic risk was eliminated. Later on remedies were reduced to such an extent that it was diluted beyond Avogadro's number (6.02×10^{-23}) (Salva, 2001).

In order to increase the efficacy of the diluted substance, Hahnemann believed that the addition of kinetic energy would increase its therapeutic action. This process of potentization or dynamization is thought to bring forth the substance's hidden medicinal powers (Razlog, 2000).

2.8.2 The Law of Similars

A medicine which, in its action on the healthy body, has demonstrated its power of producing the greatest number of symptoms *similar* to those observable in the case of disease under treatment, does also, in doses of suitable potency and attenuation, rapidly, radically and permanently remove the totality of the symptoms of this morbid state. All medicines cure, without exception, those diseases whose symptoms most nearly resemble their own (Hahnemann, 1997).

2.8.3 Preparation of Homoeopathic Medicines

The preparation of homoeopathic medicine is done according to certain specifications depending on the type of vehicle used and scale of dilution required. The most commonly used scales are the decimal, centesimal and fifty millesimal scales.

In the case of preparing a liquid carrier with a centesimal scale the process will entail adding one part of a medically active substance to ninety-nine parts of carrier substance (alcohol or water) and then rapidly beating it against an elastic body ten times. The result of the described process will be a medication in the first centesimal potency (1CH). To increase the potency, one need to repeat the abovementioned steps, using the prepared potency as the medically active substance (German Homoeopathic Pharmacopoeia, 2001).

2.8.4 Organotherapy

Organotherapeutic medicines are prepared from healthy organs, tissues or metabolic factors. The collected materials are processed either fresh, or from a freeze-dried state. Organotherapeutic substances are then further prepared according to the standard potentizing methods (Driehsen *et al.*, 1997).

The first principle of organotherapy is concerned with cellular specificity (glandular or tissue related) thus: *The organ acts upon the organ.* The second principle of organotherapy is the triphasic activity of potencies of homologous preparations, thus: *Low potencies (4CH) stimulate, Medium potencies (7CH) regulate, and High potencies (9CH) are depressant.* There is however a variability in the action of different potencies in relation to individual sensitivity of patients, and some patients may experience the opposite effect (Anon. A, 2004).

The debate on the relationship between prions and new variety Creutzveldt-Jakob disease has lead some countries to ban the use of organ remedies. Prions are thought to be agents that cause diseases. The theory of pathology is that it is protein-based and nucleic acid (DNA) free. Its evolution is linked to a sheep disease called Scrapie. Cows fed processed remains of infected sheep and developed Bovine Spongiform Encephalopathy, the chain continues up to human consumption. Conflicting theories and lack of agreement amongst scientists make the mechanism of action unclear (Bolton and Somerville, 2004).

2.8.5 Homoeopathy for Animals

As with humans, the philosophy and principles of homoeopathy apply to animals. Correct homoeopathic prescribing for animals would require one to be guided by the totality of the signs and symptoms presented and not only by the name of a disease. Totality of symptoms refers to the presence of physical signs and symptoms as well as causes and individualizing factors like modalities, time of aggravation and behaviour.

One aspect that differs from a conventional human homoeopathic practice is that animals are unable to talk, thus it is impossible to elicit information (like the nature of the pain etc.). An animal is treated according to the symptoms shown, its behavior, its build and the specific characteristics of the animal (Verkade, 2001:11).

Concerns about the use of chemical substances to promote optimum farming capacity has led to consideration of alternative treatment of common problems (Salva, 2001; Verkade 2001). One example would be that the milk of a sick cow treated with antibiotics is unusable for several weeks for health reasons, whilst it remains wholesome after homoeopathic treatment (Salva, 2001)

Various homoeopathic studies conducted on animals have produced positive results and warrants further research in agriculture.

Studies done by Leistner (2003) and Mullinder (2003) on broiler chickens regarding the treatment of aspergillosis and vaccinosis respectively have both shown positive results. Leistner (2003) concluded that subjects included in treatment groups have shown a statistically significantly higher mean live mass compared to that of the control group. Furthermore the homoeopathic remedies appeared to have positively affected both feed conversion rates and performance efficiency factors. Mullinder (2003) reached similar conclusions regarding his study.

A study done by Van Niekerk (2002) on pre-weaned piglets regarding enteric disease have shown promising results. Although this study could not produce a significant difference in mortality rate between the experimental group and the control group, a difference in weaning weights reflected positively on the experimental group.

Jeannes (2001) conducted a study on dogs regarding the treatment of intestinal parasites. Conclusions drawn from this study was that results of the homoeopathic substance administered compared favourably to that of conventional treatment. Although it was established that the homoeopathic substance administered had a slower clearance rate, the cost of treatment was shown to be 57,4% less compared to that of conventional treatment.

The abovementioned studies have proven homoeopathy to be an effective and economical healing modality regarding animal health.

2.9 Remedy Selection

2.9.1 The Basis of Remedy Selection

According to Verkade (2001:15) the first step towards successful prescription starts with good observation. One need to take note of discharges, temperature, swellings, mobility, appetite, thirst and behavior.

The cause of morbidity is also important. Origin of disease can be linked to injury, poisoning, specific disease, severe weather conditions, vaccination, shock etc.

Modalities like temperature, motion and preferences are to be taken into account. Periodicity and duration of disease are two aspects that should not be neglected.

All abovementioned aspects are aids to comply with the law of similars as set out by Hahnemann (1997).

2.9.2 Repertorization

A repertory is a place (book or software) where information is stored or categorized so that it can be retrieved more easily. It is an index of symptoms, with a listing of all of the remedies known to be associated with each particular symptom. Thus it is a symptom-remedy cross-reference (Razlog, 2000; Rowe, 1998).

The purpose of the repertory is to help you find the correct remedy for a given case (Rowe, 1998).

A rubric is a key word identifying symptoms in the repertory (Razlog, 2000). Remedies listed under a particular rubric are graded according to how strong, clear and commonly they are associated with that particular symptom (Rowe, 1998).

The process of choosing symptoms and combining these to choose the right remedy is called *repertorization* (Rowe, 1998)

2.9.2.1 Rubrics Considered

The rubrics that were taken into account when deciding the most appropriate remedy were based upon the signs associated with hypocalcemia as well a related disorders.

Thus gynecological conditions such as prolapsed uterus, retained placenta, metritis, mastitis, decreased milk production and general complaints during delivery were taken into consideration. Other important signs considered were ketosis, diminished appetite, a slow inactive stomach, bloating and lack of vital heat.

Characteristic signs like cold ears, sunken eyes, general muscle twitching and slowness of neurological function could not be ignored.

Repertorization is used as a tool to bring up suggestions rather than giving an absolute correct answer. The results thus are open to personal interpretation.

Although the repertorization (Appendix A) was done from Schroyens (2001), which was designed for human use, the aspects taken into account were based on objective observations made by leaders in the dairy science industry.

2.9.3 *China officinalis*

Through the process of repertorization and comparison of remedies, China officinalis featured prominently as a remedy suitable for the symptoms of hypocalcemia.

2.9.3.1 Brief History of *China officinalis*

Cinchona bark (origin of quinine, used to treat malaria) was to Hahnemann what the falling apple was to Newton and the swinging lamp to Galileo. Dissatisfied with the explanation of the action of the bark, Hahnemann took it himself and a symptom picture similar to malaria ensued (Murphy, 1995). Thus it was quinine that revealed homeopathy to Hahnemann (Tyler, 1992). Hahnemann did not *develop* malaria, but experienced symptoms *similar* to malaria. Hence *the law of similars* was discovered (Hahnemann, 1997).

2.9.3.2 Toxicology of *China officinalis*

The toxicology of quinine (derived from *China officinalis*) has been well established:

- Death caused by overdose usually follows renal failure, acute hemolytic anaemia, and respiratory arrest (Haddad *et al.*, 1998). It has been mentioned before that mortality in hypocalcemia may be caused by suffocation. The role of the kidneys in calcium metabolism via vitamin D has also been reported.
- Medically it is used for chloroquine-resistant malaria and nocturnal cramps in patients with myotonia (Haddad *et al.*, 1998).
- On skeletal muscle, it decreases tetanic response, excitability of the motor endplate, and calcium distribution (Haddad *et al.*, 1998; Martindale, 1993).

- Inappropriate insulin release caused by quinine may also lead to symptomatic hypoglycaemia (Martindale, 1993; Schonwald, 2001).
- Cardiovascular toxicity includes conduction disturbances and arrhythmias (Haddad *et al.*, 1998).

2.9.3.3 Homoeopathic Indications of *China officinalis*

The dynamics of this remedy in homoeopathic potency cover the hypocalcemic condition to a large extend. The most prominent indication for China officinalis is debilitated, weakened subjects caused by loss of vital fluids. Mechanisms for losing vital fluids include hemorrhaging, diarrhoea, vomiting, *overlactation and galactorrhoea* (Macleod, 1995; Murphy, 1995; Morrison, 1993; Tyler, 1992; Verkade, 2001; Vermeulen, 2002). In addition to the abovementioned correlation, China officinalis is indicated as follows:

- The face appears to be sunken with hollow eyes and dilated pupils. The subject presents with a hot head and cold extremities (Murphy, 1995; Vermeulen 2001). Coldness of the ears is also indicated (Schroyens, 2001:433).

- Respiratory symptoms are indicated by labored, slow respiration (Macleod, 1995). Vermeulen (2001) reports oppression of the chest as though from fullness in the stomach.

- Action on the cardiovascular system is noted to be a small, hard, rapid and irregular pulse. The heart becomes feeble and tachycardia develops. The weakened circulation in turn leads to complete relaxation and collapse of this system (Macleod, 1995; Tyler, 1992; Vermeulen, 2001).

- The gastrointestinal tract is greatly affected by China officinalis. Colic flatulence is characterized by being ameliorated when bending double

(Morrison, 1993; Murphy, 1995; Vermeulen, 2001). A great degree of bloat is present in the abdomen. Murphy (1995) describes the abdomen as being tympanic and Morrison (1993) indicates flatulent distention of the abdomen (almost to the point of bursting). A lack of tone is mostly responsible for the constipation indicated in this remedy seeing that defecation is difficult despite having soft stool (Murphy, 1995; Vermeulen, 2001). The stomach is slow and inactive with weak digestion (Murphy, 1995; Vermeulen, 2001). Anorexia is present with an aversion to all food. The appetite is described to be entirely lost (Murphy, 1995; Schroyens, 2001; Vermeulen, 2001). Despite having a diminished appetite, the subject may crave sour or sweet foods (Vermeulen, 2001).

- The musculoskeletal system presents with great debility, trembling and numbness. Epilepsy, chorea and paralysis also reflect *China officinalis* (Murphy, 1995, Verkade, 2001). According to Tyler (1992) emaciation is an indicated sign.

- Signs associated with the reproductive system include prolapsed uterus, retained placenta, inflammation of the uterus and complaints during delivery (Schroyens, 2001).

- Clinical indications given include constipation, debility, flatulence, labour, convulsions and breast-feeding disorders (Murphy, 1995; Verkade, 2001).

2.9.4 *Parathyroid*

Given the vital role of the parathyroid glands in calcium metabolism as discussed previously (2.5.1), the use of it in homoeopathic potency relates directly to hypocalcemia and it's physiology.

The use of *Parathyroid* has been based upon the organotherapeutic principle as set out by Anon. A (2004). Thus the use of the remedy in the fourth potency intends its action to stimulate the parathyroid gland. Despite the implication of Parathyroid 4CH not being based on the law of similars Julian (1990) and Murphy (1995) indicate the remedy *Parathyroid* as follows:

- Generally the remedy presents with weakness, hypotonia, fatigue and emaciation.

- The behaviour of the subject reflects mental confusion, agitation, and hypomania.

- Neuromuscular symptoms include paresis of the limbs, muscular hypotonia, and painful, difficult, dragging movements.

- Gastrointestinal features are presented as anorexia, slow digestion, belated gastric attacks, and atonic constipation.

- The cardiovascular system presents with tachycardia.

2.10 Remedies Considered

As has been discussed (2.9.2.1), repertorization will bring up suggestions to aid in the selection of the correct remedy. The choice of treatment ultimately lies with the individual and may not reflect the remedy that scores highest. In light of this, other remedies identified through repertorization that may have beneficial effects will be shortly discussed.

Remedies considered to match the clinical picture of hypocalcemia were *Calcarea phosphorica, Magnesia phosphorica,* and *Nux vomica.* Ultimately, only *China officinalis* and *Parathyroid* were selected for research purposes.

2.10.1 *Calcarea phosphorica*

The remedy, by direct implication of calcium and phosphorus, was considered to be indicated for hypocalcemia. It corresponds to hypocalcemia in the following aspects:

- According to Vermeulen (2001) and Murphy (1995) *Calcarea phosphoricum* is generally indicated for chronic wasting diseases with a tendency to emaciation. General lack of vital heat is an indication. As with *China officinalis*, a loss of fluids may aggravate the presenting condition.

- Peevish, restless and fretful behavior may be observed *when Calcarea phosphoricum* is indicated (Vermeulen, 2001).

- The ears may be cold at the tips (Murphy, 1995; Vermeulen, 2001).

- Gastrointestinal symptoms include flatulence with an inability to raise gas, weak digestion, an enlarged abdomen and mal-assimilation (Murphy, 1995; Tyler, 1992; Vermeulen, 2001).

- Musculoskeletal symptoms are represented by trembling limbs, cramps, and coldness of limbs (Morrison, 1993; Murphy 1995; Vermeulen, 2001).

- Frequent, short and difficult breathing is a respiratory indication (Vermeulen, 2001).

- The mammary glands may be sore and painful on pressure. Prolapsed uterus is indicated as well (Murphy, 1995; Tyler, 1992; Vermeulen, 2001).

- Clinical indications related to hypocalcemia are epilepsy, colic, and prolapsed uterus (Murphy, 1995).

2.10.2 *Magnesia phosphorica*

The elements calcium and magnesium are directly connected to the hypocalcemic state. The remedy *Magnesia phosphorica*, by direct implication, has also been taken into consideration. The remedy is homoeopathically indicated as follows:

- The gastrointestinal system is mainly indicated in case of flatulent colic which forces the subject to bend double (Morrison, 1993; Murphy 1995, Tyler 1992). The abdomen appears bloated, and constipation due to flatulence is indicated (Tyler, 1992; Verkade, 2001; Vermeulen 2001). *Magnesia phosphorica* is also said to have cured cows of meteorism (Tyler, 1992).

- Musculoskeletal symptoms indicated are twitching, spasms and muscular weakness (Verkade, 2001; Vermeulen 2001). *Magnesia phosphorica* is regarded to be a great antispasmodic remedy (Tyler, 1992; Verkade, 2001). According to Vermeulen (2001) the remedy is also indicated by spasms without febrile symptoms.

- Clinical indications include paralysis, twitching and shaking of limbs leading to muscular weakness (Verkade, 2001).

2.10.3 *Nux vomica*

Nux vomica relates to many key symptoms associated with hypocalcemia. The remedy is homoeopathically indicated as follows:

- Gastrointestinal symptoms are characterized as flatulence, bloated epigastrium, aversion to food, constipation, and distended abdomen

associated with dyspnoea (Murphy, 1995; Tyler, 1992; Verkade, 2001; Vermeulen 2001).

- The musculoskeletal system is indicated by dragging feet while walking, trembling legs, unsteady gait, chorea, twitching, tremors, and muscle spasms anywhere in the body (Morrison, 1993; Murphy, 1995; Vermeulen, 2001).

- Genitourinary symptoms include prolapsed uterus and inefficient labor pains (Murphy, 1995; Vermeulen, 2001).

- Respiratory symptoms are associated with shallow respiration and oppressed breathing (Murphy, 1995; Vermeulen, 2001).

- Cardiac symptoms are represented by presenting with a rapid and irregular pulse (Vermeulen, 2001).

- Clinical indications include bloat, ketosis, constipation, and prolapsed uterus (Morrison, 1993; Murphy, 1995; Verkade, 2001).

2.10.4 Schüssler Biochemic Tissue Salts

Besides its homoeopathic application, *Calcarea phosphorica* and *Magnesia phosphorica* are also available as tissue salts. Tissue salts were pioneered by a German homoeopathic physician, Dr Schüssler, who believed that all diseases are caused primarily by a disturbed mineral balance within individual cells of the body (Driehsen, 1997; Powell, 1977). Despite being potentised, the action of tissue salts are based upon micronutrient supplementation rather than the law of similars (Hahnemann, 1997).

3 METHODOLOGY

3.1 Sample Group

3.1.1 Sample Size

The study was conducted on eighteen subjects randomly divided into three groups of six. The allocation of medication was done double blind. Three containers were marked A, B, and C. The researcher had no prior knowledge of the exact contents of the respective containers. The cows in group A received the simplex remedy *China officinalis* 12CH. The cows in group B received the complex remedy *China officinalis* 12CH – *Parathyroid* 4CH. The cows in group C served as the control group, and received a medically inactive alcohol preparation.

3.1.2 Location of the Sample Group

All the subjects were located on one farm (Welkom farm in the Christiana district).

3.1.3 Breed of the Sample Group

All the subjects were Holstein dairy cows.

3.1.4 Parity of Research Subjects

All the subjects used in the study were multiparous dairy cows.

3.2 Duration of the Study

The study was conducted over a period of approximately one hundred and fourteen days, starting two weeks before the expected day of parturition and ending at one hundred days of lactation. The date of parturition was taken as the first day of lactation. To exclude seasonal changes as a variable, treatment of all the individuals were completed within a period of three months.

3.3 Treatment and Administration

The respective medications were stored in an amber glass bottle and the carrier substance was a solution of twenty-percent alcohol.

3.3.1 Period and Frequency of Administration

Administration of medication was done over a period of approximately four weeks, starting two weeks before the expected date of parturition and ending two weeks after parturition. During this period subjects received one dose of their respective medications twice a week.

3.3.2 Method of Administration

One dose was comprised of ten drops of medication added to five millilitres distilled water. The abovementioned dose was drawn into a sterile syringe and the content was inserted orally. Treated subjects were ticked off a checklist used to keep record of all dates of contact.

3.4 Data Capture

3.4.1 Blood Samples

3.4.1.1 Data Acquired from Blood Samples

Each sample drawn was used to obtain readings on serum calcium, magnesium and phosphate levels. Values were expressed in milli-molecules per litre (*mmoll/l*).

3.4.1.2 Method of Collection

Five millilitres of venous blood was drawn from each cow. For each sample drawn a sterile needle was used and the blood stored in a sterile, unused tube. Tubes were labeled beforehand to prevent any confusion as to the origin of each sample.

3.4.1.3 Time of Collection

Blood samples were taken from subjects on three occasions. The first sample was taken within four days after parturition; the second sample was taken at one week after parturition, and the third sample taken at two weeks after parturition. Due to logistical problems, it was not possible to obtain the first sample of subject C3.

3.4.1.4 Processing and Storage of Samples

After collection, blood samples were centrifuged in order to separate the blood serum from the cellular content. The serum was then decanted into a new

sterile tube and labeled. All serum samples were frozen and sent for chemical analysis at the end of the study.

3.4.2 Milk Production

Values for milk production were obtained from routine record keeping on the farm. Weekly readings for each of the subjects were taken. The first fourteen weeks were taken as one hundred days of lactation. Subjects who did not outlive this period were recorded to have produced no milk for the remaining period.

3.4.3 Incidence of Mastitis

The values for the incidence of mastitis were also obtained from routine record keeping used on the farm.

4 RESEARCH RESULTS AND ANALYSIS

4.1 Parameters of the Research Study

Five parameters were used to measure the effect that the respective remedies may have on the calcium metabolism of dairy cows.

As shown in the literature study, lowered calcium levels result in elevated levels of cortisol. Cortisol is immuno-suppressant; thus infections like mastitis are common. The first parameter is therefore to compare the incidence of mastitis of the three groups.

The literature study also revealed that inadequate calcium regulation leads to decreased milk production. The second parameter is therefore to compare the milk production of the subjects during the first hundred days of lactation.

The shift in value of serum calcium, phosphate, and magnesium levels during clinical and subclinical hypocalcemia lends importance to the measurement of these elements. The third, fourth and fifth parameters therefore are serum calcium, phosphate and magnesium levels respectively.

Although the mortality rate was not directly linked to the study, its occurrence is also reported.

4.2 Research Subjects

Research subjects were selected according to the criteria explained in the methodology, and randomly divided into three groups of six. A total of eighteen dairy cows were used in the study. The cows in group A received the simplex remedy *China officinalis* 12CH. Cows in group B received the complex remedy *China officinalis* 12CH – *Parathyroid* 4CH, and cows in group C received a medically inactive substance.

4.3 Data of Subjects Used in the Study

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	**4**	4	22.2	22.2	22.2
	5	6	33.3	33.3	55.6
	6	2	11.1	11.1	66.7
	7	5	27.8	27.8	94.4
	8	1	5.6	5.6	100
	Total	18	100	100	

Table 4.3.1 Parity of subjects used in the study

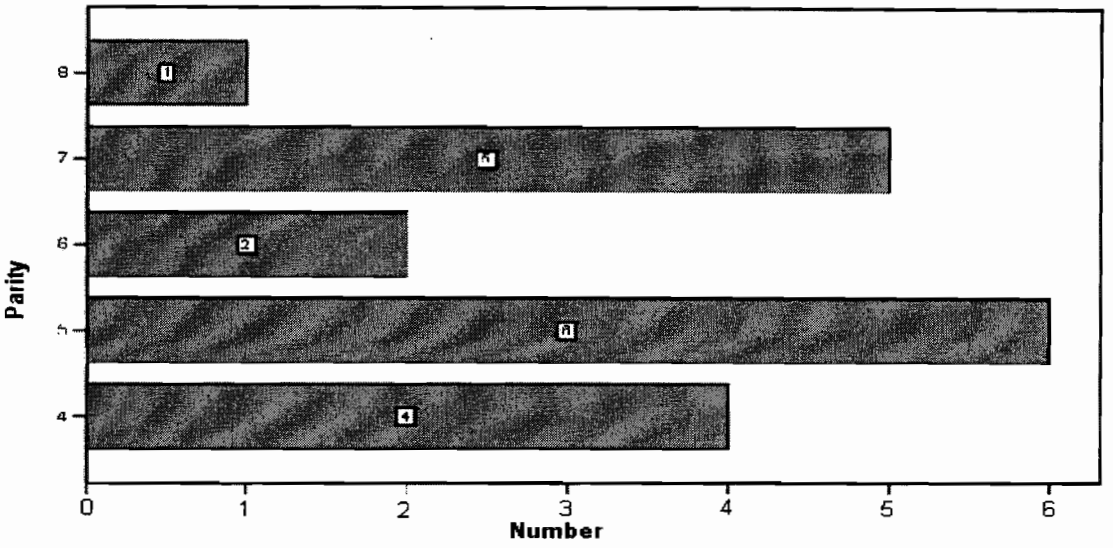

Figure 4.3.1 Parity of subjects used in the study

			Parity					Total
			4	5	6	7	8	
Group	A	Count	1	1	2	1	1	6
		% within Group	16.70%	16.70%	33.30%	16.70%	16.70%	100.00%
	B	Count	1	3	0	2	0	6
		% within Group	16.70%	50.00%	0.00%	33.30%	0.00%	100.00%
	C	Count	2	2	0	2	0	6
		% within Group	33.30%	33.30%	0.00%	33.30%	0.00%	100.00%
Total		Count	4	6	2	5	1	18
		% within Group	22.20%	33.30%	11.10%	27.80%	5.60%	100.00%

Table 4.3.2 Crosstabulation of parity of the subjects used in the study

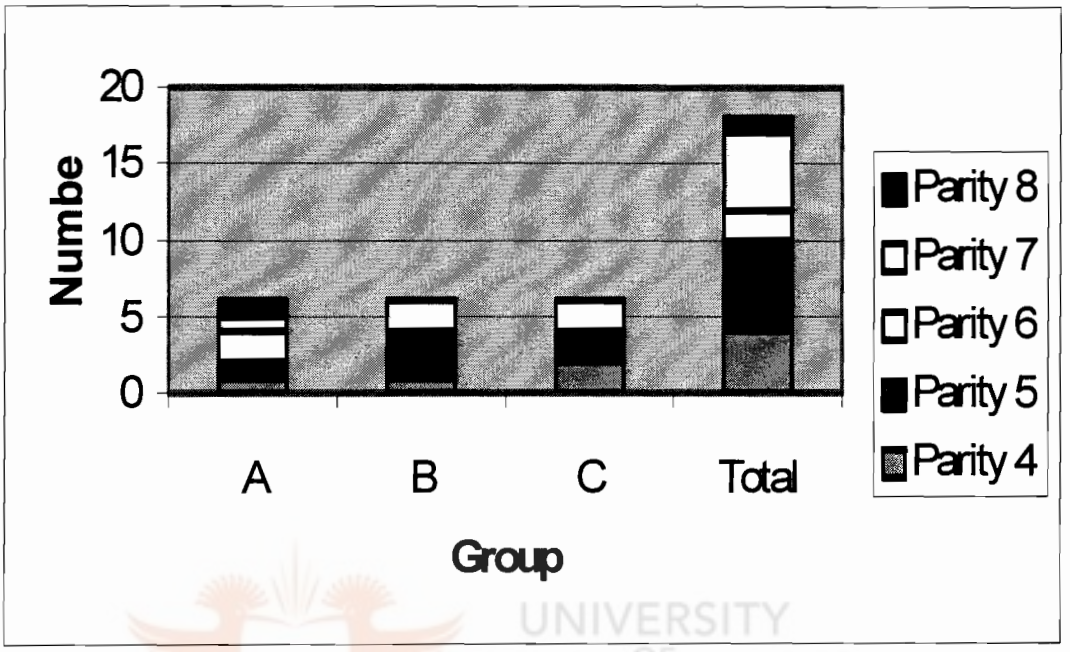

Figure 4.3.2 Distribution of parity of subjects

		Average Parity
Group	A	6.00
	B	5.50
	C	5.33

Table 4.3.3 Average parity of cows in each group

4.4 Incidence of Mastitis and Analysis

The incidence of mastitis was recorded as a yes or no value. The week of occurrence was also recorded.

In total, five of the eighteen cows contracted mastitis (Table 4.4.1 and Figure 4.4.1). One incidence occurred during the sixth week of lactation, two occurred during the ninth week, and two occurred during the eleventh week of lactation (Table 4.4.2).

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	13	72.2	72.2	72.2
	Yes	5	27.8	27.8	100
	Total	18	100	100	

Table 4.4.1 Total incidence of mastitis

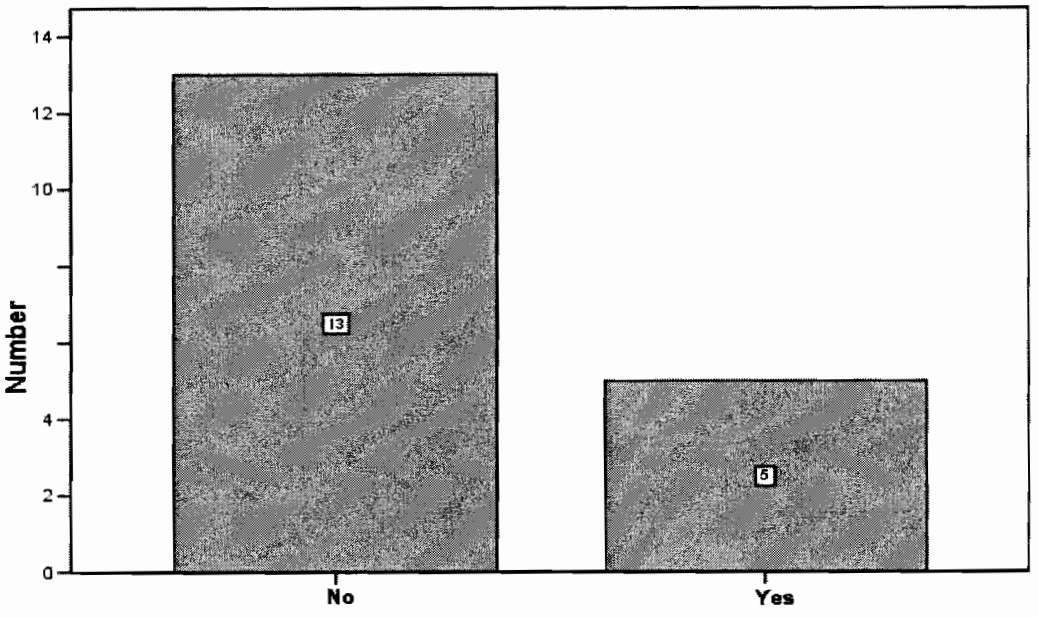

Figure 4.4.1 Total incidence of mastitis

	Week	Frequency	Percent	Valid Percent	Group
Valid	**6**	1	5.6	20	A
	9	2	11.1	40	A, B
	11	2	11.1	40	A, C
	Total	5	27.8	100	
Missing	**System**	13	72.2		
Total		18	100		

Table 4.4.2 Time of occurrence of mastitis

			Mastitis		Total
			No	Yes	
Group	**A**	**Count**	3	3	6
		% within Group	50.00%	50.00%	100.00%
	B	**Count**	5	1	6
		% within Group	83.30%	16.70%	100.00%
	C	**Count**	5	1	6
		% within Group	83.30%	16.70%	100.00%
Total		**Count**	13	5	18
		% within Group	72.20%	27.80%	100.00%

Table 4.4.3 Crosstabulation of incidence of mastitis

4.4.1 Group A

A total of three cows contracted mastitis during the research study (Table 4.4.3). One incidence occurred during the sixth week, one during the ninth week, and one during the eleventh week of lactation (Table 4.4.2).

Calculation:

3 cows with mastitis ÷ 6 cows in group A = 0.50 x 100 = 50% incidence.

4.4.2 Group B

One cow contracted mastitis during the research study (Table 4.4.3). The incidence occurred during the ninth week of lactation (Table 4.4.2).

Calculation:

1 cow with mastitis ÷ 6 cows in group A = 0.166 x 100 = 16.7% incidence.

4.4.3 Group C

One cow contracted mastitis during the research study (Table 4.2.3). The incidence occurred during the eleventh week of lactation.

Calculation:

1 cow with mastitis ÷ 6 cows in group A = 0.166 x 100 = 16.7% incidence.

4.4.4 Comparison of the Incidence of Mastitis

The incidence of mastitis in group A was 50%, whilst the incidence of group B and C were 16.7%. The 33.3% difference represents two cows. In a bigger group, a difference of 33.3% would have been significant. Taking into account the size of the respective groups, no significant conclusions are to be made from these results. One explanation may be that the average parity of the cows in group A is higher than that of the cows in group B and C (Table 4.1.3). The incidence of mastitis carries great economical impact.

Besides hypocalcemia, factors like hygiene, immune system efficiency and environmental factors may also have impacted on the incidence of mastitis.

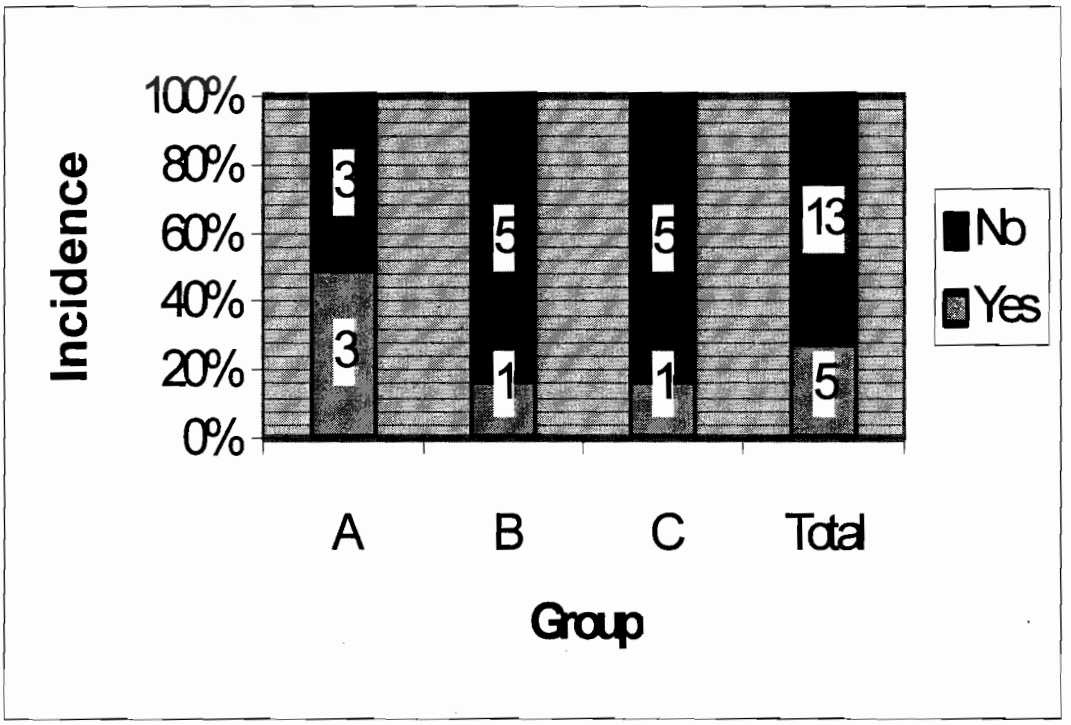

Figure 4.4.4.1 Comparison of the incidence of mastitis

4.5 Milk Production

Routine measurement of milk production was done on the first day of every week. The first fourteen readings are reflected in the following tables. The unit of measurement is *litres*. A capital D refers to the time of death of the affected individuals. Naught values indicate that no milk has been produced for that specific reading. Causes of naught values may be due to death, mastitis, heat stress or low output by the a specific individual.

4.5.1 Analysis of Milk Production

The production of milk was statistically analysed by using the Analysis Of Variance (ANOVA) method.

The ANOVA method is an analysis of variance between groups and is a parametric test. It is used when testing the difference between subgroups of a variable. This means that it determines whether there is a statistically significant difference in the weekly mean production of milk amongst the three groups. For a positive result, the p-value has to be smaller than 0.05.

Cows that died were removed from the equation; thus calculations for mean values of milk production were adapted thereafter.

4.5.2 Group A

The calculation of the mean weekly milk production of group A had to be adapted during the fourteenth week of lactation due to the death of one cow (Table 4.5.2.2). The milk production results (Table 4.5.2.1) were used to calculate the mean weekly milk production. Subject **A3** (week eleven) and **A6** (week six) developed mastitis

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A1	23	24	30	33	40	38	40	38	42	39	40	39	41	D
A2	23	25	31	33	39	35	36	36	12	20	27	28	29	27
A3	10	11	14	11	12	15	18	20	15	20	0	0	0	0
A4	21	25	31	31	31	34	32	36	34	32	30	28	28	29
A5	16	16	23	21	24	31	24	25	20	29	24	30	26	28
A6	10	9	11	12	2	0	0	0	0	0	0	0	0	0

Table 4.5.2.1 Milk production results of group A

Week	Mean	Standard Deviation	Valid N
1	17.17	6.11	N=6
2	18.33	7.31	N=6
3	23.33	8.96	N=6
4	23.50	10.31	N=6
5	24.67	15.20	N=6
6	25.50	14.90	N=6
7	25.00	14.63	N=6
8	25.83	14.54	N=6
9	20.50	15.28	N=6
10	23.33	13.56	N=6
11	20.17	16.52	N=6
12	20.83	16.64	N=6
13	20.67	16.85	N=6
14	16.80	15.35	N=5

Table 4.5.2.2 Mean weekly milk production for group A

4.5.3 Group B

The calculation of the mean weekly milk production of group B had to be adapted during the seventh and twelfth weeks of lactation due to the death of two cows (Table 4.5.3.2). The milk production results (Table 4.5.3.1) were used to calculate the mean weekly milk production. Subject **B4** (week five and six) failed to produce milk due to illness (unknown).

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
B1	11	19	22	28	37	36	D	0	0	0	0	0	0	0
B2	13	0	0	0	0	0	0	0	16	16	11	D	0	0
B3	28	28	30	27	29	33	34	29	35	34	32	33	38	28
B4	22	29	36	36	0	0	16	20	22	22	24	27	25	22
B5	27	30	33	30	30	37	37	36	35	36	36	32	39	19
B6	23	24	23	28	32	21	34	13	21	24	25	34	24	15

Table 4.5.3.1 Milk production results of group B

Week	Mean	Standard Deviation	Valid N
1	20.67	7.12	N=6
2	21.67	11.36	N=6
3	24.00	12.98	N=6
4	24.83	12.59	N=6
5	21.33	16.75	N=6
6	21.17	17.36	N=6
7	24.20	15.88	N=5
8	19.60	14.01	N=5
9	25.80	8.70	N=5
10	26.40	8.41	N=5
11	25.60	9.56	N=5
12	31.50	3.11	N=4
13	31.50	8.10	N=4
14	21.00	5.48	N=4

Table 4.5.3.2 Mean weekly milk production for group B

4.5.4 Group C

The milk production of group C (Table 4.5.4.1) was used to calculate the mean weekly milk production (Table 4.5.4.2).

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C1	27	26	31	35	36	39	23	31	26	33	33	24	33	36
C2	20	19	26	26	22	27	30	32	31	32	14	13	19	21
C3	12	32	31	32	21	18	24	25	32	26	29	23	29	20
C4	19	34	39	37	32	29	33	36	34	26	31	35	35	23
C5	28	32	35	38	33	38	30	19	30	25	28	27	32	25
C6	29	31	36	38	37	31	25	31	36	33	30	27	27	33

Table 4.5.4.1 Milk production results of group C

Week	Mean	Standard Deviation	Valid N
1	22.50	6.66	N=6
2	29.00	5.59	N=6
3	33.00	4.60	N=6
4	34.33	4.68	N=6
5	30.17	6.97	N=6
6	30.33	7.74	N=6
7	27.50	4.04	N=6
8	29.00	6.03	N=6
9	31.50	3.45	N=6
10	29.17	3.87	N=6
11	27.50	6.83	N=6
12	24.83	7.17	N=6
13	29.17	5.74	N=6
14	26.33	6.62	N=6

Table 4.5.4.2 Mean weekly milk production for group C

4.5.5 Comparison of Milk Production

The ANOVA model (Table 4.5.5.1) tested negative by having no p-value (sig.) smaller than 0.05, thus there are no significant differences in the weekly mean milk production between the three groups for any specific week of lactation.

		Sum of Squares	df	Mean Square	F	Sig.
W1	**Between Groups**	88.111	2	44.056	0.999	**0.392**
	Within Groups	661.667	15	44.111		
	Total	749.778	17			
W2	**Between Groups**	357.333	2	178.67	2.508	**0.115**
	Within Groups	1068.67	15	71.244		
	Total	1426	17			
W3	**Between Groups**	349.778	2	174.89	1.944	**0.178**
	Within Groups	1349.33	15	89.956		
	Total	1699.11	17			
W4	**Between Groups**	418.778	2	209.39	2.191	**0.146**
	Within Groups	1433.67	15	95.578		
	Total	1852.44	17			
W5	**Between Groups**	238.778	2	119.39	0.639	**0.541**
	Within Groups	2801.5	15	186.77		
	Total	3040.28	17			
W6	**Between Groups**	252.333	2	126.17	0.649	**0.537**
	Within Groups	2915.67	15	194.38		
	Total	3168	17			
W7	**Between Groups**	33.582	2	16.791	0.109	**0.898**
	Within Groups	2160.3	14	154.31		
	Total	2193.88	16			
W8	**Between Groups**	245.731	2	122.87	0.85	**0.448**
	Within Groups	2024.03	14	144.57		
	Total	2269.77	16			

W9	**Between Groups**	363.141	2	181.57	1.662	**0.225**
	Within Groups	1529.8	14	109.27		
	Total	1892.94	16			
W10	**Between Groups**	102.163	2	51.081	0.56	**0.584**
	Within Groups	1277.37	14	91.24		
	Total	1379.53	16			
W11	**Between Groups**	172.349	2	86.175	0.614	**0.555**
	Within Groups	1963.53	14	140.25		
	Total	2135.88	16			
W12	**Between Groups**	273.333	2	136.67	1.063	**0.373**
	Within Groups	1670.67	13	128.51		
	Total	1944	15			
W13	**Between Groups**	346.771	2	173.39	1.265	**0.315**
	Within Groups	1781.17	13	137.01		
	Total	2127.94	15			
W14	**Between Groups**	250.8	2	125.4	1.202	**0.334**
	Within Groups	1252.13	12	104.34		
	Total	1502.93	14			

Table 4.5.5.1 ANOVA model for milk production

4.6 Analysis of Serum Levels

The serum calcium, phosphate and magnesium levels were statistically analysed by using two methods. The ANOVA method and the Kruskal-Wallis test.

The Kruskal-Wallis test performs a hypothesis test of the equality of population medians for two or more populations.

These tests used the mean values of each respective group to determine a statistically significant difference. In both tests, a result smaller than 0.05 would indicate a positive result.

In group C where the first reading of one subject was not recorded, the calculation to determine a mean value was adapted accordingly.

4.7 Serum Calcium Levels

All values are expressed in *mmol/l*. The first serum calcium reading for each individual is located in the row corresponding to **Ca 1**, The second reading corresponds to **Ca 2**, and the third reading corresponds to **Ca 3**. The normal ratio for serum calcium levels is between 2.00 *mmol/l* and 3.00 *mmol/l*.

4.7.1 Group A

The mean serum calcium levels for the respective samples taken in group A were calculated by using the readings produced by each individual in the group (Table 4.7.1.1).

	A 1	A 2	A 3	A 4	A 5	A 6	Mean	Valid N
Ca 1	1.92	1.96	1.51	1.99	1.85	1.7	1.82	6
Ca 2	1.80	2.15	1.69	1.98	1.79	1.75	1.86	6
Ca 3	2.06	2.17	1.64	2.20	1.95	1.83	1.98	6

Table 4.7.1.1 Serum calcium levels of group A

4.7.2 Group B

The mean serum calcium levels for the respective samples taken in group B were calculated by using the readings produced by each individual in the group (Table 4.7.2.1).

	B 1	B 2	B 3	B 4	B 5	B 6	Mean	Valid N
Ca 1	1.55	1.89	1.71	1.11	2.02	1.29	1.60	6
Ca 2	2.01	2.11	1.89	1.86	2.15	1.88	1.98	6
Ca 3	2.02	1.96	1.95	1.96	2.22	1.85	1.99	6

Table 4.7.2.1 Serum calcium levels of group B

4.7.3 Group C

The mean serum calcium levels for the respective samples taken in group C were calculated by using the readings produced by each individual in the group (Table 4.7.3.1).

	C 1	C 2	C 3	C 4	C 5	C 6	Mean	Valid N
Ca 1	1.56	1.73		1.91	2.23	2.04	1.89	5
Ca 2	1.89	1.91	2.16	2.04	2.17	2.42	2.10	6
Ca 3	1.83	2.01	2.10	2.05	2.27	2.17	2.07	6

Table 4.7.3.1 Serum calcium levels of group C

4.7.4 Comparison of Serum Calcium Levels

The ANOVA model (Table 4.7.4.1) tested negative by having no p-value (sig.) smaller than 0.05, thus there are no significant differences in the mean serum calcium levels between the three groups for each respective measurement.

		Sum of Squares	df	Mean Square	F	Sig.
Ca 1	**Between Groups**	0.276	2	0.138	1.832	**0.196**
	Within Groups	1.054	14	0.075		
	Total	1.33	16			
Ca 2	**Between Groups**	0.17	2	0.085	3.031	**0.078**
	Within Groups	0.422	15	0.028		
	Total	0.592	17			
Ca 3	**Between Groups**	0.032	2	0.016	0.566	**0.579**
	Within Groups	0.419	15	0.028		
	Total	0.451	17			

Table 4.7.4.1 ANOVA model for serum calcium levels

The Kruskal Wallis test (Table 4.7.4.2) failed to reveal any statistically significant results by having no p-values (Asymp. Sig.) smaller than 0.05.

	Chi-Square	df	Asymp. Sig.
Ca measurement 1	2.659	2	**0.265**
Ca measurement 2	5.933	2	**0.051**
Ca measurement 3	1.066	2	**0.587**

Table 4.7.4.2 Kruskal-Wallis test for serum calcium levels

4.8 Serum Phosphate Levels

All values are expressed in *mmol/l*. The first serum phosphate reading for each individual is located in the row corresponding to **P 1**, The second reading corresponds to **P 2**, and the third reading corresponds to **P 3**. The normal ratio for serum phosphate levels is between 1.20 *mmol/l* and 2.30 *mmol/l*.

4.8.1 Group A

The mean serum phosphate levels for the respective samples taken in group A were calculated by using the readings produced by each individual in the group (Table 4.8.1.1).

	A 1	A 2	A 3	A 4	A 5	A 6	Mean	Valid N
P 1	2.11	2.00	3.74	2.12	2.51	2.73	2.53	6
P 2	2.32	2.25	2.73	2.96	1.79	1.97	2.34	6
P 3	2.12	2.28	2.74	2.38	2.16	3.18	2.48	6

Table 4.8.1.1 Serum phosphate levels of group A

4.8.2 Group B

The mean serum phosphate levels for the respective samples taken in group B were calculated by using the readings produced by each individual in the group (Table 4.8.2.1)..

	B 1	B 2	B 3	B 4	B 5	B 6	Mean	Valid N
P 1	2.95	2.41	2.23	2.78	2.07	3.04	2.58	6
P 2	2.29	1.88	2.65	2.43	2.15	2.63	2.34	6
P 3	2.03	2.34	2.73	3.06	2.19	2.95	2.55	6

Table 4.8.2.1 Serum phosphate levels of group B

4.8.3 Group C

The mean serum phosphate levels for the respective samples taken in group C were calculated by using the readings produced by each individual in the group (Table 4.8.3.1).

	C 1	C 2	C 3	C 4	C 5	C 6	Mean	Valid N
P 1	2.45	2.55		2.32	2.20	2.42	2.39	5
P 2	2.28	2.26	2.46	2.43	2.00	3.65	2.51	6
P 3	2.43	2.39	3.00	2.95	2.03	3.02	2.64	6

Table 4.8.3.1 Serum phosphate levels of group C

4.8.4 Comparison of Serum Phosphate Levels

The ANOVA model (Table 4.8.4.1) tested negative by having no p-value (sig.) smaller than 0.05, thus there are no significant differences in the mean serum phosphate levels between the three groups for each respective measurement.

		Sum of Squares	df	Mean Square	F	Sig.
P 1	**Between Groups**	0.107	2	0.054	0.251	**0.782**
	Within Groups	3.001	14	0.214		
	Total	3.108	16			
P 2	**Between Groups**	0.124	2	0.062	0.299	**0.746**
	Within Groups	3.107	15	0.207		
	Total	3.23	17			
P 3	**Between Groups**	0.077	2	0.038	0.223	**0.802**
	Within Groups	2.585	15	0.172		
	Total	2.662	17			

Table 4.8.4.1 ANOVA model for serum phosphate levels

The Kruskal Wallis test (Table 4.8.4.2) failed to reveal any statistically significant results by having no p-values (Asymp. Sig.) smaller than 0.05.

	Chi-Square	df	Asymp. Sig.
P measurement 1	0.515	2	**0.773**
P measurement 2	0.214	2	**0.899**
P measurement 3	0.609	2	**0.737**

Table 4.8.4.2 Kruskal-Wallis test for serum phosphate levels

4.9 Serum Magnesium Levels

All values are expressed in *mmol/l*. The first serum magnesium reading for each individual is located in the row corresponding to **Mg 1**, The second reading corresponds to **Mg 2**, and the third reading corresponds to **Mg 3**. The normal ratio for serum magnesium levels is between 0.60 *mmol/l* and 1.20 *mmol/l*.

4.9.1 Group A

The mean serum magnesium levels for the respective samples taken in group A were calculated by using the readings produced by each individual in the group (Table 4.9.1.1).

	A 1	A 2	A 3	A 4	A 5	A 6	Mean	Valid N
Mg 1	0.73	0.82	0.80	0.86	0.63	0.58	0.74	6
Mg 2	0.71	0.89	0.63	0.82	0.88	0.53	0.74	6
Mg 3	0.81	0.95	0.68	0.94	0.72	0.66	0.79	6

Table 4.9.1.1 Serum magnesium levels of group A

4.9.2 Group B

The mean serum magnesium levels for the respective samples taken in group B were calculated by using the readings produced by each individual in the group (Table 4.9.2.1).

	B 1	B 2	B 3	B 4	B 5	B 6	Mean	Valid N
Mg 1	0.68	0.78	0.85	0.84	0.89	0.69	0.79	6
Mg 2	0.74	0.81	0.84	0.95	0.81	0.72	0.81	6
Mg 3	0.89	0.81	1.01	1.00	0.87	0.80	0.90	6

Table 4.9.2.1 Serum magnesium levels of group B

4.9.3 Group C

The mean serum magnesium levels for the respective samples taken in group C were calculated by using the readings produced by each individual in the group (Table 4.9.3.1).

	C 1	C 2	C 3	C 4	C 5	C 6	Mean	Valid N
Mg 1	0.68	0.71		0.88	0.70	0.75	0.74	5
Mg 2	0.85	0.74	0.88	0.99	0.91	0.78	0.86	6
Mg 3	0.76	0.85	0.93	1.01	0.89	0.86	0.88	6

Table 4.9.3.1 Serum magnesium levels of group C

4.9.4 Comparison of Serum Magnesium Levels

The ANOVA model (Table 4.9.4.1) tested negative by having no p-value (sig.) smaller than 0.05, thus there are no significant differences in the mean serum magnesium levels between the three groups for each respective measurement.

		Sum of Squares	df	Mean Square	F	Sig.
Mg 1	**Between Groups**	0.009	2	0.005	0.511	**0.610**
	Within Groups	0.126	14	0.009		
	Total	0.135	16			
Mg 2	**Between Groups**	0.04	2	0.02	1.675	**0.220**
	Within Groups	0.18	15	0.012		
	Total	0.22	17			
Mg 3	**Between Groups**	0.038	2	0.019	1.793	**0.200**
	Within Groups	0.159	15	0.011		
	Total	0.197	17			

Table 4.9.4.1 ANOVA model for serum magnesium levels

The Kruskal Wallis test (Table 4.9.4.2) failed to reveal any statistically significant results by having no p-values (Asymp. Sig.) smaller than 0.05.

	Chi-Square	df	Asymp. Sig.
Mg measurement 1	0.733	2	**0.693**
Mg measurement 2	2.273	2	**0.321**
Mg measurement 3	2.15	2	**0.341**

Table 4.9.4.2 Kruskal-Wallis test for serum magnesium levels

4.10 Mortality Rate

The incidence of mortality was recorded as a yes or no value. The week of occurrence was also recorded.

In total, three of the eighteen cows died (Table 4.10.1 and Figure 4.10.1). One incidence occurred during the seventh week of lactation, one occurred during the twelfth week, and one occurred during the fourteenth week of lactation (Table 4.10.2). Cause of death was not established.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	15	83.3	83.3	83.3
	Yes	3	16.7	16.7	100
	Total	18	100	100	

Table 4.10.1 Total mortality rate

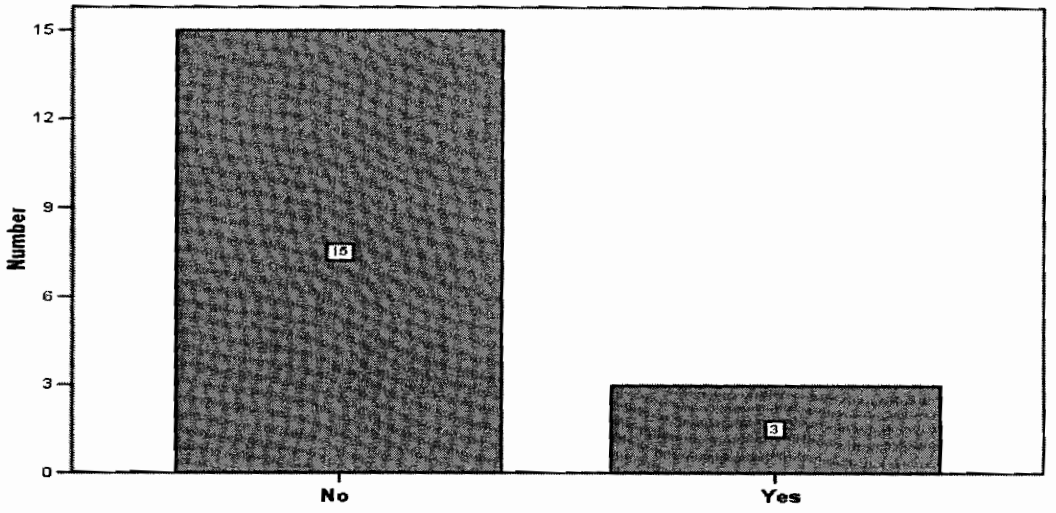

Figure 4.10.1 Total mortality rate

	Week	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	1	5.6	33.3	33.3
	12	1	5.6	33.3	66.7
	14	1	5.6	33.3	100
	Total	3	16.7	100	
Missing	System	15	83.3		
Total		18	100		

Table 4.10.2 Time of mortality

			No	Yes	Total
Group	A	Count	5	1	6
		% within Group	83.30%	16.70%	100.00%
	B	Count	4	2	6
		% within Group	66.70%	33.30%	100.00%
	C	Count	6	0	6
		% within Group	100.00%	0.00%	100.00%
Total		Count	15	3	18
		% within Group	83.30%	16.70%	100.00%

Table 4.10.3 Crosstabulation of mortality rate

4.10.1 Group A

One cow in group A died during the research study (Table 4.10.3). The death occurred during the fourteenth week of lactation.

Calculation:

1 cow dead ÷ 6 cows in group A = 0.167 x 100 = 16.7% incidence.

4.10.2 Group B

Two cows in group B died during the research study (Table 4.10.3). The incidences occurred during the seventh and twelfth week of lactation.

Calculation:

2 cows dead ÷ 6 cows in group B = 0.333 x 100 = 33.3% incidence.

4.10.3 Group C

No cows in group C died during the research study (Table 4.10.3).

Calculation:

0 cows dead ÷ 6 cows in group C = 0 x 100 = 0% incidence.

4.10.4 Comparison of the Incidence of Mortality

The incidence of mortality for group A was 16.7% while that of group B was 33.3%. Compared to group C it represents one and two cows respectively (Figure 4.9.4.1).
Taking into account the size of the respective groups, no significant conclusions are to be made from these results.

Seeing that the deaths occurred at the latter part of the study and at least five weeks after the last administration of medication, the cause is unlikely to be the homoeopathic substance administered. The fact that the results of measured parameters reflected no statistically significant differences is also an indication that no particular group were put at a disadvantage by the respective treatment they received.
Seeing that Homoeopathy is regarded as a holistic modality of medicine, one would expect it to impact positively on the health of the subjects. The fact that it failed to prevent death demonstrates that either Homoeopathy was not applied correctly in this study or that it does have its limits.

No cases of clinical hypocalcemia were reported to be the cause of any mortality.

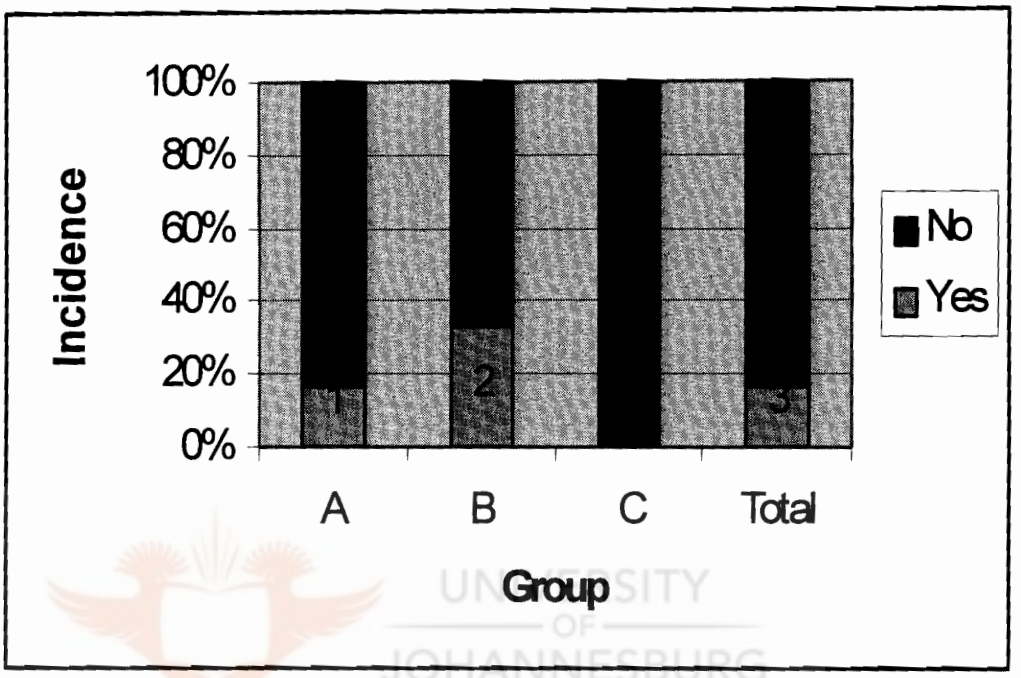

Figure 4.10.4.1 Comparison of mortality rates

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The aim of this study was to determine and compare the effects of two homoeopathic remedies, one simplex (*China officinalis* 12CH) and one complex remedy (*China officinalis* 12CH – *Parathyroid* 4CH), on the calcium levels of multiparous dairy cows.

Agricultural research is of vital importance to ensure sustainability and stability in South Africa. Currently the dairy industry is under great strain due to cost of production accompanied by international competition.

The costs involved in homoeopathic treatment are minimal and side effect free. The use of this modality may reduce costs in terms of prevention and treatment of diseases.

5.2 Conclusions

Based on the results of this research study, it has been found that neither *China officinalis* 12CH nor *China officinalis* 12CH – *Parathyroid* 4CH Complex managed to produce statistically viable results indicating a beneficial action upon the physiological mechanisms for regulating serum calcium levels in multiparous dairy cows in order to prevent clinical or subclinical hypocalcemia and related parameters.

Conventional medicine often makes use of animal experimentation before implementing it for human use. In the case of homoeopathy the reverse is true (Shelton, 2004). Many cases of successful homoeopathic treatment of animals have been reported.

Although there is nothing to suggest that the treatment caused it, the fact that subjects died during the study is unfortunate. It however serves as an example that although Homoeopathy can be extremely effective when used correctly, it is not a panacea, cheating disease and death at every opportunity.

Seeing that homoeopathic information regarding animal diseases are not as well established and complete as human literature, more research studies need to be done to prove the efficacy of homoeopathy for the treatment of ailments in the dairy industry.

Although this particular study failed to produce significant results, homoeopathy may still have a lot to offer the dairy industry.

5.3 Reasons for Failure

Reasons why the study failed in its objectives may be due to inexperience on the side of the researcher in this field of study as well as focussing on wrong aspects of the problem:

The law of similars (Hahnemann, 1997) requires one to match the symptomatology of the disease process to that of the action of the remedy. The fact that remedy selection was based upon *anticipated* symptoms rather than *presenting* symptoms might have impacted negatively upon this study.

As discussed in the literature review regarding treatment protocol for clinical hypocalcemia, it is known that cows in stage three are prone to relapse after receiving intravenous calcium. It is at this period that homoeopathic intervention may prove to be valuable.

Administration of the homoeopathic remedy before parturition prevented one from getting a baseline reading for blood serum levels and hindered comparison of the results. A baseline reading would have made comparison of results more effective.

Repertorization of the problem was based on books related to human pathology. Homoeopathic information related to animal diseases is not as well established and complete as the abovementioned literature.

Ignoring remedies like *Calcarea phosphorica, Magnesia phosphorica* and *Nux vomica* or combinations of these remedies discussed in the literature review may have hindered a positive outcome in this study. Information gained after commencement of the study revealed that a complex of *Calcarea phosphorica* and *Magnesia phosphorica* may be effective as a prophylactic against hypocalcemia (Verkade, 2001).

Wrong choice of potency may have impacted the results of the study. Except for the use of *Parathyroid* in the fourth centesimal potency for specific reasons, higher potencies from 30 CH to 200 CH may have been more effective.

The breed of dairy cow used in the study may also have impacted on the study. It is known that Jersey cows are more prone to hypocalcemia than any other dairy breeds (Holstein). Using a herd with a higher incidence of hypocalcemia may have aided in better comparative capabilities.

5.4 Recommendations

Future studies regarding hypocalcemia may make the following changes:

- Conduct the study on subjects directly after conventional treatment of clinical hypocalcemia to ascertain efficacy on prevention of relapse. This

however may be very time consuming and time specific, but positive results will prove valuable.

- Choice of remedy should not be restricted to one or two. Inclusion of bigger complex remedies or more experimental groups may be considered. Special emphasis should be put on the inclusion of *Calcarea phosphorica* and *Magnesia phosphorica* (either in potency or tissue salt). The use of more experimental groups would be ideal for a joint project shared between many students.
- Keeping in track with the law of similars (Hahnemann, 1997), and the dietary cation-anion balance used as a preventative strategy against hypocalcemia (Roginski *et al.*, 2003), the use of a cationic salt may prove to be homoeopathic if potentised. Salts consisting of cations like potassium or sodium and anions like fluoride, chloride or phosphate may be considered.
- Use higher potencies.
- Take one blood sample before administering medication to establish a baseline.
- Use the Jersey dairy cow breed for research rather than the Holstein breed.
- Use the annual incidence of hypocalcemia and mortality of the herd as baseline before commencing the study to improve analytical capability.

Future research studies regarding this topic should also consider aspects regarding methodology:

- An increased number of subjects used in the study will contribute to more reliable analysis of results.
- The method of administration may be reconsidered. According to Verkade (2001) the use of spray-bottles can be used to administer single doses of medication. This would ensure a more even dispersion of medication and an enlarged area of absorption.
- The frequency of administration may be increased from twice a week to every second day to ensure proper stimulation.

- Alternatively, groups can be split into separate holdings and receive medication through their water supply.
- The number of blood samples taken per subject may be increased in order to improve the accuracy of analysis of results.

5.5 Other Areas of Importance

One disease that is closely related to hypocalcemia in terms of pathology and economic impact is mastitis. Separate studies focussing on prevention and treatment should also be considered.

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Appendix A

Repertorization of Hypocalcemia

	Stomach, Appetite diminished. p653	Gen, Lack of vital heat. p1621	Ear, Coldness. p433	General, Emaciation. p1594	Gen, Trembling - spasmodic p1693/5	Mind, Slowness. p202	Stomach, Inactivity/Slow. p676/70	Female, Prolapsus, uterus. p947	Female, Retained placenta. p947	Female, Inflammation, uterus. p913	Female, Complaints during deliv p910	Chest, Inflammation, mammae. p1040	Chest, Milk decreased. p1042.	Eye, sunken. p411	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**T**
Ars	1	2	1	3	2	1		1	2	3				2	18
Calc	1	3	2	3	2	2	1	2		1	1	1		1	20
Calc p		3	2	2	1			2							10
Carb v	1	2	1	2	1	2	2		1		1	2		2	17
Caust	2	3		2	2	1					3		1		14
Chin	1	2	1	3	2	2	3	2	1	1	2			3	23
Con	2	2		1	2	3		2		1		2			15
Kali c	1	3	2	2	3			2	2	1	2			2	20
Lach	2	2	2	2	2	1		2		3		2		1	19
Lyc	2	2	1	3	2	1	2	1		3	2	2		2	23
Mag p		3		1	1										5
Merc	1	2	1	2	2	1		1		2		2		2	16
Nux v	1	3		3	2	1	2	2	2	2	2			1	21
Phos	1	3		3	2	3		2	1	2		2		2	21
Sep	1	2		1	2	3	2	3	3	2	1			1	21
Sil	1	3		3	2		3	2		2		3			19
Sulph	1	2		3	2			2		2	1	3		2	18

Appendix B

HEAD OFFICE

Natura Building
8, 18th Street
Hazelwood
PRETORIA 0081

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MENLO PARK 0102

Tel: (012) 346-1230
Direct line: (012) 346-0014
Fax: (012) 346-3760

NATURA

CAPE TOWN REGIONAL OFFICE

Suite 5
Milpark Centre
Ixia Street
MILNERTON 7441

P O Box 86
HOWARD PLACE 7450

Tel: (021) 555-1144
Fax: (021) 555-1148

14 October 2003

Jurgens Staats
jurgenss@webmail.co.za

Dear Jurgens

A Comparative study on the effect of China officinalis 12CH and *China officinalis* 12CH-*Parathyroid* 4CH Complex on Blood Calcium Levels of Multiparous Dairy Cows

Further to my letter of 3 October 2003, the following:

1. The remedies will be provided in 20% alcohol.

2. Labelling:
   Product A – Hypocalcemia. Dosage: Add ten (10) drops to 5ml distilled water. Give orally twice weekly. (For office use only – China officinalis 12CH)
   Product B – Hypocalcemia. Dosage: Add ten (10) drops to 5ml distilled water. Give orally twice weekly. (For office use only – China officinalis 12CH; Parathyroid 4CH)
   Product C – Hypocalcemia. Dosage: Add ten (10) drops to 5ml distilled water. Give orally twice weekly. (For office use only – Placebo).

3. Storage instructions: Store below 25°C. Store away from direct sunlight. Keep out of reach of children.

4. Costing: The subtotal will be R250-00 + VAT = R35-00 = Total: R285-00.

Please be so kind as to advise us if this is acceptable to you.

Kind regards

Dr Elzabé Stoffberg
National Manager: Professional Division

Appendix C

CONSENT FORM

Hereby I, Chris Fawe give permission to Jurgens Staats (2000265) to conduct his research project: **A Comparative Study on the Effect of *China officinalis* 12CH and *China officinalis* 12CH-*Parathyroid* 4CH Complex on Blood Calcium levels of Multiparous Dairy Cows at** Welkom Kta. Should any of the subjects become ill or die during the study due to natural causes neither he nor the Technikon Witwatersrand will be held responsible.

Signed: C Fawe

Date: 18/11/03