EFFECT OF PROBIOTIC (RE3) SUPPLEMENT ON GROWTH PERFORMANCE, DIARRHEA INCIDENCE AND BLOOD PARAMETERS OF N'DAMA CALVES

BY

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DECLARATION

"I hereby declare that this project is the result of my own research and that no part of it has been presented for another degree in this university or elsewhere. I also declare that the preparation and presentation of this project were supervised in accordance with guidelines on supervision of project laid down by the University of Science and Technology, Kumasi, Ghana".

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DEDICATION

I dedicate this work to my daughter Fatima Abdul Aziz, father Alhaj Yunus Musa and my aunt Hajia Fati Abdul Aziz.

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ABSTRACT

A sixteen week trial was conducted at the Research Farm (Technology Village) of the Department of Animal Science, School of Agriculture, University of Cape Coast, Cape Coast. This study sought to investigate the effects of Probiotic (RE3) products on growth performance, diarrhea incidence and blood parameters of N"dama calves. Sixteen N"dama calves of an average initial weight of 44.2 kg were randomly assigned to one of four dietary treatments according to their body weight, age and sex (T1- Control (No RE3 supplementation, T2- 0.03 ml RE3 per kg body weight, T3- 0.06 ml RE3 per kg body weight and T4- 0.09 ml RE3 per kg body weight) in a Completely Randomize Design (CRD) and there were 3 replicates per treatment. The calves were allowed access to feed and water *ad libitum*. The body weight of the calves was recorded with a weighing balance at the start of the experiment and thereafter regularly at two weeks interval. Weighing was done early morning before the calves are allowed to access feed and water and were also observed in their pens for occurrence of diarrhea and faecal scores recorded. Blood samples were obtained from each calf at the end of the study through jugular vein puncture. Supplementation of RE3 to calves had showed a beneficial effect by reducing the incidence of diarrhea. The highest faecal score was recorded in T1 and the least faecal score was recorded in T3. There were significant difference in the faecal score between the treatment group and the control after two weeks of the experiment ($P<0.05$). There was no significant difference in the average daily gain of the animals ($P>0.05$). Hematological and biochemical indices of calves were all within the normal range except in treatments (1, 3 and 4) which recorded high

WBC count with no significant difference ($P>0.05$).

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cattle production in Ghana consists of mainly an open grazing system where animals are allowed to graze on natural pasture from the early hours of the day and returned to the kraal in the evening. For the duration of the grazing, the animals are at times taken to the watering point such as rivers and streams to be refreshed. A major problem of the system is that the quality and quantity of fodder available from the natural pasture shows seasonal fluctuation (Evitayani *et al.*, 2005; Grimaud *et al.*, 2006). Well-known with tropical pastures, in the period of dry season there is an acute shortage of feed supply and what is accessible is of low quality being less in protein and high in fiber (Evitayani *et al.*, 2005; Grimaud *et al.*, 2006). Cattle grazing such pastures are less productive and thus causing mortality in neonatal calves due to severe starvation, disease and pest infestation and malnutrition.

There is therefore, the need to explore alternative management system for the production and sustenance of calves and ruminant livestock in Ghana. In order to be successful, management of calves should be in such a manner that stimulates utilization of nutrients to support growth and lessen the risk of disease. Sick calves do not grow well or express full genetic potential thus treating them becomes very expensive and time consuming. Consequently, sickness and death among calves can represent huge losses in the ruminant livestock industry.

Antibiotics and synthetic antimicrobial agents are frequently used to treat sick calves, improve growth and promote feed efficiency. Nonetheless, unceasing use of sub-therapeutic levels of antibiotics in the livestock industry results in development of resistance strains with associated

increase in the cost of control (Dibner and Richards, 2005). Attempt to minimize the use of veterinary drugs including adherence to hygienic standards and the use of probiotics as feed additives have been successful (Dibner and Richards, 2005). The main effects of these feed additives are enhanced resistance to colonization with pathogenic bacteria and improved pathogen load and health status of the animal (Choct, 2009).

Numerous countries as well as the United Kingdom (UK), Denmark and Sweden have accordingly prohibited the use of antibiotic growth promoters (AGP) in animal production and have enacted strict legislations on the use of antibiotics in animal production (Buchanan *et al*., 2008). The European Union (EU) also in 2006 banned antibiotics used in animal production in all member countries (Vondruskova *et al*., 2010). Furthermore, the United States of America (USA) has since December 2013 banned the use of all medically important antibiotics in animal production (FDA, 2013). Scientists have now therefore strengthened research into products such as prebiotics and probiotics among others that can successfully replace these in-feed antibiotics in growth and health promotion in animal production without being detrimental to the health of humans and animals as well.

Patterson and Burkholder (2003) defined prebiotic as a non-digestible food ingredient that usefully affects the host by selectively exciting the growth and/or activity of one or a limited number of bacteria. Miguel *et al.* (2004) reported that probiotic administration to calves and pigs is capable of reducing multiplication of coliform of large intestine and colon of the animals. Prebiotics in recent times have been reported to have characteristics of enhancing the immune system (Okamoto *et al*., 2003). Furthermore, Okamoto *et al.* (2003) reported that feeding performance as well as the overall animal health can be improved by reducing the number of potentially disease causing

bacteria that attach themselves to the intestinal wall of the animal which in turn reduces their effect on the host animal.

Direct-fed microbial (DFM) or probiotics which, according to Fuller (1989), are viable microorganisms which improves the growth and health of farm animals have been tested by several researchers as a possible substitute to growth stimulating antibiotics.

Probiotics as defined by Heyman and Menard (2001) are live microbial feed additives which positively influence the host animal by enhancing its intestinal microbial balance. According to Timmerman *et al.* (2005), probiotics have the ability of improving the immune system of the young animal as well as defending the animals against enteropathic disorders. In addition, probiotic supplementation to animals enhances feed effectiveness and weight gain (Lesmiester *et al*., 2004).

Even though the method of action of probiotics have not been obviously acknowledged, Riddell *et al*. (2010) reported that lactic acid bacteria are thought to impede the development of pathogenic bacteria through lowering the pH in large intestine by production of lactic acid and via competitive attachment. One of the major species of beneficial micro-organism in the gut of monogastric animals are *Lactobacilli* (Blaut, 2002).

At early stage of life, the fore-stomach of ruminants is similar to that of monogastric animals and therefore supplementation with *Lactobacillus* has been shown to improve digestibility of nutrients and ultimately growth in pre-weaning calves (El Adawy *et al*., 2000; Soliman *et al*., 2000). Sadik (1989) reported that the plasma globulin of buffalo heifers can be significantly improved by *lactobacillus* concentrate. Moreover, supplementation of yeast culture can improve the value of plasma globulin in lactating buffalo (Mohanna, 2000), growing crossbred sheep (Salem *et al*.,

2000) as well as young sheep (El-Ashry *et al*., 2003). According to Shim (2005), haematological traits like white blood cell count and haemoglobin in weaned pigs were not affected by multi-strain probiotic, prebiotic and synbiotic. Nevertheless, Heinrich *et al*. (2003) also reported that synbiotic supplementation are likely to influence the immune parameters like lymphocytes, neutrophils and leukocyte of weaned pigs compared to single administration of *Lactobacillus.* In contrast, Morill *et al*. (1995) reported no positive effect on blood parameters such as immunoglobulins in calves supplemented with probiotics. Similarly, Riddell *et al*. (2010) also reported no significant effect on plasma IgG1 concentration of neonatal calves supplemented with probiotics. Probiotic supplementation to young calves seems to be a rational approach to intestinal health, improved performance and reducing the dependence on antibiotics.

There is a dearth of information on the effect of direct-fed microbial (RE3) on animal growth or feed efficiency in calves in Ghana. Enhanced intestinal bacterial flora may reduce the risk of diarrhea, improve performance, particularly when animals are exposed to significant immunological, environmental or other stressors considering the animal production system in Ghana.

RE3, is a direct-fed microbial product produced and distributed in Ghana by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada and its subsidiary in Ghana, has been observed to improve growth performance and efficiency in broiler and growing pigs (Bonsu *et al*., 2012; Okai *et al*., 2010); laying performance (Bonsu *et al*., 2014) and reduce the cost of production (Dei *et al*., 2010). Several research carried out on the effect of probiotic (RE3) using different models of animals have also produced many results. Osei *et al.* (2008) reported that probiotics can improve weight gain in sheep as well as delayed weight loss under feed-stress conditions. Okai (2010) also reported improvement in growth and efficiency of feed

consumption in monogastric animals such as poultry and pigs, better egg production and characteristics as well as reduced mortality in laying hen.

Probiotics (RE3) have also been reported to increase body weight gain and feed efficiency and decrease the incidence of diarrhea and mortality when administered to calves (Donovan *et al*., 2002).

The objective of this study was to evaluate the effect of probiotic (RE3) supplementation on growth performance, reduced diarrhea incidence and hematological and blood chemistry of N‟dama calves.

1.2 Organization of the study

The research is organized into five chapters. Chapter One presents background to the study and its objective. Chapter Two reviews existing relevant literature related to the study. Chapter Three outlines the methodology employed to accomplish the objective of the study. The Results and Discussion of the study are reported in Chapter Four. Finally, the conclusions and recommendations of the study are presented in Chapter Five.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter presents the literature relevant to the theme of the study. It looks on the breed of cattle in Ghana, features of N‟dama cattle, cattle production in Ghana, management of calves, antibiotics and antimicrobial drugs use in cattle production, antibiotic resistivity, alternative to antibiotics in animal production, probiotics, prebiotics, RE3 and haematology.

2.2.1 Breeds/Types of Cattle in Ghana

The most noticeable cattle breed in Ghana is the West African Shorthorn (WASH). Generally, WASH is a term used to describe small humpless breed of cattle (Aboagye, 2002). These cattle breeds are usually white and black in colour and sometimes yellowish brown and white. WASH is an aboriginal strong cattle breed, short and broad. The most abundant cattle in Ghana largely seen towards the northern boundary and particularly towards the north-eastern part where the challenge of tsetse fly is much fewer are the Zebu breed (Hutchinson, 1962). Additionally, the Zebu breeds are also susceptible to trypanosomiasis. Other cattle breeds found in Ghana are Sokoto Gudali, White Fulani, Muturu, Sanga among others. N‟dama is the next breed of significance in Ghana.

2.2.2 Features of N’dama Cattle

The N‟dama breed is the most characteristic *Bos Taurus* and trypanotolerant breed in West Africa (ILCA, 1979). It originates from the Fouta Djallon regions of Guinea, and is now found in the whole of coastal West and Central Africa (DAGRIS, 2005). The N'dama breed is compact with large and strong head and set on short legs of fine bone; has thick and deep neck with fairly broad

back. The N‟dama breed is also of medium size, being 100 cm and 120 cm height at shoulder for cows and bulls respectively (Spencer and Eckert, 1988). The N‟dama cattle are identified for their beef conformation. Spencer and Eckert (1988) reported an average birth weight of 19 and 22 kg in two high and zero trypanosomiasis risk areas respectively. The average adult weights range from 320 to 360 kg and 250 to 270 kg for males and females, respectively (Mason, 1996). The dressing percentage is around 50% and the meat has a very good taste without much fat (Maule, 1990; Rege, 1999). Female N‟dama cattle are poor milkers, producing only 2 – 3 litres of milk per day during 7 – 8 months period (Payne, 1990).

2.2.3 Cattle Production Systems in Ghana

Basically, there are three main livestock production system in Ghana, namely;

- The extensive/grazing system
- Semi intensive/grazing with system of supplementary feeding
- Intensive/zero grazing system

In the extensive system, the herdsmen normally Fulanis moves the animals from place to place more specially in the hamattan season in search of pastures. Animal growth under extensive system of production is very low and calf mortality risk is 4 – 23% (Otte and Chilonda, 2002).

In the semi-intensive system, which is the most prevalent in Ghana, the animals are allowed to go out to graze on natural pasture from the early hours of the day and returned to the kraal in the evening. During the grazing period the animals are sometimes taken to the watering point to be refreshed. Sometimes supplements and water is also made available in the pens for the animals after grazing.

In the intensive system, animals are restricted in well constructed houses and supplied with feed, water and medication. There are two main types of the intensive system. In the first type, forages are cultivated and divided into paddocks. The animals are allowed to graze from paddock to paddock and provided with water. Shelter is provided on the pasture to protect the animals from the heat of the sun (Hill, 1988). On the other hand, Sonkor (2001) reported that animals may be housed in a structure with a hard slatted floor covered with beddings. In this system animals do not graze but grass is harvested and brought to them in their houses hence the name zero grazing or cut and carry.

2.2.4 Trend of cattle production in Ghana

In Ghana, livestock production plays important role in agricultural sector. Basically, livestock production largely contributes towards the meeting of food requirements of farmers, providing farm power for ploughing and serves a source of income. Furthermore, in farming communities, cattle serve as an asset used in determining wealth and also for securing loans. The animals additionally supplies farmyard manure for maintenance of soil fertility and improve soil structure for crop production. The contribution of livestock industry to agricultural GDP is about seven percent as reported by (SRID, 2001). This excludes compost and farm power provided to the crop segment. Animals such as cattle, sheep and goat are frequently slaughtered for a variety of occasions and functions like festivals, birth rites, funeral rites and marriage ceremonies.

As at 2000, local meat production recorded was 66,283 Mt (Table 2.1). Beef recorded the highest percentage meat production of about 28% followed by poultry 21%, mutton 19% and chevon 17%. Pork recorded the least meat production (15%) in that same year (SRID, 2001).

However, domestic meat production improved from about 76, 582 Mt in 2005 to 101,895 Mt in 2008 (Table 2.2). That is about 33% increase over the period. Poultry contributed the highest

percentage (32%) of total domestic meat production followed by beef (19%) (MOFA, 2009). The least percentage (15%) was recorded by mutton.

Ghana largely depends on livestock imports, meat and milk to meet animal protein requirement. However, the amounts of livestock imported from neighbouring countries are difficult to estimate since they are not recorded.

Table 2.1: Production of domestic meat (tons) and off take rates (percent) from 1996-2000

species	1996	1997	1998	1999	2000	off take
Cattle	15,411	17,160	17,325	18,029	18,570	11
Sheep	9,315	10,886	11,232	11,940	12,298	30
Goat	8,408	9,879	10,370	11,216	11,552	30
Pigs	11,680	11,360	11,104	11,172	10,056	80
Poultry	10,466	11,104	12,710	14,534	13,807	
Total	55,280	60,389	62,741	66,891	66,283	

Source: SRID (2001)

Table 2.2 Production of domestic meat (ton) from 2003 to 2008

species	2003	2004	2005	2006	2007	2008

Cattle	18,486	18,686	18,874	19,140	19,346	19,553
Sheep	13,568	14,004	14,450	14,912	15,390	15,831
Goat	13,884	15,308	15,300	15,588	16,364	17,180
Pigs	10,181	9,979	9,744	16,027	16,498	17,002
Poultry	21,116	22,982	22,709	27,224	29,630	32,249
Total	77,235	80,959	81,077	92,891	97,228	101,815

Sources: MOFA (2009)

2.2.5 Marketing channels for cattle and agents

Cattle marketing structure operates reasonably well and usually involves nomadic traders, middlemen as well as butchers. Generally, the flow of ruminant livestock is from the three northern regions of Ghana as well as the Volta (MOFA, 2009). These animals are transported to the southern part of the country in articulator trucks. Additionally, the nomad traders occasionally moves from place to place buying animals from producers and transport them to market centers where they sell them to individual consumers, small scale producers as well as butchers. Kumasi abattoir serves as the market center for livestock trading in Ashanti Region. There is a high increase of prices of ruminants livestock more especially cattle and sheep during festive occasions such as Muslim festivals and Christmas where demand is very high.

2.2.6 Constraint to increased cattle production

Some of the factors that affect the growth of ruminant livestock production in Ghana include inadequate nutrition, lack of enhanced breeding stock, poor vaccination programs, insufficient water supply, lack of funds among others (MOFA, 1998). However, the country has the potential to increase livestock production when proper measures are put in place. This will result in superior meat and milk production to satisfy the country‟s animal protein requirements (MOFA, 1998).

Another area of concern that affects the cattle production in Ghana is pest and disease infestation. Diseases such as, Brucellosis, Anthrax, Tuberculosis, Foot and Mouth Disease, Contagious Bovine Pleuropneumonia and Trypanosomiasis are of major concern (MOFA, 2009). Influx of Helminth is also a major setback in ruminant production nationwide. Ill-health in cattle is usually connected with helminthes and these result in poor productivity and high mortality rate in calves (MOFA, 2009).

Poor feeding, housing and disease control also contributes to low productivity and mortality in young calves. There is therefore the need to explore alternative measures in cattle production to ensure sustainability of the industry.

2.3 Management of Calves

2.3.1 Housing

Housing is an essential tool in the management of calves. Calves that are housed individually tend to start eating early than group housing. Moreover, housing calves in a well constructed pen helps in monitoring any sign of diseases in calves for early treatment so as to maximize production. However, calves that are not housed are exposed to harsh environmental conditions and this may decrease performance. Calves are usually housed in pens which are partly roofed and fenced to check excessive heat as a result of sunshine and also to protect the calves against predators, rainwater and wind speed. Rulofson *et al.* (1993) reported that for diarrhea monitoring and observance of feed intake, there the need to house calves individually. Emanuelsson *et al.* (2000) also reported that keeping calves individually in pens reduces the risk of contracting respiratory diseases than keeping calves in group pens with automatic milk feeding. Furthermore, calves kept in individual pens according to Olsson *et al.* (1994) have low risk of

diarrhea outbreaks than calves kept in group pens. It is essential to allow enough air circulation in calves pen due to ammonia build up in the pen. Simensen (1981) found that calves kept in separate houses have the lowest level of ammonia build ups and air humidity than group housed calves. Therefore, the need to house calves in a well-ventilated and concreted pen is crucial in the proper management of calves.

2.3.2 Feedstuffs and Rumen Development of Calves.

The digestive system of the newborn calf develops very rapidly during the first two to three weeks of life. Heinrichs and Lesmeister (2005) reported that liquid feed bypasses the reticulorumen to the omasum and abomasum through the oesophageal groove where digestion is similar to that of the monogastric animal. Rumen development and production of microbes are influenced greatly by the level of feed intake and the kind of feedstuffs. Ingestion of solid feed tends to excite rumen microbial development and production of volatile fatty acids. In contrast, feeding calves with milk replacers or milk tends to slow down rumen development (Heinrichs and Lesmeister, 2005).

2.3.2.1 Liquid feeds and rumen development

Liquid feeds such as milk or milk replacer make up the preliminary diet of new born calves. Heinrichs and Lesmeister (2005) reported that milk or milk replacer restrict the development of calf‟s rumen due to its chemical constituent. Quite a lot of researches have shown least growth of rumen in calves on solely liquid feed. This is because the absorption of volatile fatty acid and metabolic activity is very small and as a result restricting epithelial development and growth of muscles (Heinrichs and Lesmeister, 2005).

Vázquez-Añón *et al*. (1993) on the other hand reported that the size of calf rumen will continue to develop relatively as the calf grows. Heinrichs and Lesmeister (2003) also reported that calves receiving liquid diet only may show normal growth; however their rumen will remain not fully grown at weaning and will become apparent. This is because the calf will remain not healthy and shows partial development as a result of its failure to assimilate solid feeds such as forages and grains.

2.3.2.2 Solid feeds and rumen development

Concentrate and forages used as solid feed for cattle stimulates the development of rumen though there may be differences in their effectiveness in doing so. According to Heinrichs and Lesmeister (2005), concentrates as solid feed have the ability of improving the development of rumen at a faster rate than forages. The production of volatile fatty acids and microbial growth increases by the intake of concentrate or grain. A study conducted by Heinrichs and Lesmeister (2003) shows that intake of concentrate influences rumen epithelial development and wall vascularization. However, forage ingestion does not encourage rumen epithelial development but rather retains a superior ruminal pH because of its bigger particle size and higher content of fiber (Heinrichs and Lesmeister, 2005). Moreover, Coverdale *et al*. (2004) reported that ingestion of forages enhances rumen muscularization, maintains strong rumen epithelial and encourages rumination and saliva movement into the rumen.

2.3.3 Energy requirements of calves

Calves requirement for energy is obtained on the basis of metabolizable energy according to (NRC, 2001). Daily net energy for maintenance (NE_M) ranges from 0.96 to1.62 Mcal in young replacements fed milk or milk replacer only and weighing between 25 and 50 kg, where NE_M =

0.086 LW $^{0.75}$. The efficiency of use of metabolizable energy (ME) from milk or milk replacer to meet maintenance requirements is set at 86 percent. Maintenance metabolizable energy (ME) is defined as 0.100 Mcal/kg $^{0.75}$ daily. Requirements for ME are calculated with the equation:

ME requirement (Mcal/d) = 0.100 BW $^{0.75}$ + (0.84 BW $^{0.355}$) (BWG$^{1.2}$).

Where, BW = body weight in Kilogram and BWG = body weight gain in Kilogram.

Calves have high and variable metabolic rate in the first week of life as a result the ME requirements may be underestimated (NRC, 1999). Calves are required to consume a substantial amount of nutrient from starter by the second week of life which is encouraged by providing water *ad libitum* and also using a well pleasant nutritious starter. This is critical to the development of an active functioning rumen. Therefore, regardless of diet, the NE requirements for maintenance and gain should not change. NRC (2001) reported that ingestion of dry matter from starter increases from about 0.8 percent to 0.1 percent of body weight at 3 weeks of age to 2.8 percent to 3.0 percent of body weight at 8 weeks of age respectively.

2.3.4 Protein requirements of calves

Protein requirements for calves are grouped into two main parts namely; maintenance and gain. According to NRC (2001), maintenance constitutes essential nitrogen losses in urine and faeces and gain relates to nitrogen stored in the tissues. Requirement of protein is expressed in apparent digestible protein (ADP). The apparent digestible protein is given by the equation:

ADP (g/d) = 6.25 [1/BV (E + G + M x D) – M x D].

Where BV = biological value (the efficiency of nitrogen use for growth above maintenance, equal to a value of 0.80), E = endogenous urinary nitrogen, G = the amount of nitrogen in gain,

M = metabolic fecal nitrogen, and D = the amount of dry matter consumed. Loss of nitrogen in hair and skin is disregarded (NRC, 2001).

2.3.5 Colostrum

Colostrum is a yellowish fluid produced by dams soon after birth rich in minerals and antibodies before the production of true milk. Colostrum is an excellent and very essential source of vitamins and minerals for newborn calves. Placental transfer of immunoglobulin (Ig) is minimal in cattle as a result of the thick epitheliochorial placentation. Calves are basically born with no immunity; hence, they are extremely dependent on the passive transfer of immunoglobulin from the colostrum of the dam (Pedersen *et al*., 2000). The immunoglobulins in colostrum are derived from plasma proteins and are transported through mammary secretory cells into the colostrum. Upon intake of the colostrum, Ig binds to receptors in the microvilli of the intestine and is absorbed by nonspecific endocytosis into the epithelial cells of the jejunum and ileum. The Ig is enclosed in a vacuole, which moves to the cell membrane and expels its contents by exocytosis into the lamina propria. From there, the Ig passes into the systemic circulation via lymphatics and venous capillaries. This transfer of the proteins ceases 24 hours after the calf is born (Pedersen *et al*., 2000). Colostrum is vital for neonatal calves as a result of its disease guard by passive immunity and its supply of energy. For the survival of calves, there is the need for high intake of quality colostrum soon after birth. Low blood IgG concentrations are directly related to calf morbidity and mortality as well as long term calf performance. Arthington *et al*. (2000) reported a positive linear relationship between colostral IgG concentrations and IgG absorption. The immunoglobulin content of colostrum is highly variable. In order for a calf to receive at least 100 grams of IgG, it must within an hour after birth ingest 3 liters of colostrum from multiparous cows (NRC, 2001). Furthermore, NRC (2001) reported that the colostrum contains numerous

growth factors and hormones that excite growth and improvement of the digestive tract and other organ systems unlike colostrum replacements.

2.3.6 Water

Water is vital for calves for optimum growth and consumption of dry feed. Since water constitutes 70-75% of the weight of a calf, it is essential to thermoregulation and osmoregulation (NRC, 2001). Regulation of body water is a problem for young calves with diarrhea. Scours in calves can result in a 10-12% reduction in body weight with loss of electrolytes and possible death (NRC, 2001). Heinrichs and Lesmeister (2005) reported that calves should be allowed to access water from an early age and this result in an increase body weight, feed intake, and reduced the incidence of diarrhea.

2.3.7 Health and disease in calves

Globally, calf and herd health is a great concern among producers, as it can be detrimental to performance and increase rearing costs (Donovan *et al*., 2002). The possibility of disease transmission is increased when calves are mixed into a group pen. The transmission can take place through many routes such as fecal-oral route, cross-suckling, and aerosolization of pathogens (Donovan *et al*., 2002). Normally it is cheaper to prevent calves from contracting disease than treating them. According to Rulofson *et al* (1993), it is cheaper to prevent disease buildup in neonatal calves than treating sick animals as this leads them off to a good start and reduces mortality. Hunter (1996) reported that healthy animals that are well nourished, provided with water, adequate shelter or shade, and not overworked or stressed are usually better equipped to cope with any disease problems that they may encounter and are also more productive. Enough air circulation in calf house is critical as this prevents ambient

temperatures becoming extremely high and thereby endangering the calves to heat stress. High-quality ventilation reduces the risk of spread of respiratory infections (Hunter, 1996). Management practices such as castration, dehorning branding, ear-tagging and tattooing should be done at least two weeks prior to weaning so as to minimize stress on calves. Calves must be vaccinated to protect them against infection. Calves must also be monitored during the hours of the day for any signs of abnormalities, respiratory problems and occurrences of diarrhea. Cattle normal body temperature is 38.6°C and hence the use of rectal thermometer in calf pen helps in early detection of diseases.

Parish *et al*. (2009) reported that early disease detection is essential for effective treatment

2.3.8 Calf diarrhea

Diarrhea is a regular challenge to the health and well-being of calves. Diarrhea in calves causes more financial losses to ruminant livestock producers than any other health conditions in their herds (Rulofson *et al.*, 1993). Scours averts the absorption of fluids from the intestines of calves. The scouring calf loses fluids and rapidly dehydrates (Rulofson *et al.*, 1993). Furthermore, loss of body fluids is related to a loss of electrolyte (important body chemicals) and the buildup of acid. Diarrhea in calves is caused mainly by infectious organisms such as viruses (Corona virus and Rotavirus), bacteria (*E. coli*, *Salmonella*, and some types of *Clostridia*) and protozoan parasites. Olsson *et al.* (1994) reported that an infectious agent causes the primary damage to the intestine, but death from diarrhea usually results from dehydration, acidosis, and loss of electrolytes.

2.3.9 Calf mortality

Mortality in calves is also a major concern for livestock producers. Calf mortality means a loss of potential breeding stock and replacement dairy cows as well as revenue. Poor management

practices, lack of proper pen-hygiene, malnutrition, starvation, diarrhea, and disease and pest infestation among others are the major causes of calf mortality in Ghana. Olsson *et al*. (1994) reported that in West Africa, the GI (gastrointestinal) parasites are one of the major problems facing domestic animals production. They are very prevalent and despite the fact that often neglected, may cause severe losses in young animals. Protozoan parasites like coccidies, and helminths with various classes of nematodes, cestodes and premature stages of trematodes consist gastrointestinal parasites.

The major causes of mortality in calves are diseases, starvation and snake bites (Kaba, 1994). Table 2.3 indicates calf mortality rates in the guinea savanna zone of Ghana from 1984 to 1992, which range from 6.9% – 10.2% and the average for the same period being 8.4%. Adequate nutrition, colostrum, vaccination, good sanitation and probiotic administration are some of the preventive measures against calf mortality.

Table 2.3: Mean Annual Calf Mortality Rate from 1984 to 1992 in the Guinea Savanna Zone of Ghana.

Year	Number of calving	Number of death	Mortality %
1984	166	17	10.2
1985	184	16	8.6
1986	154	13	8.4

1987	163	12	7.3
1988	169	16	9.4
1989	160	11	6.9
1990	183	13	7.1
1991	297	26	8.7
1992	147	14	9.3

Source: Kaba (1994)

2.4 Antibiotics and Antimicrobial Drugs used in cattle production

Antibiotics are pharmaceutical drugs that are used to treat infections caused by bacteria and other microorganisms in animals. Antimicrobial agents are used in livestock and cattle production for therapeutic, prophylactic, and to support growth and enhance efficiency of feed utilization (NRC, 1999). The therapeutic uses of antibiotics in clinically ill animals involve using medicinal amount of antimicrobial agents for a quite short time period. Conversely, antimicrobial agents used for acute illness may be given to the entire animal group and not just to sick animal. Numerous antimicrobial agents that are used therapeutically are administered in water to animals kept under industrial settings (NRC, 1999). This may result in individual cattle getting insufficient amount. Among the antimicrobial agents approved for therapeutic use in animals, many are identical or similar to drugs used in human medicine (USDA, 2003).

Little amount of antimicrobial agents are added to healthy animals feed in animal production to support growth, essentially by enhancing efficiency of feed. Antimicrobial agents may be used prophylactically at either low doses or therapeutic doses to prevent diseases that are regular to animals raised under industrial conditions (NRC, 1999). Additionally, sub-therapeutic doses of

antimicrobial agents added to animal feed also results in promoting growth in animals. This may result in shorter time to butchery at less expense to the producer as well as improving profits and decreasing consumer costs (Mathews, 2001).

2.4.1 Antibiotic resistivity

Microbial resistance is an adaptation mechanism exhibited by bacteria and other microbes to all forms of biochemical stress (Hooper *et al.*, 2001). In consequence, microbes in general have a way of protecting themselves from the harmful environment in which they find themselves. There are several mechanism through which bacteria becomes resistant. Some bacteria have the ability of transferring resistance genes and can confer antibiotic resistance on earlier vulnerable bacteria. Furthermore, certain bacteria also have the ability of neutralizing the effectiveness of antibiotics; other bacteria can also release the antibiotics before it can have a result. Some of the mechanism exhibited by bacteria resistance to antibiotics and other toxins according to Džidic *et al.* (2008) and Hooper *et al*. (2001) include the following:

- Modification of cell wall permeability such that antimicrobials cannot enter to cause harm.
- Inactivation of the antibiotics by enzyme hydrolysis before they reach target sites.
- Some bacteria also absorb insignificant quantities of these antimicrobial substances and are therefore not harmed by them.

Microorganisms that cause diseases are increasingly becoming resistant to currently available antibiotics and thereby causing problems in human health. Numerous diseases such as, TB, STDs and infections of the ear have become complex to cure with antibiotics. Several livestock industries use antibiotics in their management practices either for therapeutic purposes, prevention

of disease and/or production purposes. Lack of adherence to withdrawal period from the use of antibiotics can lead to resistance to drugs used in treating human illness

2.4.2 Alternatives to Antibiotics in Animal Production

Antibiotics are normally used as feed additives for calves for growth promotion. Low concentrations of antibiotics are administered to large numbers of healthy animals for long period of time to increase the rate and efficiency of growth. These low levels of antibiotics are below the minimum inhibitory concentration of most pathogens. Antibiotic resistance in microorganisms has been connected to the constant use of antibiotics which could be transmitted from animals to human. However, short term application of antibiotics reduces this risk (Buchanan *et al*., 2008). Because of the increase in resistance and the outbreak of diseases caused by these resistant bacteria strains, it has become important to stop the use of medically important antibiotics in animal production (Buchanan *et al*., 2008). Thus, there has been the need to find alternatives to antibiotic growth promoters and antibiotics used in animal production and feeding. Several strategies according to Doyle (2006) have been proposed among which the use of other feed additives is prominent. Doyle (2006) again reported that for any additive to effectively replace antibiotics in growth promotion, that additive should be able to improve feed efficiency, increase growth rate and also lower the occurrence of certain diseases. Hardy (2002) also reported that some feed additives that are being tested as possible alternatives to antibiotic growth promoters include prebiotics and Direct-Fed Microbial (DFM) or probiotics.

2.5 Prebiotics and Probiotics

2.5.1 Prebiotics

Prebiotics are indigestible carbohydrates which are not metabolized in the small intestine and fermented in large intestine. Miguel *et al*. (2004) reported that the population of coliform in the large intestine and colon of neonatal calves and pigs can be reduced by administration of prebiotics. Prebiotics in recent times have been found to have immune improving characteristics (Okamoto *et al*., 2003). Addition of chitosan oligosaccharide to the diet of broilers was found to be effective in improving ileal digestibility of nutrient and efficiency of feed (Huang *et al*., 2005). Huang *et al*. (2007) reported that higher serum IgG, IgM and IgA concentration was observed in broilers supplemented with oligochitosan prebiotic compared with the control animals. Furthermore, Kong *et al*. (2007) also showed that piglets supplemented with prebiotics containing Chinese herbal ultra-fine powder had higher average daily gain, efficiency of feed consumption and decreased occurrence of diarrhea. Moreover, Deng *et al*. (2007) stated that the intestinal microflora of piglet was improved when supplemented with prebiotic containing polysaccharides of cassiae seed. They concluded that addition of this prebiotic to the diet of piglet enhanced *Lactobacillus* count and decreased *Escherichia coli* count in the colon.

2.5.2 Probiotics

Probiotics are defined as live microbial feed supplements which beneficially affects the host by improving its intestinal microbial balance (Heyman and Menard, 2001). Probiotics have been reported to improve immune system as well as protect young animals against enteropathic disorders (Timmerman *et al*., 2005). Lesmiester *et al*. (2004) also reported that probiotic has the ability of improving feed efficiency and weight gain. According to Krehbiel *et al*. (2003),

probiotic supplementation to young calves can reduce occurrence of diarrhea and development of diseases after weaning or transport as well as milk yield improvement in lactating cows. Furthermore, Davidson *et al.* (2000) reported that the production performance of ruminant livestock can be improved by microbial products identified to encourage rumen metabolic growth through transforming rumen function and fermentation activities of its microflora. Also, according to Davidson *et al*. (2000), the health benefits in humans from frozen yogurt supplemented with bacterial probiotics include improved lactose utilization, anti-carcinogenic activity, and control of intestinal infections.

An effective probiotic must have the ability to become part of the normal microflora in the intestine and should survive passage through the gastrointestinal tract and be able to adhere and colonize the intestinal tract. Organisms that can produce a substance that can inhibit growth or kill existing organisms in the intestine have a distinct advantage because there are so many other organisms in the intestine (Oh *et al*., 2000).

2.5.3 Mode of action of probiotics bacteria

Even though the mechanism of action of probiotics have not been obviously acknowledged, Riddell *et al*. (2010) reported that lactic acid bacteria are thought to impede the development of pathogenic bacteria by lowering the pH level in the large intestine through production of lactic acid as well as competitive attachment. Blaut (2002) reported that Lactobacilli are one of the major species of beneficial micro-organism in the gut of monogastric animals. Some species of lactic acid bacteria that have been used as probiotics include *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, and Lactobacillus reuteri,* and some *Bifidobacterium species.* The fore-stomach of ruminants in early stage is comparable to that of monogastric animals and therefore supplementation with *Lactobacillus*

enhances digestibility of nutrients (El Adawy *et al*., 2000; Soliman *et al*., 2000) and eventually growth in pre-weaning calves. *L. acidophilus* is a homo fermenter, which means it ferments lactose efficiently to lactic acid (Cruywagen *et al*., 1996). Again, a probiotic strain has a distinct advantage if it is a good competitive inhibitor. Lactic acid bacteria produce a number of antimicrobial materials like organic acids, hydrogen peroxide, and bacteriocins and thus making it an excellent probiotic. According to Oh *et al*. (2000), *L. acidophilus* strains exhibit more acid and bile tolerance than other lactic acid bacteria. In particular, *L. acidophilus* 30SC was able to survive in bile because it was able to deconjugate bile acids. It was also active over a wide pH range and stable in heat treatments. *L. acidophilus* and *Bifidobacterium* can tolerate a pH of 3 and 2 to 8% concentrations of bile acid (Tejada-Simon *et al*., 1999).

Bacillus subtilis is another probiotic that showed beneficial results. *Bacillus* species have particular mechanisms that hinder gastrointestinal tract infection by pathogens or producing antimicrobials. Kritas *et al*. (2006) examined the effects of probiotic containing *B. licheniformis* and *B. subtilis* on young lambs and milking ewes under field conditions which tend to reduce the mortality of young lambs and increased the daily milk yield of ewes. Jenny *et al*. (1991) reported that calves fed *B. subtilis* had higher body weight gain compared to calves fed the control or another treatment of a mixed microbial concentrate.

Several other bacterial strains have been tested for use as feed additives. *Bifidobacterium pseudolongum* added to the diet of pre-weaned calves was reported to have improved body weight gain and decreased incidence of diarrhea (Donovan *et al*., 2002). Furthermore, *Bifidobacterium pseudolongum* showed a positive effect on the body weight gain and feed conversion efficiency of calves (Abe *et al.,* 1995). They proposed that this was a result of improved intestinal environment based on lower faecal scores. Administration of this probiotic

was as effective as antibiotics in protecting against diarrhea. At birth there are no bacteria in the intestine, so the probiotic administered at this time will likely have a better opportunity to colonize in the intestine over the pathogenic bacteria. *L. acidophilus*, *Bifidobacterium* has been reported to stimulate immunity in animals (Abe *et al*., 1995). This same microbe added to frozen yogurt has provided various health benefits in humans as well (Davidson *et al.,* 2000).

2.5.4 Probiotics in Calf Feeding Systems

Several researches revealed that the inclusion of probiotics in calf feeding systems significantly enhanced ADG, feed intake and feed conversion ratio. The oral administration of probiotic containing *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus* in milk replacer tends to improve body weight gain, feed conversion ratio and intake of feed in neonatal calves (Abe *et al.,* 1995). In contrast, Windschitl *et al*. (1991) in an experiment using Holstein calves reported that the body weight gain of calves 4 to 7 months old and feed efficiency did not significantly improved by the inclusion of *Lactobacillus acidophilus*, *Bacillus subtilis* and *Aspergillus oryzae*. Nonetheless, several researchers also reported that probiotics have the ability to reduce incidence of diarrhea in calves. According to Abe *et al.* (1995), supplementation of probiotics containing *Bifidobacterium pseudolongum* and *Lactobacillus acidophilus* to Holstein calves decreases the occurrence of diarrhea. Taras *et al*. (2006) also reported a reduced diarrhea incidence in piglets supplemented with probiotic containing *Enterococcus faecium*. This result is an indication that probiotics may improve the resistance of pathogenic bacteria that causes diarrhea. Additionally, supplementation of probiotics containing *Lactobacillus acidophilus* to the diet of calves was found to improve the average daily intake of calves in the treatment group than the control group (Cruywagen *et al*., 1995). Conversely, they reported no significant difference in the occurrence of

diarrhea in both the treatment groups and the control. In contrast, Higginbotham and Bath (1993) reported no significant difference in average daily gain and faecal score when *Lactobacillus acidophilus* was added to milk replacer and waste milk of Holstein calves. Gill *et al*. (1987) working with cattle also reported an increase in average daily gain and an improvement of feed efficiency in the probiotic treated groups compared with control groups. Moreover, Galyean *et al*. (2000) also working with finishing beef steers fed steam flaked corn diet reported that a mixture of probiotic bacteria may be more effective for use in feeding cattle on a high concentrated diet as a substitute of one specific microorganism. There is therefore the need to supplement calves with RE3 for increase production.

2.5.5 RE3 as a Probiotic Product

RE3 is a probiotic obtained from natural and organic resources consisting of alfalfa plant, barley grain, wheat etc and are free from toxic chemicals and can be administered orally or mixed thoroughly with animal diet before feeding. The product was introduced in Ghana to reduce the cost of animal production and maximizes profit, improve the animal immune system and reduce the use of antimicrobial drugs in livestock industry.

RE3 is produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada. RE3 (Table 2.4) is in the liquid form. Several researches conducted in Ghana on the effects of RE3 on different farm animals‟ shows positive results. Okai *et al*. (2010) observed significant ($P < 0.05$) improvements in average daily gain (ADG), feed conversion ratio (FCR) in weaned pigs. Bonsu *et al*. (2012) also reported better FCR and reduced serum cholesterol of RE3 supplementation in broilers. Dei *et al*. (2010) found that addition of RE3 to the diet of broilers reduced the cost of production as a result of reduced medication such as coccidiostats. Additionally, Osei *et al*. (2013) explained that RE3 supplementation in gestating rabbits resulted

in heavier bunnies. Wallace *et al*. (2012) also indicated that the addition of RE3 to rabbit diets resulted in significantly ($P < 0.05$) better FCR of (4.739 versus control 5.062), higher white blood cell (WBC) and lymphocyte levels respectively (13.33×10^9 µL and 8.37×10^3 µL versus control 6.97×10^9 µL and 3.73×10^3 µL) . The addition of RE3, again, resulted in higher levels ($P < 0.05$) of blood platelet in birds (Agyarko, 2013). Recently, Bonsu *et al*. (2014) reported that the inclusion of RE3^{TM} to layer chicken diets resulted in the laying of heavier eggs with a reduction ($P < 0.05$) in feed consumption as compared to the control. There is no reported work currently on the use of RE3^{TM} on calves and that necessitate this current study The composition of RE3 is shown on Table 2.4.

Table 2.4: Composition of RE3

Constituents	Amount
Water	99.9%
Microorganisms	
Lactobacillus spp.	1.0×10^8 CFU/g
Bacillus spp.	4.0×10^{12} CFU/g
Saccharomyces cerevisiae	11×10^5 CFU

Source: Amoah (2010).

2.6 Haematology

Haematology as defined by Merck Veterinary Manual (2012) is the study of the numbers and morphology of the cellular elements of the red blood cells, white blood cells, and the platelets and their use in the diagnosis and monitoring of disease. Mmereole (2008) and Isaac *et al.* (2013) reported that the study of haematology is very important in selecting animals that are genetically resistant to diseases and environmental conditions. Furthermore, Khan and Zafar (2005) reported that the haematological parameters are excellent indicators of the animal physiological status. Therefore calves with superior blood composition are more likely to exhibit good performance.

2.6.1 Haematological Parameters

The blood and blood forming organs are connected to haematological parameters (Waugh and Grant, 2001; Bamishaiye *et al*., 2009). The blood parameter changes in relation to the physiological status of animal. Haematological parameters commonly used are red blood cells, white blood cells, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration (Carlson, 1996; Chineke, 2006).

2.6.2 Red Blood Cell

According to Wikipedia (2013), the red blood cell count refers to a blood examination that shows the number of red blood cells in the whole blood of an animal. Bunn (2011) reported that the red blood cell examination can facilitate anaemia diagnosis in animals and other conditions affecting the red blood cells. Red blood cells help in the transportation of haemoglobin which subsequently transports oxygen to other parts of the animal body. Awodi *et al*. (2005) reported that the major functions of the red blood cells or erythrocytes are to serve as carrier

haemoglobin. During internal respiration, oxyhaemoglobin are formed when haemoglobin reacts with oxygen in the blood.

Merck Veterinary Manual (2012) reported that the normal reference values for red blood cell for cow is 5.0 x 10^6/mm^3 – 10.0 x 10^6/mm^3. Congenital heart disease and dehydration resulting from excessive diarrhea and low level of oxygen in the blood are connected with high red blood cell. Additionally, a very low red blood cell level is a sign of anemia, bone marrow failure due to toxins, malnutrition and haemorrhage (Gernsten, 2009; Bunn, 2011).

2.6.3 White Blood Cell

The white blood cells (WBC) also known as leucocytes are cells of the immune system involved in protecting the animal body against disease, foreign materials, and infections (Chineke *et al*., 2006). According to Maton *et al*. (1997), there are five different types of white blood cells and are all produced and derived from hematopoietic stem cell. White blood cells are found throughout the body of an animal. The normal values for white blood cell, neutrophiles and lymphocytes reported by Merck Veterinary Manual (2012) for cow are 4 – 12 (K/µL), 0 – 5% and 2.5 – 7.5% respectively.

High level of white blood cell is an indication of infectious diseases. According to Valencia (2012), an increase level of white blood cell may possibly be as a result of infection, disorder of the immune system as well as trauma. Additionally, a decrease level of white blood cell is an indication of a decrease in disease-fighting cells circulating in animal‟s body (Mayo, 2013). Animals with very low white blood cells are easily exposed to infections. According to Bagby (2007), low level of white blood cell may be as a result of deficiency of the bone marrow, liver or spleen diseases and exposure to radiation.

2.6.4 Haematocrit/Packed Cell Volume

Packed Cell Volume also referred to as Haematocrit as defined by Purves *et al.* (2003) is the fraction of the red blood cells in the whole blood after centrifuging. Isaac *et al*. (2013) reported that the Packed Cell Volume is involved in the carrying of nutrients absorption and oxygen transport. Furthermore, Wikihow (2013) reported that the Packed Cell Volume of animals can be used to assess the condition of the animal as well as the degree of anaemia. Additionally, Wikipedia (2013) reported that low level of haematocrit is an indication of persistent iron deficient anaemia resulting in unusual haemoglobin synthesis throughout erythropoiesis. Moreover, a very high haematocrit level is connected with dehydration and diarrhea (Chineke *et al*., 2006). RAR (2009) documented the normal haematocrit or Packed cell volume values for cow to be 24 – 48%.

2.6.5 Haemoglobin

Haemoglobin is very important in animal respiration. They carry oxygen in the blood from the lungs to the rest of the body for tissue respiration which then releases energy to the animal. According to Costanzo (2007), the animal haemoglobin can bind up to four oxygen molecules. Loss of blood, bone marrow disorders and nutrient deficiencies are connected with low level of haemoglobin MedicineNet (2012). Additionally, elevated levels of haemoglobin are also linked to exposure to high altitudes and dehydrations (MedicineNet, 2012). Merck Veterinary Manual (2012) documented 10 – 15 (g/dl) as the normal levels of haemoglobin for cow.

2.7 Glucose in calves

Glucose is the free sugar which circulates in the blood. The level of blood sugar that is circulating the animal body rises soon after intake and absorption. Glucose concentration in the blood of a fasting animal is found to be about 80 mg per 100 ml (Rastogi, 2008). Nevertheless,

the glucose level is almost maintained constant and changes with narrow limit except during abnormal conditions like hyperglycemia or hypoglycemia (Angelov *et al.,* 1996). According to Rastogi (2008), the major source of energy for the body is blood sugar and it is obtained from the breakdown of carbohydrates derived from daily intake and regulated via the process of glycogenolysis and gluconeogenesis. The blood sugar level is maintained by diet absorption and insulin. Rastogi (2008) reported that presence of too much insulin drops the sugar level below normal resulting in hypoglycemia. Furthermore, Rastogi (2008) also reported that absence of enough insulin slows down transport of blood sugar thereby causing it to rise resulting in a condition known as hyperglycemia. Measurement of blood sugar level is essential in the evaluation of carbohydrate related disorder (Rastogi, 2008). The glucose level in the blood of calves depends on the age of the calf, feeding regime and type of feed. Ilgaža and Birğele (2003) reported that the glucose level in the blood of calves becomes more stable when giving concentrate mixed diet and forages and it is not affected with feeding and time after feeding. According to Angelov *et al.* (1996), the level of glucose in the calf blood at birth is very low estimated to be 1.68 ± 0.19 mmol/L. But considerably higher amount of glucose in the blood of neonatal calves prior to the first feed intake is estimated to be between 4.1mmol/L to 4.3 mmol/L. Tighe and Brown (2003) reported a range value of 2.1 – 3.8 mmol/L and Tietz (1995) reported 3.33-6.11 mmol/L. These differences could be associated with the animal breed, climatic conditions, nutrition of pregnant cows and other circumstances that affect the sugar level in the blood of new born calves (Ilgaža and Birğele, 2003).

2.8 Total protein, Albumin and Globulin

The concentration of plasma proteins remains constant even during dietary variations and abnormal conditions. Prolonged malnutrition, however, affects the protein concentration. Quoted values for normal range of total protein in serum are 6.3 – 7.8 g/dl (Rastogi, 2008), 70 – 94 g/L, (Tighe and Brown, 2003) and 6.0-7.8 g/dl (Singh, 2003). Reported normal range of albumin in serum are 3.2 – 5.1 g/dl (Rastogi, 2008), 34 – 43 g/L (Tighe and Brown, 2003) and 3.5-5.0 g/dl (Singh, 2003). Normal ranges of globulin in serum are 2.5 g /100 ml (Rastogi, 2008) and 2.3 - 3.5 g/dl (Singh, 2003). In certain abnormal conditions the albumin content of the plasma is lowered. Concentrations below 2 g/100 ml are always associated with oedema. Furthermore, globulins carry the lipid fraction of proteins and contain antibodies for generating immune responses (Rastogi, 2008). Albumin/globulin ratio in blood is 1.2 - 1.5 (Singh, 2003). Hyperproteinemia or hyperalbuminemia usually occurs during multiple myeloma caused by dehydration, excessive water loss, as in severe vomiting and diarrhorea (Rastogi, 2008). Hypoproteinemia or hypoalbuminemia usually occurs in malnutrition, nephritic syndrome, malabsorption and severe liver cirrhosis (Rastogi, 2008). Details of the chemical constituents of cattle blood are shown in Table 2.5.

Table 2.5: Chemical Constituents of Blood in Cattle

Parameters	values
Blood Glucose (mg %)	35 – 55
Total Plasma Protein (g %)	6.74 – 7.46
Albumin (g %)	3.03 – 3.55
Globulin (g %)	3.00 – 3.48
Serum Total Cholesterol (mg %)	50 - 230

Source: Pampori (2003)

In conclusion, several studies demonstrate that probiotics (RE3) are beneficial to the calf, especially in the early stages of its life by improving growth, decreasing diarrhea incidence and improving overall animal health. Several strains of bacteria have been tested, with the most beneficial being *Lactobacillus* and *Bifidobacterium.* These probiotics may minimize or eliminate the use of antibiotics in feed and; thus, are extremely promising for the cattle industry.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Animals and Dietary Treatments

Sixteen N‟dama calves were used in a sixteen week experiment to determine the effect of probiotic supplementation on growth performance and reduced diarrhea incidence of N‟dama calves. All calves were born at the University of Cape Coast Teaching and Research Farm, Cape Coast, Ghana, between, December, 2013 and February, 2014 and housed at the UCC-Research Farm for the entire duration of the study.

Calves were maintained in a well-ventilated and properly managed shed pen measuring 2m x 2m. The body weight of the calves was recorded with a weighing balance at the start of the experiment and thereafter regularly at two weeks interval. Weighing was done early morning before the calves were allowed to access feed and water. Calves were ear-tagged in order to facilitate their identification and handling.

3.2 Source of RE3 and storage

The RE3^{TM} (multi-strain) used in this experiment was obtained from the Basic Environmental Systems and Technology (BEST), Canada. The DFM (RE3^{TM}) was composed by *Lactobacilli* (1×10^{8} cfu/g), *Bacillus* (1×10^{12} cfu/g), *Saccharomyces cerevisiae* (yeast, 1×10^{5} cfu/g) and fermentation product. The product was stored at a clean dry place.

3.3 Experimental Design and Probiotic Administration

Sixteen N‟dama calves (bulls n=4, heifers n=12) of an average initial weight of 44.2 kg were randomly assigned to one of four dietary treatments according to their body weight, age and sex. The treatments are as follows:

T1- Control (No RE3 supplementation).

T2- 0.03ml RE3 per kg body weight

T3- 0.06ml RE3 per kg body weight

T4- 0.09ml RE3 per kg body weight

The experiment was laid out in a Completely Randomize Design (CRD) and there were 4 replicates per treatment with 3 females and 1 male in each treatment.

3.4 Feeding and Health Management of Calves

Calves were sent out for grazing every morning from 9.00 am to 2.00 pm on the paddocks within the University of Cape Coast Research Farm. The calves were also allowed to suckle milk from their dams and were allowed free access to water throughout the experiment. The calves were sprayed with the acaricide Fenrovet[1] at dilution rate of 100 ml per 20 liters of water to control tick

[1] Mobedco, The Arab Pesticides and Veterinary Drugs Mfg. Co., Amman, Jordan [2] Hebeiyuanzheng Pharmaceutical Co. Ltd., China

infestation in the second month of the experiment. Experimental calves and the control were also dewormed with Albendazole[2] for the control of worms.

3.5 Sample Collection

3.5.1 Diarrhoea Presence and Weight Gain

Each day before feeding, calves were observed in their pens and faecal scores recorded according to the method of Larson *et al*. (1977). For faecal fluidity, scoring was done as follows:

1 = normal, 2 = soft, 3 = runny and 4 = watery.

Body weight of calves were recorded at the beginning the experiment using Salter Brecknell[1] scale and at 14, 28, 42, 56, 70, 84, 98 and 112 d of age.

3.5.2 Blood Samples

The samples of blood were obtained from each calf at the end of the experiment through jugular vein puncture. Seventy percent (70%) alcohol was used to disinfect the vein location to prevent contamination. Five ml of the blood was taken from each animal and 2.5 ml of the blood taken was transferred into EDTA tubes to prevent clotting. The remaining 2.5 ml was transferred into a plain tube not containing EDTA to cause clotting. The blood samples were then placed in an icechest containing ice and sent to Noguchi Memorial Institute for Medical Research, Accra, Ghana for analysis.

[1] Salter Brecknell Scale (5kg – 300kg): Made in USA

3.6 Laboratory analysis

3.6.1 Haematological parameters

Sysmex analyser[1] was used to analyse blood samples transferred into EDTA tubes for the determination of the following parameters: Haemoglobin (Hb), White blood cell (WBC), Red blood cell (RBC), Haematocrite (HCT), Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Red cell distribution width (RCDW), Neutrophiles, Lymphocyte and Platelets. The methods used were the ones outlined by Gersten (2009).

3.6.2 Blood chemistry

The clotted blood was further centrifuged at 2,000 rpm for 10 min at 4 ^{o}C to separate the serum from the whole blood.The serum was used for the analysis of blood glucose and total protein using Selectra E analyser[2].

[1] Sysmex Haematology Analyzer. Made in China.

[2] Selectra E Chemistry Analyser. Made in Netherland

3.7 Statistical Analysis

All data collected were analyzed as complete randomize design (CRD) using SAS (2012), and Duncan's Multiple Range Test was used to compare treatment means. The initial weight of the calves was included in the model as a covariate to account for differences in starting weight.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Effects of RE3 on Body Weight

The effect of Probiotic (RE3) on average daily gain (ADG) of calves for the experimental period is shown in Table 4.1.

Table 4.1 Effect of RE3 supplementation on average body weight gain (kg) of N'dama Calves.

	Treatment					
Parameters	T1	T2	T3	T4	SEM	P-Value
Final BW (kg)	85.1	85.3	86.1	88.6	0.84	0.07
Initial BW (kg)	47.0	44.5	41.5	43.8	1.13	0.92
ADG (kg)	0.34	0.37	0.39	0.40	0.01	0.07
INGS (ml)	0.00	3.00	5.00	8.00		

T1 = (control) no RE3 supplementation, T2 = 0.03ml/ Kg body weight.

T3 = 0.06ml/Kg body weight, T4 = 0.09ml/ Kg body weight.

ADG= Average Daily Gain, SEM = Standard Error of Mean. INGS= Ingested Supplement.

As shown above (Table 4.1), calves initial body weight at the initiation of the experiment was averaged 44.2 kg body weight. Average weights ranged from 41.5 kg for T3 to 47.0 kg for T1.

Average daily gain (ADG) of all treated calves (T2, T3 and T4) did not show much difference (P>0.05) from the control, however, there was a slight improvement in the performance of calves supplemented with RE3. The lowest value of ADG was recorded for the control group T1 (0.34 kg) as compared to those on the treatment groups T2 (0.37), T3 (0.39) and T4 (0.40)). The positive effects of probiotics supplementation could be due to decrease in the multiplication of harmful bacteria resulting from improvement in gut environment and enhanced nutrient utilization (Miles, 1993).

The experimental animals have higher numerical value (final body weight) than the control at the end of the experiment (Table 4.1). However, the difference observed between the two groups was not statistically significant (P > 0.05). Again feed intake was not measured because the calves were allowed to graze freely and no special diet was formulated for the animals to feed on. Among the experimental animals as shown in table 4.1, the ingested supplement of RE3 was higher in treatment T4 and least in treatment T2 and this is as a result of body weight differences. In an experiment using lambs, El-Shamaa (2002) reported no significant difference in animals with higher ingested supplement as compared to those with lower ingested supplement. This current result is in agreement to the result of Morrill *et al*. (1995), Kamra *et al*. (2002) and Gorgulu *et al*. (2003) who reported no significant difference in average daily gain in the control and experimental groups. Saijpaul *et al.* (2005) also observed that supplementation of *Lactobacillus acidiphilus* 0.1% of body weight in rabbit diet had no effect on either body weight gain or on digestibility of different nutrient when supplemented for a period of one month. Likewise, no effect on body weight gain was observed when Rao (2007) supplemented

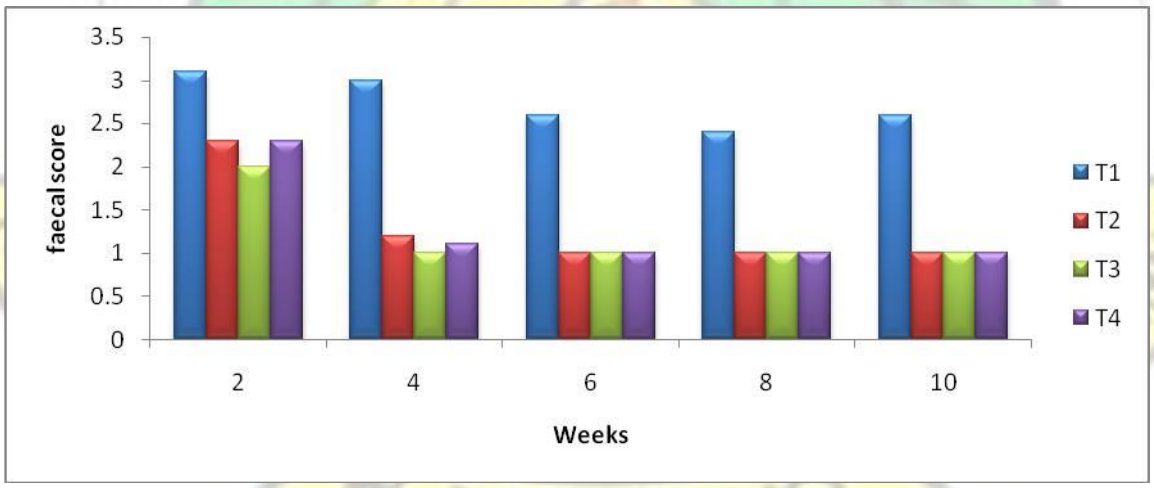
Lactobacilli in male *Muzaffarnagari* lambs (age 10 days to six months). Vishal and Baghel (2010) in an experiment using Buffalo calves concluded that supplementation with *Lactobacillus acidiphilus* is more beneficial in initial stages of calves‟ lives when fibre level in the diet is low and that the effect is reduced when the rumen develops, for then fibre intake may increase and utilization of crude fibre is not improved by the bacteria *Lactobacillus acidiphilus.*

In contrast, the positive effect of probiotics was found in Abe *et al*. (1995) with calves to 25 days of age. Hossani *et al*. (2010) reported that the groups with probiotic and antibiotic had significantly higher body weight than the control group ($P < 0.05$), which is consistent with Higginbotham and Bath (1993), who also conducted experiments in the first month of birth. Abdala *et al*. (2002) also reported a significant difference in the growth of the probiotic group between 21stand 42ndday.

4.2 Effect of RE3 on diarrhea

Mean diarrhea score by calves supplemented with or without probiotic is shown in Figure 4.1.

Figure 4.1. Mean faecal score of N‟dama calves supplemented with or without probiotics



T1 = (control) no RE3 supplementation, T2 = 0.03ml/ Kg body weight T3 = 0.06ml/Kg body weight, T4 = 0.09ml/ Kg body weight.

Faecal Score Scale: 1 = normal, 2 = soft, 3 = runny and 4 = watery

Calve diarrhea was evaluated using the faecal score and recorded according to Larson *et al*.‟s recommendation (1977). For faecal fluidity, scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery during the experimental period. A faecal score greater or equivalent to 3 was used as an indicator of diarrhea in the study. Diarrhea incidence was recorded in both the treated calves and the control calves in the first two weeks of the experiment (Figure 4.1). The highest faecal score was recorded in T1 (3 = runny) and the least faecal score was recorded in T3 (2 = soft). There were significant difference ($p < 0.05$) in the faecal score between the treatment group and the control after two weeks of the experiment; subsequently, faecal score became constant in the treatment groups and never exceeded the normal value (Figure 4.1). RE3 was found to reduce the incidence of diarrhea and was effective after two weeks of application. The reduced incidence of diarrhea may be as a result of an improved intestinal bacterial flora in calves supplemented with RE3.

Probiotics was found to suppress the occurrence of diarrhea in calves fed milk replacer (Timmerman *et al*., 2005). Additionally, Cruywagen *et al.* (1995) on the otherhand reported no positive effect of the inclusion of probiotic in milk replacer on diarrhea incidence. Probiotics may therefore help to enhance intestinal health of the calves when experiencing challenges. Transporting animals to a long distance may disturb their intestinal flora and this may cause diarrhea. Addition of probiotics to the diet of these animals may help reduce the occurrence of diarrhea by stabilizing their intestinal flora. Probiotics can reduce diarrhea in neonatal calves after weaning and/or transport as well as morbidity cases and can also advance milk yield in

lactating cows (Krehbiel *et al*., 2003). Furthermore, Gorgulu *et al.* (2003) also reported that calves supplemented with probiotics were superior with respect to diarrhea than the control groups and concluded that probiotics supplementation before weaning could boost calf health and reduce mortality and cost of buying drugs. The same conclusion was reported by Marcin *et al.* (2003) for piglets and calves. Their finding is in agreement with this present study.

4.3 Effect of RE3 on blood parameters

4.3.1 Hemoglobin, Packed Cell Volume and Red Blood Cell (RBC)

Effect of Probiotic (RE3) supplementation on the level of HGB, PCV and RBC‟s count on N‟dama calves is shown in Table 4.2.

Table 4.2: Effect of probiotics (RE 3) supplementation on haematological profile of N‟dama calves.

	Treatment				
Parameters	T1	T2	T3	T4	SEM

WBC (K/µL)	14.38	10.37	13.13	17.80	7.84
RBC (M/µL)	9.87	8.02	8.64	9.31	0.84
HGB (g/dL)	13.03	11.15	11.95	12.33	1.08
HCT (%)	42.23	35.85	37.68	38.95	3.80
MCV (fL)	42.73	44.23	44.13	41.78	1.47
MCH (ps)	13.20	13.88	13.98	13.23	0.40
MCHC (g/dL)	30.90	31.38	31.73	31.73	0.54
NEUT (%)	4.18	4.43	5.00	3.83	2.41
LYM (K/µL)	9.40	7.10	9.30	13.03	1.16
MXD (%)	5.75	4.58	10.50	5.45	2.73

T1 = (control) no RE3 supplementation, T2 = 0.03ml/ Kg body weight. T3 = 0.06ml/Kg body weight, T4 = 0.09ml/ Kg body weight.
SEM = Standard Error Mean, RBC = Red blood cell, HGB = Haemoglobin, HCT = Haematocrit, MCV =Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin WBC = White blood cell concentration, LYM = Lymphocyte, NEUT = Neutrophiles, MXD = Mixed cell count.

In general, probiotic supplementation had no significant effect on any of the haematological traits measured (P>0.05). Red blood cell (RBC) contains haemoglobin, an iron-rich protein which picks up oxygen as the blood passes through the lungs and transports and releases it to organs and tissues throughout the body. RBC is produced in the bone marrow, and is continuously being formed and broken down. They live for approximately 120 days in the circulatory system and are eventually removed by the spleen (Aderemi, 2004). A deficiency of red blood cells result in a condition known as anaemia and can lead to weakness, shortness of breath and irritability (LSA, 1994). Anemia is defined by Benjamin (1978) as a decrease below normal in RBC, Hb, and PCV. It was

observed from Table 4.2 that the control calves (T1) had higher percentage HCT (42%) than the experimental calves and it may be due to dehydration resulting from frequent diarrhea. Chineke *et al* (2006) reported that elevated haematocrit is most often associated with dehydration which is a decreased amount of water in the tissues, diarrhea etc. There were no significant difference in the percentage of blood volume composed of red blood cells (the haematocrit), MCV, MCH, and MCHC among the treatments and the control group (Table 4.2). This current study is in agreement with the findings of Adams *et al* (2008) who reported no variations between probiotic treated calves and their control counterparts in overall haematocrit. RE 3 as a probiotic can thus be said to sustain the normal haematopoietic function of calves as no significant differences ($P > 0.05$) were established among all the treatment groups and that the values obtained in this study were within the normal references for cow, RBC (M/µL) 5 – 10 , HGB (g/dL) 10 –15, HCT (%) 26 – 46, MCV (fL) 39 – 55, MCH (ps) 13 – 17, and MCHC (g/dL) 30 – 36 respectively (Merck Veterinary Manual, 2012) which indicates better health condition.

4.3.2 White Blood Cell (WBC)

A white blood cell (WBC) plays a vital role in protecting the body against disease causing bacteria, viruses and fungi. There were no significant differences ($p>0.05$) observed in WBC count among the treatment and the control groups (Table 4.2). However, the study showed a higher WBC value for T1 (14.38 K/µL) T3 (13.13 K/µL) and T4 (17.8 K/µL) whilst T2 had value that fell within the normal range for cow (10.37 K/µL). The high value recorded in the treatments (T1, T3 and T4) may be as a result of tick infestation which occurred in the last two weeks of the experiment. High WBC count has been reported to be usually associated with microbial infection or the presence of foreign bodies or antigens in the circulatory system

(Ahamefule *et al*., 2006). The normal values for WBC, NEUT, LYP and MXD reported by Merck Veterinary Manual (2012) for cow were 4 – 12 (K/µL), 0 – 5, 2.5 – 7.5, and 5 – 10 (%) respectively. Valencia (2012) reported that a high white blood cell count could be caused by infection, immune system disorders, and stress among others.

4.4 Blood Glucose

Table 4.3: Effect of probiotics (RE 3) supplementation on the blood glucose and total protein of N‟dama calves with or without probiotics.

	Treatment				
Parameters	T1	T2	T3	T4	SEM
Blood glucose (mg/dl)	65.68	73.88	78.08	80.85	0.30
Total Protein (g/l)	61.03	60.88	56.98	60.88	1.22

T1 = (control) no RE3 supplementation, T2 = 0.03ml/ Kg body weight, T3 = 0.06ml/Kg body weight, T4 = 0.09ml/ Kg body weight, SEM = Standard Error Mean.

The results (Table 4.4) revealed that there were no treatment effects in the blood glucose of the animals in both the control and the experimental calves. The glucose level is an indicator of physiological condition of the animals. It was observed from the above table that T4 and T3 recorded the highest value of glucose (80.85 mg/dl) and (78.08 mg/dl) respectively whilst the least value was recorded in T1 (65.68 mg/dl) but the difference observed was not statistically significant ($P > 0.05$). Glucose represents the synthesis of carbohydrates and is in the form in which carbohydrates is supplied to cell from body fluids. The values obtained in this study were within the normal references for cow 59 – 105 mg/dl and 61 – 81g/l respectively as documented in Merck Veterinary Manual (2012) which indicates better health condition of the animals.

4.5 Total Protein

The results (Table 4.4) revealed that there were no treatment effects in the total protein of calves in both the control and the experimental groups. Proteins are important building blocks of all cells and tissues. Proteins are necessary for growth, development, and health of animals. Deficiency of protein impairs both humoral and cell mediated immunity, thus predisposing an animal to diseases (Aderemi, 2004). Blood contains two classes of protein, albumin and globulin. Albumin proteins keep fluid from leaking out of blood vessels. Globulin proteins play an important role in the immune system. High total protein in animal may indicate inflammation or infections and bone marrow disorders (Aderemi, 2004). Furthermore, Aderemi (2004) agaun reported that low total protein may also indicate bleeding, liver disorder, kidney disorder and malnutrition. It was also observed that T3 recorded the least value of total protein (56.98 g/l) than T1, T2 and T4. The value obtained was within the normal references for cow 61 – 81g/l as documented in Merck Veterinary Manual (2012) which indicates better health condition of the animals. This is consistent with the findings of Adams *et al* (2006) who also found no differences between calves receiving probiotics and control animals.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Probiotic (RE3) supplementation generally improved average daily gain, reduced the incidence of diarrhea and did not adversely affect the levels of blood haematological and biochemical indices of N‟dama calves compared with the controls.

5.2 Recommendation

Farmers should be educated on the use of probiotics (RE3) in animal production in order to reduce the use of antibiotics in animal industry which has negative effect on the consumers‟

health. Further studies should focus on factors such as microbial composition of the probiotic (RE3) supplementation, application dose and time and their interaction with environmental condition to assess the effect on animal growth performance. Similar studies should be carried out using large number of animals to assess the effect of RE3 on animal growth performance.

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APPENDICES

Appendix 1: Covariate Analysis and Table of Means

Dependent Variable: FNWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1028.669113	257.167278	30.14	<.0001
Error	11	93.850262	8.531842		
Corrected Total	15	1122.519375			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	80.8738840	26.9579613	3.16	0.0682
INITWT	1	998.6372377	998.6372377	117.05	<.0001

Dependent Variable: ADGg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	12503.58975	3125.89744	4.60	0.0200
Error	11	7481.00963	680.09178		
Corrected Total	15	19984.59937			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	6446.902292	2148.967431	3.16	0.0682
INITWT	1	4033.137473	4033.137473	5.93	0.0331

Dependent Variable: FREDIAH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	217.8423269	54.4605817	3.72	0.0377
Error	11	161.1576731	14.6506976		

Corrected Total	15	379.0000000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	216.6157078	72.2052359	4.93	0.0208
INITWT	1	2.3423269	2.3423269	0.16	0.6969

Dependent Variable: RBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.21949060	2.05487265	0.67	0.6276
Error	11	33.84895315	3.07717756		
Corrected Total	15	42.06844375			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	7.26487225	2.42162408	0.79	0.5259
INITWT	1	0.47717185	0.47717185	0.16	0.7013

Dependent Variable: HGB

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.77296914	2.19324229	0.39	0.8106
Error	11	61.62453086	5.60223008		
Corrected Total	15	70.39750000			

Source	DF Type III SS	Mean Square	F Value Pr > F
TRT	3 6.68629223	2.22876408	0.40 0.7573
INITWT	1 1.45046914	1.45046914	0.26 0.6209

Dependent Variable: HCT

Source	DF	Sum of Squares	Mean Square	F Value Pr > F
Model	4	105.0653186	26.2663296	0.38 0.8181
Error	11	759.3646814	69.0331529	
Corrected Total	15	864.4300000		

Source	DF	Type III SS	Mean Square	F Value Pr > F
TRT	3	76.02489524	25.34163175	0.37 0.7782
INITWT	1	18.43031860	18.43031860	0.27 0.6156

Dependent Variable: MCV

Source	DF	Sum of Squares	Mean Square	F Value Pr > F
Model	4	18.1640342	4.5410085	0.45 0.7730
Error	11	111.8734658	10.1703151	
Corrected Total	15	130.0375000		

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	17.32598307	5.77532769	0.57	0.6476
INITWT	1	1.51653420	1.51653420	0.15	0.7067

Dependent Variable: MCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.13006057	0.53251514	0.80	0.5475
Error	11	7.28431443	0.66221040		
Corrected Total	15	9.41437500			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	2.12310817	0.70770272	1.07	0.4019
INITWT	1	0.07818557	0.07818557	0.12	0.7376

Dependent Variable: MCHC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.93077784	0.48269446	0.35	0.8420
Error	11	15.38359716	1.39850883		
Corrected Total	15	17.31437500			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	1.62359713	0.54119904	0.39	0.7646

INITWT	1	0.09890284	0.09890284	0.07 0.7952

Dependent Variable: perMXD

Source	DF	Sum of Squares	Mean Square	F Value Pr > F
Model	4	102.8421710	25.7105427	1.15 0.3828
Error	11	245.4078290	22.3098026	
Corrected Total	15	348.2500000		

Source	DF	Type III SS	Mean Square	F Value Pr > F
TRT	3	73.45969611	24.48656537	1.10 0.3910
INITWT	1	17.59717098	17.59717098	0.79 0.3935

Dependent Variable: NEUT

Source	DF	Sum of Squares	Mean Square	F Value Pr > F
Model	4	6.63267525	1.65816881	0.22 0.9209
Error	11	82.36669975	7.48788180	
Corrected Total	15	88.99937500		

Source	DF	Type III SS	Mean Square	F Value Pr > F
TRT	3	2.28478501	0.76159500	0.10 0.9573

INITWT	1 3.69580025	3.69580025	0.49 0.4969	

Dependent Variable: MXD

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value Pr > F**
Model	4	0.87622227	0.21905557	0.51 0.7267
Error	11	4.68127773	0.42557070	
Corrected Total	15	5.55750000		

Source	**DF Type III SS**	**Mean Square**	**F Value Pr > F**
TRT	3 0.23071810	0.07690603	0.18 0.9073
INITWT	1 0.52872227	0.52872227	1.24 0.2888

Dependent Variable: NEUT

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value Pr > F**
Model	4	6.63267525	1.65816881	0.22 0.9209
Error	11	82.36669975	7.48788180	
Corrected Total	15	88.99937500		

Source	**DF Type III SS**	**Mean Square**	**F Value Pr > F**
TRT	3 2.28478501	0.76159500	0.10 0.9573
INITWT	1 3.69580025	3.69580025	0.49 0.4969

Dependent Variable: BLDGLU

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value Pr > F**
Model	4	632.802886	158.200721	0.21 0.9274

Error	11	8281.621489	752.874681
Corrected Total	15	8914.424375	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	438.2016982	146.0672327	0.19	0.8983
INITWT	1	107.5310108	107.5310108	0.14	0.7127

Dependent Variable: TOTPROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	53.7380092	13.4345023	0.71	0.5995
Error	11	206.9994908	18.8181355		
Corrected Total	15	260.7375000			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	40.24795741	13.41598580	0.71	0.5644
INITWT	1	6.87050917	6.87050917	0.37	0.5579

Dependent Variable: WBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	104.8280244	26.2070061	0.67	0.6275
Error	10	391.1693089	39.1169309		
Corrected Total	14	495.9973333			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	99.34128175	33.11376058	0.85	0.4993
INITWT	1	4.19235774	4.19235774	0.11	0.7501

Dependent Variable: LYM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	76.6167216	19.1541804	1.10	0.4121
Error	9	156.3268498	17.3696500		
Corrected Total	13	232.9435714			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	58.79086775	19.59695592	1.13	0.3883
INITWT	1	11.96065021	11.96065021	0.69	0.4281

Dependent Variable: LYM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	76.6167216	19.1541804	1.10	0.4121
Error	9	156.3268498	17.3696500		
Corrected Total	13	232.9435714			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	58.79086775	19.59695592	1.13	0.3883
INITWT	1	11.96065021	11.96065021	0.69	0.4281

Dependent Variable: INITWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	61.687500	20.562500	0.16	0.9181
Error	12	1498.750000	124.895833		
Corrected Total	15	1560.437500			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	61.68750000	20.56250000	0.16	0.9181

Appendix 2: Data Input

```
TRT $              REP INITWT FNWT ADGg WBC RBC  HGB  HCT MCV  MCH
     MCHC          LYM perMXD NEUT LYM MXD NEUT BLDGLU
     TOTPROT FREDIAH;
T1 1 50 84.6 308.93 10.5 11.23                    15.3 49 43.6 13.6
         31.2 66 5.7 28.3 6.9 0.6 3 63.8 62.3 83
T1 2 38 73.2 314.29 11.4 8.68 11.3 35.1 40.4 13 32.2
        60.5 6.7 32.8 6.9 0.8 3.7 86.9 60 75
T1 3 40 82.2 376.79 20.5 10.4 13 43.5 41.8 12.5 29.9
         68.1 4.6 27.3 14 0.9 5.6 83.4 60 89
T1 4 60 100.4                 360.71 15.1 9.15 12.5 41.3 45.1 13.7
       30.3 64.7 6.1 29.2 9.8 0.9 4.4 28.6 61.8 77
T2 1 40 83.5 388.39 4.9 6.65 8.3 25.9 38.9 12.5 32
        86.8 3.3 9.9 4.3 0.2 0.4 67.5 60.4 76
T2 2 38 79.4 369.64 18.7 9.45 12.6 40.2 42.5 13.3 31.3
         58.6 7.2 34.2 11 1.3 6.4 61 61.6 72
T2 3 40 84.8 400.00 . 9.76 14.6 49.4 50.6 15 29.6
         91.8 1.7 6.5 . 2.6 9.9 95.1 62.4 76
T2 4 60 96.5 325.89 7.5 6.21 9.1 27.9 44.9 14.7 32.6
         80.3 6.1 13.6 6 0.5 1 71.9 59.1 71
T3 1 38 81.4 387.50 11.8 8.46 11.6 36.4 43 13.7 31.9
       71.7 6.9 21.4 8.5 0.8 2.5 68.7 58.4 72
T3 2 30 78.1 429.46 8.9 5.24 7.9 25 47.7 15.1 31.6
         . 23.9 76.1 . 2.1 6.8 74.4 45.7 70
T3 3 60 100.2              358.93 15.1 10.21        14.1 45.8 44.9
        13.8 30.8 52.2 6.2 41.6 7.9 0.9 6.3 80 61.5 71
T3 4 38 81.6 389.29 16.7 10.64                14.2 43.5 40.9 13.3
         32.6 69 5 26 11.5 0.8 4.4 89.2 62.3 75
T4 1 40 83.4 387.50 7.4 8.15 11.2 33.2 40.7 13.7 33.7
       72.8 5.3 21.9 5.4 0.4 1.6 45.2 62.7 73
T4 2 50 92 375.00 21.7 9.36 12.3 38.9 41.6 13.1 31.6
       71 5.6 23.4 15.4 1.2 5.1 48.9 61.1 72
T4 3 30 79.3 440.18 25.8 9.91 13.2 42.8 43.2 13.3 30.8
       74.6 5.1 20.3 19.2 1.3 5.3 94.9 63 70
T4 4 55 99.5 397.32 16.3 9.83 12.6 40.9 41.6 12.8 30.8
       74.5 5.8 19.7 12.1 0.9 3.3 134.4       56.7 74
```

Appendix 3: Recording the weight of calves

Appendix 4: Taking blood samples from calves