# Immunoglobulin response and growth performance of new born Holstein calves fed Garlic (*Allium sativum*) powder and Probiotics as feed additives.

BY

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## DECLARATION

I Kekana Thapelo Wilton (Student no: 11615279), hereby declare that this dissertation was submitted in partial fulfilment of the requirements for the degree of M. Sc. in Agriculture (Animal Science) submitted to the Department of Animal Science, School of Agriculture at the University of Venda has not been submitted previously for any degree at this or other university. It is original in design and execution, and all reference material contained therein has been duly acknowledged.

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# DEDICATION

This work is dedicated to my beloved family: my parents Mr. Maesela, J. and Mrs Mashaledi, F. Kekana, my brothers: Thabo, J. and Tiišetšo, K. Kekana, sisters: Sadie, P., Phomolo, S., Kgothatso, S. and Malefa, P. Kekana, and to my beloved friend Molatelo, R., Mokoelele.

# ABSTRACT

Garlic, *Allium sativum,* contains secondary metabolites which promote proper immunity by stimulating the level of immunoglobulin (Ig) and the efficiency of rumen function. The objectives of the study were to investigate the effects of feeding Garlic powder or Probiotics or a combination of both on level of IgG and growth performance of Holstein calves. The calves were randomly allocated, according to birth weight, to four dietary treatments, each with 8 calves. The treatments were: control, no additive (C); supplemented with either 5g/d Garlic powder (G) or 4 g/d Probiotics (P) alone or a combination of 5g/d Garlic powder and 4 g/d Probiotics (GP) with the total viable count of 1.3 x 107 cfu/g. Garlic and Probiotics were diluted in the daily milk allocation from day 4. Commercial starter feed (17.5% CP) and fresh water was available *ad libitum* from day 4 until day 42 of age. Calves fed G and GP had higher ($P<0.05$) IgG than C and P calves. Calves fed GP tended ($P = 0.056$) to have higher final BW compared to C (60.3 kg vs. 56.0 kg). Garlic, Probiotics or their combination did not affect ($P>0.05$) calves' serum glucose, heart girth (HG), average daily gain (ADG) and body length (BL). Calves in GP and P groups had lower ($P<0.05$) faecal score than control and garlic. The results of the current study revealed complementary effects of Garlic and Probiotics due to improved nutrients intake, body weight, and serum IgG level and reduced diarrheal incidences when fed to calves during the first 42 days of life.

**Key words**: Garlic powder, Probiotics, IgG, growth, diarrhoea and Holstein calves

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## LIST OF ABBREVIATIONS

ADG : Average daily gain
BL : Body length
BW : Body weight
C : Control
CP : Crude protein
DFM : Direct fed microbial
DM : Dry matter
DMI : Dry matter intake
EO : Essential oils
FCR : Feed conversion ratio
FTP : Failure transfer of passive immunity
G : Garlic
GIT : Gastro intestinal tract
GP : Garlic plus Probiotics
HG : Heart girth
IgG : Immunoglobulin G
ME : Metabolisable energy
N : Nitrogen
P : Probiotics

# CHAPTER ONE: INTRODUCTION

## 1.1 Background

Dairy producers are faced with some unique and formidable challenges for successful calf rearing. Calf hood diseases can have a major impact on economic viability of cattle operation due to cost of calf losses and treatments and long term effects on performance. Adequate resistance to infection in the immediate post-parturient period is essential for health and survival of calf (Woods and Rousell, 1993). Lawrence and Pierce, (1983) reported that inadequate ruminal development and calves being born hypogammaglobulinemic leads to increased health problems on calves and consequently delays the weaning age (Bernadette and Fallon, 1999). Though, it is well documented that colostrum is an important source of immunoglobulin (Ig) and provides immunity to disease for the new-born calf (Fallon *et al.*, 1986; Fallon *et al.*, 1989; Earley *et al.*, 1999) within 24 hours, inadequate serum concentration of Ig in new-born calves will results in increased disease susceptibility (Besser and Gay, 1994; Weaver *et al.,* 2000; Rogers and Capucille, 2000, Radostis, 2001). This is because the level of serum Ig achieved at 3 days in the calf depends on the total mass of Ig absorbed. This is a function of the Ig concentration of colostrum and the total amount of colostrum ingested during the period of maximum absorption (Bernadette and Fallon, 1999).

Absorbed serum Ig level recommended is >10 g/l and the inadequate level is < 10g/l and is declared as failed transfer of passive immunity (FTP) and is associated with high mortality rate of calves (Welss *et al.*, 1992; Quigley *et al.*, 1998). Prevalence of FTP in calves has been reported to range from 11% to 31% leading to an attack of common diseases in calves such as diarrhoea, pneumonia and respiratory problems in the world's cattle industry. Approximately 50% of mortality that occurred in pre-weaned calves throughout the world was directly related to inadequate acquisition of passive immunity (Perino, 2001).

Rauprich *et al.* (2011) postulated that maximizing the first colostrum intake does not necessarily improve the efficiency of the gastrointestinal tract in neonatal calves in first 4 to 4 days. Control of the pathogens in animal production is largely based on prevention of exposure, improving immunity, and chemotherapeutic (drugs) agents that are administered to animals (Bernadette and Fallon, 1999). Many antimicrobial substances are used to control pathogenic agents. However, their use in animal feeds is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria (Calsamiglia *et al.*, 2007). Additionally it has been estimated that the elimination of antibiotics from ruminant feeds will result in an increased production costs of about 3.5-5.0% (Carro and Ranilla, 2002, cited in

Cardozo *et al.,* 2005). Therefore the search for alternatives, including yeast, organic acids, plant extracts, Probiotics and antibodies, has therefore become necessary (Calsamiglia *et al.*, 2006). Garlic (*Allium sativum)* supplementation through feed, in particular, has many favourable experimental and clinical effects, which include the stimulation of the immune function, enhanced foreign compound detoxification, and the restoration of physical strength and resistance to various stresses (Prasad and Saharma, 1981; Amagase *et al.,* 2001).

Many investigations reported Garlic as highly inhibitory to *Escherichia coli* (Santo *et al.*, 1990; Kumar *et al.,* 1999), as antifungal and antibacterial, (Santo *et al.*, 1990; Kumar *et al.,* 1999), as enzyme inhibitory (Kumar *et al.*, 1999). Garlic is a medicinally important plant species that is helpful in the treatment and prevention of a number of diseases such as cancer, coronary heart diseases, obesity, hypercholesterolemia, hypertension and gastrointestinal (Izzo *et al.*, 2004). Although Garlic is known for its therapeutic properties (anti-parasitic, insecticidal, anti-cancer, anti-oxidant, immunomodulatory, anti-inflammatory, hypoglycaemic), its antimicrobial activity against a wide spectrum of gram-positive and negative bacteria is its most prominent activity and has been thoroughly studied (Reuter *et al.*, 1996).

Busquet *et al.* (2005, 2006) have consistently shown that Garlic oil iced the proportions of acetate and VFA, and decreased the proportions of propionate and butyrate in a continuous culture. Busquet *et al.* (2005b, c) suggested that the anti-methanogenic effect of Garlic and its active components was the result of the direct inhibition of *Archaea* microorganisms in the rumen. They added that these results suggest that Garlic may improve the efficiency of energy and N utilisation in the rumen. Gupta *et al.* (2005) and Aiad *et al.* (2005) found that improvement in the digestibility coefficients of different nutrients is probably due to improved gross activity of rumen microflora, increased immunity alternation in numbers and species of microorganisms in the rumen. On conclusion, Garlic can increase cellulolytic bacteria, increased total VFA's concentration and the animals' rations and higher dry matter (DM), total digestible nutrients (TDN) intake and higher gain rate (Gupta *et al.,* 2005; Aiad *et al.*, 2005).

However, based on the aforementioned Garlic's results, little or no information could be found of Garlic on pre-weaned cattle calves. It is hypothesized that supplementing Garlic as a feed additive to neonatal Holstein calves would improve their immunity by increasing the level of IgG and performance by stimulating feed intake, thus accelerating body weight gain. Therefore the aim of the study was to evaluate the effects of Garlic powder on the health and performance of new born Holstein calves.

## 1.3 Problem statement

Despite the improved knowledge concerning management of colostrum feeding and actual rates of success, passive transfer of immunity still need considerable improvement because new born calves continue to be attacked by diseases in their early days of life due to low immunity as a result of FTP. Meanwhile, maximising colostrum intake does not necessary improve the efficiency of the gastrointestinal tract in neonatal calves (Rauprich *et al.,* 2000). Slow rumen development becomes a limiting factor to stimulating feed intake for accelerated body weight gain in calf rearing. Antibiotics have shown good results on boosting the level of immunoglobulin and modifying the ruminal function in livestock (Abu-Tarbouch *et al.,* 1999). However, they are banned in the European Union for they appear in the final products (Calsamiglia *et al.*, 2007). In addition, Field *et al.* (1999) stated that though antibiotics can increase the level of lymphocytes and modulate rumen function, they are capable of altering the CD4 and CD8 T-cells. T-cells are the type of lymphocytes associated with the component of an immune response known as cell-mediated immunity. For these reasons, secondary metabolites from plants such as Garlic which are under investigation serve as alternatives to antibiotics to modulate rumen fermentation and stimulate immunity of animals.

## 1.3 Justification

Modern improved animal production requires the use of safe and effective additives to stimulate feed consumption, destroy harmful microorganisms in the diet, improve immunity and be used as rumen manipulators (Ahmed *et al.*, 2009). Dairy new-born calves face a greater challenge of diseases due to low immunity, with a low feed intake resulting in a retarded growth rate. Garlic has been used as both food and medicine in many cultures for thousands of years. Investigations have shown that Garlic plays an important pharmacological role as an antibacterial, antiviral, and antifungal agent (Amagase *et al.,* 2001; Grela and Klebaniuk, 2007). Garlic can also help to prevent heart disease and cancer (Agarwal, 1996; Amagase *et al.*, 2001). Based on previous study by Tatara *et al.* (2005), feeding Garlic extract to sows has been found to improve the gastrointestinal tract development and body weight and also maintained immune function. Therefore by using available Garlic powder as feed additive at early stages of a calf's life would improve their immunity, stimulate the feed intake as well as reduce diarrheal incidence. Limited (or no) information on performance and health of Holstein calves fed Garlic powder as feed additive is available. Such information will enhance the profitable dairy cattle industry, leading to the improved economic and social status of dairy farmers.

## 1.4 Objectives

The overall objective of the study was to investigate the use of Garlic (*Allium sativum*) powder or Probiotics or both as feed additives on the health and performance of neonatal Holstein calves.

The specific objectives are to evaluate the effects of Garlic powder and Probiotics feed given to calve within day 42 on the:

1. Level of IgG in the blood,
2. Level of blood glucose,
3. Growth performance (weight gain, heart girth and body length) and
4. Diarrhoea incidence of new-born Holstein calves within 42 day of age.

## 1.5 Hypothesis

Null hypothesis: Gallic powder or Probiotics or both have no effects on immunoglobulin G and glucose level in blood, growth performance and diarrhoea incidence of Holstein calves within day 42 of life.

## 2.1 General introduction

The replacement heifer enterprise typically represents the 20% of the expenses on the dairy farm. This makes it the second largest expense on the dairy farm, trailing only the feed cost for lactating cows (Drackely *et al.,* 2004). Unfortunately neonatal mortality in dairy calves remains a major problem. With the constant mortality rate in dairy calves (USDA, National monitoring system, 2003), costs are incurred from day one, with no economic returns until the heifer enter the milking herd. Therefore goals of the replacement heifers should be to minimise expenses while ensuring healthy, vigorous heifers that grow rapidly and enter the dairy herd at 22 to 24 months at proper body size. To get the heifer off to a fast start during the pre-weaned period many additives are supplemented in to the diets, administered orally or injected to provide the foundation for healthy, well-grown and economical heifers. Since interactions between nutritional status, immunology and disease resistance are extremely complex, a number of feed additives have been shown to affect aspects such as immunity and nutrition in cattle. As such various additives are investigated nutritionally and therapeutically to enhance the livestock production.

## 2.2 Health in calves

### 2.2.1 Bovine Immunity

Immunity is defined as all the structures and cells involved in providing immune protection. Lymphocytes (white blood cells) are the primary cell type involved in an immune response, and the wide distribution of lymphocytes throughout the animal body provides them ready access to invading microorganisms and newly introduced antigens (Rowen *et al.*, 2003). These lymphocytes will produce antibodies (immunoglobulin) (Table 2.1). The produced lymphocytes are capable of removing the harmful cell as cellular response. Lymphocytes are essential in immunity because they are leucocytes (white blood cells) that develop the recognition to antigens, cytoxic attack on infected cells, immunologic memory and also regulate the specific immune response. Immunoglobulin (Ig) are produced by lymphocytes which are proteins that can bind themselves to an antigen, this includes both antibodies (circulating immunoglobulin) and those found in the cell membranes. The bovine Ig falls into four classes based on the chemical and functional characteristics. Immunoglobulin G which accounts for 75% is the most important and predominant circulating throughout the body.

**Table 2.1**: Characteristics of four groups of immunoglobulin in bovine

| class | General characteristics |
|---|---|
| IgG | Most abundant immunoglobulin in circulation. Protective against bacteria and viruses, neutralises toxins, and activates complement. Provides for passive immunity to new-borns. |
| IgA | Primarily found in secretions associated with epithelial surfaces and mucous membranes where it provides localised protection against bacteria and viruses. |
| IgE | Contributed to allergic responses by binding to mast cells and basophils to promote release of mediators of allergic reactions. Also contributes to protection against parasites. |
| IgM | First immunoglobulin produced by plasma in response to exposure to antigens. Contributes to natural antibodies such as those associated with blood cell typing. |

Adapted from Rowen *et al.,* (2003)

Immunoglobulin functions to: (1) bind free circulating antigens to reduce their potential for harm (neutralisation) and promote their removal by phagocytes process; (2) bind antigens associated with bacterial cell walls and promoting their phagocytosis; (3) bind antigens and activate complements to promote inflammation and destruction of harmful microbes; and (4) activate an agglutinin and clump particulates so that they can be more effectively phagocytised. Immunoglobulin G molecules are synthesised and secreted by plasma B-cells of lymphocytes. Immunoglobulin G antibodies are predominantly involved in the secondary immune response (IgG is the main Ig involved in primary response). The presence of specific IgG generally corresponds to maturation of the Ig response.

The IgG isotype because of its size is the only isotype that can pass through the placenta in small amounts, providing protection to the foetus in-uteri (Korhonen *et al.,* 1995; Butler, 2005). There are 2 isotypes of IgG: IgG1 and IgG2 (Larson *et al.* 1980; Williams *et al.*, 1990). IgG1 accounts for 80% of IgG and is predominantly absorbed from the calf's intestine. The two types of IgG combined ensure passive immunity in the neonate. Milk from the second milking post-partum contains only on average 55% of the IgG levels found in milk from the first milking. It is thus stated that when referring to the capacity to build passive immunity in the new born calf, only milk from the very first milking can be referred to as true colostrum. All milk from subsequent milking should be referred to as transitional milk (Butler, 1983; Radostis *et al.*, 2001; McGuirk and Collins, 2004).

### 2.2.2 Colostrum intake and prevalence failure transfer of passive immunity (FTP)

Calves are born hypogammaglobulinemic (without any health defence mechanism) and require consumption of colostrum as a source of Ig during the neonatal period. This means calves don't receive placental transfer of Ig from the dam; therefore colostrum is an important source of IgG (Quigley *et al.*, 2002). Colostrum is a mixture of lacteal secretions and constituents of blood serum, such as Ig and other proteins that accumulate in the mammary gland during the pre-partum dry period and are collected via milking of the early-lactating cow.

Colostrum and the subsequent milk provide a complete diet which is essential to the survival of the neonate while the calf cannot consume solid food (Piccone *et al.*, 2009). In addition colostrum is rich in nutrients such as fats, peptides, fat-soluble vitamins, minerals and a variety of enzymes (Campana and Baumrucker, 1995). In addition, colostrum contains high levels of bioactive components such as growth factors, hormones, lactoferrin, lysozyme and lactoperoxidase. With the exception of lactose these compounds are plentiful in the first colostrum but they decrease rapidly to the levels found in mature milk (at approximately day 8 post-partum) (Piccione *et al.*, 2009). After a calf has ingested the colostrum, absorption of intact macromolecules across the intestinal epithelium into the neonatal circulation will be possible for approximately 24 hours after the calf is born. This absorption of proteins occurs non-selectively by the process called pinocytosis, which is a movement of proteins into the epithelium. Proteins such as Ig are transported through the cell and into the lymphatic and subsequently, to the blood.

Absorption of Ig appears to increase along the length of the small intestine, where the lower small intestine is the site of maximal IgG absorption (Fetcher *et al.*, 1983). Therefore, absorbed amount of immunoglobulin and nutrients will determine the level of serum IgG and visible impacts on body weight gain and growth (Bernadette and Fallon, 1999). It is, therefore, essential to deliver an adequate supply of colostrum which is rich in IgG within this narrow window of opportunity, approximately 24 hours post-partum to ensure best results (Moore *et al.*, 2005). In order to obtain proper passive immunity, the calves have to consume at least 153 g of colostral IgG before 2 h of life, and more if colostrum feeding was delayed. Meanwhile, good quality colostrum is colostrum containing more than 50 g of IgG/l and added that, calves would require at least 3l of good quality colostrum within the first 2 h of life to acquire proper passive transfer (Godden, 2008).

If an inadequate amount of Ig from colostrum is absorbed in the rumen, this will lead to FTP which is declared when the measured level of serum Ig is less than 10g per 1000ml of blood. Calves with FTP (serum concentration of IgG <10 g/1000 ml) are at greater risk of neonatal morbidity, with an odds ratio of 6.4. In this, no other independent variables, such as calf gender, birth date, age of the dam, and dam body condition scoring, are significantly associated with failure transfer of passive immunity (Wittum and Perino, 2001). Failure transfer of passive immunity may be caused by: (1) the lowered rate of intestinal absorption due to favourable low temperatures for high producing dairy breeds such as Holstein (Rogers *et al*., 2000; Olson *et al.,* 1980) and (2) the often insufficient availability of colostrum feeding in dairy production (Roy, 1980; White and Andrews, 1986). The prevalence of shortage of colostrum in dairy as a factor on FTP and retarded growth was emphasized by Eyli and Blum, (1998). Eyli and Blum, (1998) postulated that calves suckled *ad libitum*, had an improved body weight gain and elevated IgG status during the first week when compared to those which were given colostrum by buckets and tit-bottles. Other factors could be related to underperformance of pre-natal is that they start producing their own Ig at approximately 10 days of age and reach the normal levels by 8 weeks of age (Eyli and Blum, 1998).

During the period from birth to 8 days of age, the calf's essential dependence on maternal colostrum reinforces the need for the calf to consume colostrum as soon as possible after birth (Roy, 1980; Corbett, 1991). With the high possibilities and prevalence of FTP during colostrum feeding, the insufficient availability of colostrum in dairy farms and the slow development of body Ig in dairy calves, neonatal dairy calves are likely to be subjected to diseases attack such as pneumonia and diarrhoea and they are at greater risk for mortality than calves with serum IgG levels of 10g/L and higher (Merrick Animal Nutrition, 2004).

### 2.2.3 Failure transfer of passive immunity can lead to diarrhoea

Mortality of young calves has been a major problem in dairy farms, and the most common disease among the calves is diarrhoea (Quigley *et al*., 2002; Kim *et al*., 2011). The United States of America National Dairy Heifer Evaluation reported that pre-weaning calf mortality was 8.4%. In the data, diarrhoea accounted for 52.2% of mortality, followed by other problems (Merrick Animal Nutrition, 2011). Calf scours is a broad, descriptive term referring to diarrhoea in calves. Calf diarrhoea is not a specific disease with a specific cause but, is a clinical sign of a disease complex with many possible causes such as management and agents (Merrick

Animal Nutrition, 2011). Diarrhoea is defined as a state when normal movement of water into and out of the digestive tract is disrupted, resulting in water loss and dehydration. At this stage loss of body fluids is accompanied by loss of body salts. The fluid and electrolyte loss produces a change in body chemistry that lead to severe depression in the calf and eventual death (Merrick Animal Nutrition, 2011).

Clinical signs of diarrhoea begin with loose faeces and can progress to a semi-comatose state which includes:

(1) Production of thin and watery faces and signs of dehydration that include sunken eyes, dry mucus membranes, rough hair. (Nabuurs *et al.*, 2001),

(2) Calf extremities becoming cold to the touch with a loss of appetite,

(3) Reduced milk and feed intake (Loerch and Fluharty, 1999),

(4) Difficulty in standing up and the animal turn to suffer the impaired immune function (Blecha *et al.*, 1984; Sheridan *et al.,* 1994).

Moreover, the protective potential of the microbial gut flora tends to decrease (Cray *et al.*, 1998). During these stress events, the trend is for the productive lactobacilli to decrease and for coliforms to increase (Fuller, 1989). Common agents of neonatal calf diarrhoea include bacteria such as *Escherichia coli* and *Salmonella*, viruses such as *Rotavirus* and *Corona virus*, and protozoa such as *Cryptosporidium* (McClure, 2001; Snoep and Potters, 2004). Apart from diarrhoea agents (bacteria and viruses), there are other several factors that contribute to the occurrence of diarrhoea in dairy which includes: herd (e.g. herd size, calf housing) and calf level (e.g. calving condition and colostrum feeding management and the navel treatment). In addition, the source of the calf, the level of IgG in the serum and body weight is factors contributing to the prevalence of diarrhoea (McClure, 2001; Snoep and Potters, 2004). Hassanpour *et al.* (2007) postulated that the high mortality due to diarrhoea in calves was associated with low serum IgG levels and low body weight.

Wells *at al.* (1996) also predicted that 30% mortality of neonatal dairy calves could be prevented within 21 days of age by proper first feeding methods, timing, and adequate colostrum volume in calves. Pare *et al.* (1993) also suggested that high levels of serum IgG in calves decreased the length of diarrhoea period; more so in calves. This is because calves acquire passive immunity against the common agents after absorbing antibodies from colostrum or colostrum supplements. The quality and quantity of colostrum ingested largely influences the level of passive protection. This implies that the presence of the antibodies in colostrum directed against specific agents requires prior exposure of the dam to the agent (Gutzwiller, 2002). Therefore to prevent the opportunistic pathogenic flora from flourishing, previously practice was to treat calves with antibiotics. Antibiotics such as ionophores were used to diminish not only the activity of pathogen flora, but also that of the productive flora. Ionophores have been used successfully to improve the efficiency of dairy production and to prevent or decrease the incidence of digestive upsets and stimulation of the immune system (Chalupa *et al.,* 1980) but the use of ionophores in animal feeds are restricted in the European Union since 2006 (Official Journal of European Union, 2006) and opened the search for the alternatives.

### 2.2.4 Garlic feeding on animal's immunity

An enormous variety of secondary compounds are produced by plants to provide protection against microbial and insect attack (Levin, 1976; Cowan, 1999; Isman, 2000). Some are toxic to animals, but others may not be, and indeed many have been used to manipulate gut function as well as the immune system in both ruminants and non-ruminant. Secondary metabolites, as essential oils (EO), have recently showed the possibility of improving the health status of ruminants.

Beneficial effects of EO in farm animals may arise from the activation of feed intake and the secretion of digestive secretions, immune stimulation (Ghosh *et al*., 2010). Garlic is a member of the Liliceae family and originated from Central Asia. Garlic is one of the earliest of cultivated plants. The primary sulphur containing constituents in whole intact Garlic are the γ-glutamyl- S alk (en) yl-L-cysteines and S-alk (en) yl-L-cysteine sulfoxides, including alliin. The γ-glutamyl peptides are biosynthetic intermediates for corresponding cysteine sulfoxides (Lancaster and Shaw, 1989). During the storage of Garlic bulbs at cool temperatures, alliin accumulates naturally. On average, a Garlic bulb contains up to 0.9% γ-glutamylcysteines and up to 1.8% alliin (Amagase *et al*., 2001).

Garlic supplementation through feed, in particular, has many favourable experimental and clinical effects, which include the stimulation of the immune function, enhanced foreign compound detoxification, and the restoration of the physical strength and resistance to various stresses (Amagase *et al.,* 2001). Garlic juice is able to increase the amount of IgG, IgA, and IgM (Ahmed *et al*., 2009) when supplemented to buffalo calves. This was observed when extraction of onion and Garlic juices were made from crashed onion and Garlic bulbs with (1: 0.125/l clean water) and supplemented at 2.5 and 7.5%kg diet/day for 8 weeks. These results were related to the role of the antioxidant nutrients (natural additive) which can modulate and regulate the early activation steps in the acquired immune response. The aforementioned increase of immunoglobulin (IgG, IgM and IgA) is credited to B-lymphocyte stimulation.

Consequently, the immunoglobulin will rise, thus initiating an immune response through helper T-cell, cytotoxic T-cells and C –T- cells (Ahmed *et al.*, 2009).

### 2.2.5 Garlic feeding on diarrhoea incidences in calves

Lower faecal score and lower faecal coliform count in the treatment entails the reduction of the diarrhoea occurrence. Garlic has been reported to reduce the coliform count in young animals due to antimicrobial activity. The antimicrobial property of Garlic and ability to reduce the amount of coliform is brought by the common mechanism of anti-bacterial activity of thiosulphinates where they react with biological molecules having free SH groups to influence or inhibit the growth of bacteria (Pedraza-Chaverrí *et al.,* 2006). The breakdown products of allicin have the ability to cross cell membranes and combine with sulphur containing molecular groups in amino acids and proteins, including bacterial enzymes, thus interfering with bacterial cell metabolism.

The recent clinical effects of Garlic on animals was also reported by Ghosh *et al.* (2010) in the study where Holstein calves were evaluated for faecal score, faecal coliform count when calves were supplemented with Garlic extracts diluted with fresh water. The Garlic extracts-water mixture (250mg/kg BW) tended to decrease the coliform count with an average faecal score of 3, when compared with the control group (Ghosh *et al.*, 2010). A further investigation of the impacts of the powdered Garlic will be necessary because Chung, (2006) has reported that although allicin in Garlic is considered the major antioxidant and scavenging compound, other remainder compounds from extraction of oil and juice may play stronger roles. In addition, newer research by Lanzotti, (2006) has characterised some polar compounds of phenolic and steroidal origin, which proffer various pharmacological properties. These latter compounds, in contrast to the thiosulphinates, are without odour, and are also heat stable. Furthermore, some of the scavenger properties of Garlic are not affected by heating or cutting (Pedraza-Chaverrí *et al.*, 2006).

## 2.3. Growth of dairy calves

### 2.3.1. Rumen development

Quigley, (2001) well postulated that rumen development occurs between 30 to 60 days after birth. At birth the rumen and reticulum are not fully developed and are not functioning. During this period milk is the primary diet for the neonatal dairy calves thus making it the primary source of nutrients. The abomasum is the primary stomach compartment and both energy and proteins will be derived from milk before solid feed intake. Though, milk prior to weaning is the primary source of nutrients, research revealed a minimal development of rumen in calves receiving milk or replacers for an extended period close to 3 months (Tamate *et al.*, 1962). However, ruminal size (Table 2.2) of the milk-fed calf, regardless of rumen development, has been shown to increase proportionately with the body size. Therefore, milk feeding can be concluded as essential precursor for rapid and efficient growth, despite it hampers the preparation of the pre-ruminant calf for weaning or utilization of grain and forage base diets (Tamate *et al.*, 1962).

After weaning, the animals are no longer feeding on milk but on solid feed plus water, thus reticular-rumen becomes an important compartment of the stomach to utilize the consumed feed by the fermentation process. This will occur prior the feed reaching the omasum. In the rumen the net result of fermentation is a change in the type of energy and protein available to the calf (Quigley, 1997). High solid feed intake stimulates rumen microbial proliferation and production of microbial end products, volatile fatty acids (VFA), which have been shown to initiate rumen epithelial development.

Ruminal development requires five processes which are; (1) establishment of bacteria in the rumen, (2) liquid in the rumen, (3) out flow of arterial from the rumen (muscular action), (4) absorptive ability of the tissue and (5) available substrate (Quigley, 1997). For a calf is born with a sterile reticular-rumen, within one day of age, a high bacterial concentration can be found mostly as aerobic bacterial. High number and types of bacteria will change as dry feed intake occurs, and then substrate available for fermentation changes (Quigley, 1997). Then the change in bacterial numbers and types in the rumen is thought to be a function of intake of substrate (Lengeman and Allen, 1959). To ferment substrate, rumen bacteria must live in a wet environment. Without sufficient water, bacteria cannot grow and ruminal development will be hampered. Most of the water entering the rumen comes from free water intake (Quigley, 1997).

Multiple chemical characteristics of solid feeds appear to influence rumen epithelial growth. Concentrates and diets containing casein, starch, cellulose, and minerals have increased the rate of rumen development when compared to forage sources. When introduced into the rumen as purified sodium salts, sodium butyrate had the greatest influence on rumen epithelial development, followed by sodium propionate; sodium acetate and glucose had minimal effects.

In addition, research has identified butyrate and propionate as the volatile fatty acids most readily absorbed by rumen epithelium, especially when present at physiological concentrations. Furthermore, the chemical composition of concentrates causes a shift in the microbial population, subsequently increasing butyrate and propionate production at the expense of acetate. Increased microbial production of stronger acids, i.e. lactate, butyrate, and propionate, also decreases rumen pH. Increased absorption and utilization of butyrate and propionate over acetate provides further evidence that the former volatile fatty acids stimulate epithelial development (Church, 1976).

**Table 2.2**: Composition of the rumen growth at various ages

| Compartment of total rumen in % | Birth | 28 days | 56 days | 84 days |
|---|---|---|---|---|
| Reticulo-rumen | 35 | 52 | 60 | 64 |
| Omasum | 13 | 12 | 13 | 14 |
| Abomasum | 49 | 36 | 27 | 49 |

Adapted from Church (1976)

### 2.3.2. Calf growth and nutrient requirement

Growth in young calves before weaning mainly occurs in the skeleton and muscle system. Tissue growth basically follows the deposition of protein in bone and muscle and with corresponding mineralization of the protein matrix in bone. Some fat (primarily phospholipids) is deposited as part of normal tissue growth, with additional surplus energy deposited within adipose tissues as triacylglycerol. Rates of growth expressed as the percentage increase of body size are highest at birth and decline steadily thereafter (Kertz *et al.,* 1998). Calves nutrition thus enters on provision of adequate energy and protein, meanwhile ensuring that all the required minerals and vitamins are consumed adequately. The requirements for ME and protein are functions of body mass and rate of gain was established by NRC system (National Research Council, 2001).

The approach entails, the needs for maintenance to be met first, with nutrients in excess of those needed for maintenance being available for growth support. The system is based on energy-allowable growth, with protein requirements calculated to provide the amino acids necessary to support the amount of growth allowed by the available energy. For maximum and faster growth in calves, they need to be fed adequate amount of milk or milk replacers and they must also consume more starter feed. Energy requirements of calves less than 100 kg body mass are established in units of ME. This is determined by subtracting losses of energy by faeces; digestive energy by methonogenesis is negligible and is ignored (National Research Council, 2001). Proteins like energy are also required for maintenance and growth as a source of amino acids. The agreed NRC recommendations for protein content in calf starter dry matter from 16% in 1978 to 18%, was based on dry matter intake of about 2.6% of body weight.

Several studies have reported on appropriate protein percentages in calf starter for optimal growth of young calves. In many starter diets containing various percentages of crude protein, ranging from 13% to 18% per dry matter, animals had a similar body weights. However, in other cases, when by increasing the protein from 17 to 18% of dry matter, live body weight gain was improved (Akayezu *et al.*, 1994). This is because the proportion of the total energy intake provided by protein has an impact upon growth rates and body related to the growth. Requirements for protein in calves are directly related to the growth rate, because maintenance requirements for protein are small (Bartlett *et al.*, 2004). The ability of the calf starter feed to supply an adequate amount of amino acids for growth of pre-ruminant calves depends on the amino acids profile of the protein, quality control during the manufacturing process, and the ability of the calf to digest protein. The utilization of protein is affected by the digestibility, amino acid balance and the presence of anti-nutritional factors in the protein (Davis and Drackley, 1978).

Dietary crude protein, but not feeding rate, has a pronounced effect on composition of the whole-body gain in young calves (Bartlett *et al.*, 2004). In terms of carbohydrates, though calves can neutralise milk sugar, but they are unable to digest starch, sucrose or maltose (Jenkins, 1982) because they do not possess maltase, amylase and sucrase. Calves can utilise starch in the form of heated or hydrolysed starch and dextrose. A variable but substantial proportion of starch digestion in young calves occurs through fermentation in the lower small intestine and large intestine. The end products of this lower tract fermentation (in the form of volatile fatty acids) are usable by the calf with efficiency equal to that of glucose (Davis and Drackley, 1998).

## 2.4 Feed additives in calves

### 2.4.1 Garlic effect on rumen microbes

Many studies have been conducted on various substances but in the end, essential oils appeared to be natural alternatives to the use of growth-promoter antibiotic additives in animal feeds because they have a wide variety of effects on the animal's health. The important activity of Garlic and its compounds is that they act as antiseptics and antimicrobials by developing their action against bacteria through interaction with the cell membrane (Davidson and Naidu, 2000; Dorman and Deans, 2000). This interaction causes conformational changes in the membrane and results in the leakage of ions across the cell membrane and the loss of the trans-membrane ionic gradient (Griffin *et al.*, 1999).

In most cases, large amounts of energy are diverted to this function and bacterial growth is reduced and, in some cases, microbial death occurs (Cox e*t al.,* 2001). This mechanism of action makes these essential oils more effective against gram positive bacteria, where the cell membrane can interact directly with hydrophobic compounds of essential oils (Chao and Young, 2000; Cimanga *et al.,* 2002). In contrast to monensin and other ionophores, the small molecular weight of most essential oils allows them to cross the external hydrophilic cell wall of gram-negative bacteria, being also active against them (Calsamiglia *et al.,* 2007).

### 2.4.2 Garlic oil, juice and powder effect on rumen fermentation

Garlic oil is a mixture of a large number of different molecules that are found in the plant or as a result of changes occurring during oil extraction and processing. These include sulphur compounds (thiosulphinates, allyl sulphides, glutamylcysteines, and allicin), enzymes, free amino acids, sterols, steroids, tri-terpenoid glycosides, flavonoids, phenols, and organoselenium compounds (Lawson, 1996). Beneficial results of Garlic oil as a rumen manipulator have consistently been showed by Busquet *et al.* (2005a,b,c, 2006) in *in vitro* fermentation trials with rumen fluid when 300ml/l Garlic oil reduced the proportions of acetate (58.5 mol/100ml), and increased the proportions of propionate (20.1 mol/100ml) and butyrate (16.9 mol/100ml) when compared to control (65.3, 17.3 and 13.1 mol/100ml), respectively. This fermentation profile is different from that of monensin, which reduces the acetate-to-propionate ratio and butyrate concentration and is consistent with changes observed when methane inhibitors are supplied to rumen microbes (Chalupa *et al.,* 1980). Cardozo *et al.* (2005) added Garlic extracts, dissolved in ethanol, to an *in vitro* batch culture system held at pH 7.0 . The additive significantly lowered total VFA concentrations at 3, 30 and 300 mg/l with no effect at 0.3 mg/l. In contrast, at pH 5.5, there was no effect at 0.3 mg/l but at 3 and 30 mg/l total VFA concentration was significantly increased and at 300 mg/l total VFA concentration was significantly decreased (Cardozo *et al*., 2005). At both pH 7.0 and 5.5, the concentration of ammonia was reduced as a result of the addition of the garlic extract.

In addition, microbial fermentation was altered in favour of propionate with the addition of the Garlic extract at pH 5.5 (Cardozo *et al.,* 2005). Busquet *et al.* (2005a) examined the effect of Garlic oil, obtained through steam distillation of Garlic bulbs, at a concentration of 31 and 312 mg/l on microbial fermentation in a continuous culture system. There was no effect of Garlic oil at 31 or 312 mg/l on total VFA production or ammonia concentration. However, the molar proportions of propionate and butyrate were significantly increased at the higher concentration of the oil. The higher proportion of butyrate and propionic will enhance the acceleration of ruminal development in new born calves for high digestibility of solid feed thus accelerating the growth rate. Literature on effect of Garlic juice in rumen is consistence. In one study, Garlic juice tended to increase the growing buffalo's digestibility, daily weight gain and feed conversion ratio, with about 21.7% above the control treatment when was supplemented at 7.55%kg diet/day (Aiad *et al.,* 2008). The high digestibility is thought to be through effects on *Ruminobactor amylophilus amylolytic* and proteolytic organism sensitive to Garlic.

Aiad *et al.* (2008) speculated that Garlic played a pivotal role in initiating attachment and colonisation to protein and starch rich substrates in the rumen. Meanwhile, Hart *et al.* (2007) confirmed that, whilst Garlic does indeed affect the colonisation of starch-rich material in the rumen, it has no specific effect on *R. amylophilus* (Duval *et al*., 2005). In addition, Ferme *et al.* (2004) reported that Garlic modified the microbial population profile in a continuous culture experiment, thus reducing the contribution of *Prevotella* species in the overall microbial populations in the rumen. *Prevotella* bacteria species are mainly responsible for protein degradation and amino acid deamination. Cardozo *et al.,* (2005) also tested the effects of Garlic oil using rumen fluid with a high concentrate feedlot diet at different pH (7.0 vs. 5.5). At pH 7.0, Garlic oil resulted in lower ammonia-N and total VFA concentrations; at pH 5.5 ammonia-N concentration was also reduced, but the total VFA concentration and propionate proportion increased.

The acetate proportion and the acetate to propionate ratio decreased compared with the control group, suggesting a shift in rumen microbial fermentation (Cardozo *et al.,* 2005). The recommendable microbial shift in the rumen is the precursor of the high performance of livestock. This is in agreement with Ghosh *et al.* (2010) when the supplementation of Garlic juice on 250 mg/kg BW/d/calf had significantly affected the average body weight gain and feed intake in Holstein cross bred calves. This is because Garlic resulted in the reduction in crypt depth in the ileum (Demir *et al.*, 2003). This in turn resulted in better absorption of nutrients, thereby enhancing better growth and feed intake.

The effects of Garlic juice and its main active components on N metabolism were variable however; Busquet *et al.* (2005c) suggested that Garlic can be able to inhibit protein deamination. This will reduce the protein digestibility which indirectly will affect the muscle building leading to slow growth rate of the calves. Garlic extracts (oil and juice) have complementary effects on animals as well as in *in vitro* but there are a few studies that fully demonstrate the effects of Garlic in animals as in *in vitro,* more specifically on performance of growth rate. Only in the study by Kongmun *et al.* (2010) could be found about Garlic powder where it was contrasted with that of coconut. The observed results where the same as that of oil on volatile fatty acids. The supplementation of Garlic powder 0, 4, 8, 16 mg after been oven dried at 60 °C, increased digestibility, proportion of propionate and butyric acids but decreased the protozoal population, methane and total volatile fatty acids.

These results are in line with those found by Busquet *et al.* (2005) *in vitro*. This suggests that the active Garlic components, diallyl disulphide and allylmercaptan compounds were responsible for the most effects. Having the ability to increase the proportion of propionic and butyric acids, the supplementation of Garlic power as feed additive will be beneficial to the neonatal calves because fermentation products in the rumen such as VFA (mainly butyric acid and to a lesser extent propionic acid) are considered to be the main stimulators of rumen epithelium development (Mentschel *et al.*, 2001).

Again, it is documented that restricting amounts of liquid feed alongside with *ad libitum* solid feed intake positively affected development of rumen weight, volume and function in calves (Heinrichs, 2005). In addition, solid feed intake starting from the 3rd day of life positively affects the development of rumen microflora, rumen fermentation, as well as its epithelium growth (Kristensen *et al.,* 2007). Garlic powder could be an alternative means as feed enhancers to improve ruminal fluid fermentation end products in terms of reducing fermentation losses, improving volatile fatty acids profile, reducing protozoal population and increasing cellulolytic bacterial population. There is however need for more studies to be done to explore this aspect (Kongmun *et al.,* 2010).

### 2.4.3 Probiotics

The term "probiotic" has been defined as "live Directed fed microbial (DFM), which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989) and has been used to describe viable microbial cultures, culture extracts, enzyme preparations or various combinations of the above (Yoon and Stern, 1995). As result, interest in the effects of DFM on animal health and performance has increased. For ruminants, microbial cultures have been used to potentially replace or reduce the use of antibiotics in neonatal and stressed calves, to enhance milk production in dairy cows, and to improve feed efficiency and daily gain in beef cattle. In terms of ruminant production systems, the efficacy of bacterial DFM has been studied most extensively in the neonatal dairy calf. Bacterial DFM species such as *Lactobacillus, Enterococcus, Streptococcus and Bifidobacterium* have been studied in young calves and the data have been reviewed (Chaucheyras-Durand and Durand, 2010).

In general, the importance of bacterial DFM (primarily *Lactobacillus species*) fed to young and/or stressed calves has been to establish and maintain "normal" intestinal microorganisms rather than as a production (i.e., gain and efficiency) stimulant. For dairy calves, primary goal is the rapid adaptation to solid feed by accelerating the establishment of ruminal and intestinal microorganisms and avoiding the establishment of enteropathogens, which often results in diarrhoea. Feeding calves viable cultures of species of *Lactobacillus* and *Streptococcus* has been reported to decrease the incidence of diarrhoea (Abu-Tarboush *et al.*, 1996). In study where calves were fed *L. acidophilus* 27SC, there was significantly a lower scour index during week 5, 7, and 8 compared with calves fed the control diet. This finding clearly revealed the beneficial effect of probiotic in reducing the incidence of diarrhoea in dairy calves.

Cruywagen *et al.* (1995) found increased ADG in calves receiving *L. acidophilus* in milk replacer. Control group lost 4% of initial BW during the first 2 weeks of the study, while *L. acidophilus* group maintained their initial BW. However there was no difference between groups for 11 occurrences of diarrhoea. It was concluded that there may be a beneficial effect of adding probiotics to milk replacer in the first 2 weeks of life. Higginbotham and Bath, (1993) performed two trials on *Lactobacillus acidophilus* in a combination of milk replacer and waste milk. In the first trial, Holstein calves were fed either nonviable *L. acidophilus* in milk or an untreated milk control for nine weeks. No differences were observed in average daily gain and faecal scores. In the second trial, viable and nonviable *L. acidophilus* additives were compared for 5 weeks. And again no significant differences in average daily gain, faecal score, and in faecal bacteria counts were observed.

Over the mixed information, about DFM on calves' nutrition, little or no information about immune system response to DFM products has not been well described in calves. Sun *et al.* (2010) has reported that *Bacillus subtilis natto* has immune-stimulatory properties in calves. Likewise, in humans and other animal species, probiotics and DFM products have exhibited immune-modulatory properties including immune stimulation for pathogen control, immune regulation to control inflammation, and enhancement of cell mediated immunity (Perdigon *et al.,* 1986; Caruso *et al.,* 1993; Isolauri *et al.*, 2001). In addition, probiotics in humans have been demonstrated to be capable of maintaining homeostasis in the GIT by regulating inflammation in the gut caused by bacteria (Isolauri, 1999; Ouwehand *et al.*, 2002). Based on these findings in multiple species, a DFM product may have particular efficacy in the neonatal calf by inhibiting pathogen establishment in the GIT and controlling inflammation associated with bacterial challenges. Bacillus organisms are currently used in DFM products for many species, including calves, despite the fact that they are not considered members of the commercial micro biota of calves (Hong *et al.,* 2005).

### 2.4.4 Yeasts cultures

Yeast cultures are other types of probiotics substances which contain yeast cells and compounds that are produced during fermentation activity such as B vitamins, polyphenols, and organic acids, and all of them are responsible for the positive effects on performance and health when incorporated into the diet of animals (Magalhães *et al.,* 2008). Yeast was also served as alternatives to no antimicrobial feed additives that enhance health and performance of young calves are continuously being evaluated as methods to minimize the need for antimicrobial additives (Heinrichs *et al.*, 2003).

Some yeast strains such as *Saccharomyces cerevisiae* based products have been shown to affect DMI, rumen pH, and nutrient digestibility (Callaway and Martin, 1997; Kumar *et al.*, 1997; Dann *et al.*, 2000). *Saccharomyces cerevisiae* have been shown to favour the establishment of fibrolytic bacteria in the digestive, (Chaucheyras-Durand and Fonty, 2001). This improvement in rumen microbial activities might partially explain the improvements observed in calf growth when yeast or yeast culture was incorporated into the diet in some studies (Lesmeister *et al.*, 2004; Galva˜o *et al.*, 2005). However, Quigley *et al.* (1992) observed no differences in intake, BW gain, or efficiency of gain in calves fed yeast culture for 12 week and suggested that the high incidence of health problems and low grain intake might have masked response to yeast treatments.

Another report (Seymour *et al.,* 1995) also observed a decrease in DMI in the first weeks for calves fed brewer's yeast, with no influence on the overall DM intake, growth rate, and feed efficiency. Magalhães *et al.* (2008) observed no change in DMI post inclusion of yeast in calves' diet, generally suggested yeast does not influence intake and body weight gain of calves in the first 70 days of age, thus making necessary to look for other alternatives additives.

In terms of health benefits from yeast, the presence of oligosaccharides in the cell wall of *S. cerevisiae* such as glucan and mannan (Reed and Nagodawithana, 1991) have been shown to little effect to immune system and to influence host-pathogen interactions in the digestive tract of humans and animals. Magalhães *et al.* (2008) measured the innate and hummoral immunity of calves fed yeast, and observed no change in both parameters. In laboratory animals, consumption of glucan from oats improved neutrophil function as measured by killing activity (Murphy *et al.,* 2007), which might have ramifications in the defence against pathogens.

Despite the minimum effects on immunity and nutrition, feeding yeast can reduce the risk of fever and diarrhoea in calves, suggesting protective effects of yeast culture subjected to high risk of morbidity. Differences in risk of enteric diseases are important, because diarrhoea associated with dehydration is the major cause of death of young calves in the first weeks of life (Davis and Drackley, 1998; NAHMS, 2007).

### 2.4.5 Antibiotics

In animal production, there are type antimicrobial substances or antibiotics developed to treat and prevent the bacterial disease (Constable, 2004). There are 2 primary reasons for administering the antimicrobial agents to calves which are: (1) to decrease the number of *E coli* bacteria in the small intestine and (2) to treat and prevent the potential bacteraemia. It therefore follows that when antimicrobial agents are administered to calves with diarrhea and other illness, the antimicrobial agent should be safe and effective against illness in both the small intestine and blood, which should be regarded as the 2 sites of infection (Constable, 2004). Therefore few animals appearing sick in the herd often provided diagnostic information that allowed successful prophylactic medication of the whole herd for a brief period, a practice which was both efficient and effective in maintaining heard health. Some of these antibiotics are: Amoxicillin, chlortetracycline, neomycin, oxytetracycline, streptomycin, sulfachloropyridazine, sulfamethazine, and tetracycline (Gustafson and Bowen, 1997).

There are other type of antibiotic by the name of ionophores which increase production efficiency of cattle through alteration of rumen fermentation and control of protozoa that cause coccidiosis (McGuffey *et al.*, 2001). Ionophores act by interrupting trans-membrane movement and intracellular equilibrium of ions in certain classes of bacteria and protozoa that inhabit the gastrointestinal tract. The actions of ionophores provide a competitive advantage for certain microbes at the expense of others. In general, the metabolism of the selected microorganisms favours the host animal (McGuffey *et al.*, 2001).

Benefits derived by cattle from the biological actions of ionophores were classified into three areas of effects as follows (Bergen and Bates, 1984).

- Increased efficiency of energy metabolism of rumen bacteria and (or) the animal.
- Improved nitrogen metabolism of rumen bacteria and (or) the animal.
- Retardation of digestive disorders resulting from abnormal rumen fermentation.

Each action provides nutritional and metabolic advantages to the ionophore-supplemented animal over a non-supplemented animal. The animal transforms these into increased production or improved efficiency.

Despite the positive results from the antibiotics it is been observed that a produced number of antimicrobial agents also produce deleterious effects on small intestinal function and morphology when administered to dairy calves (Constable, 2004). Some of the observed results are:

- The addition to milk replacer powder of potassium penicillin and procaine penicillin increased the incidence and duration of diarrhoea and decreased growth rate compared with untreated controls in a total of 36 milk-fed calves (Knodt and Ross, 1953).
- Administration of neomycin sulphate for the 1st 4 days of life) tended to increase the proportion of calves developing diarrhoea compared with the proportion in an untreated control group (Shull and Frederick, 1978).
- Administration of neomycin sulphate, chloramphenicol, ampicillin trihedral, or tetracycline hydrochloride for 5 days increased the occurrence of diarrhoea and decreased glucose absorption through unknown mechanisms compared with untreated controls (Rollin *et al.,* 1986)
- Whereas 2 other studies found that tetracycline hydrochloride did not induce diarrhoea or alter glucose absorption (Murley *et al.*, 1952; Aslan *et al.,* 1989).
- Additionally administered antibiotics in intra-mammary infusions in dairy animals may increase the incidence of unacceptable residues in milk, and such residues are

reduced by establishing and adhering to withdrawal periods before consumption of milk or slaughter (Constable, 2004).



For the above mentioned reasons, the use of many but not all of antibiotics in animal industry is banned in the European Union with the concern of public protection (Calsamiglia *et al.*, 2007).

With the approved use of the antibiotics such as monensin in calf health there are 4 critical measures of success of these antimicrobial therapy and are, in decreasing order of importance, (1) mortality rate, (2) growth rate in survivors, (3) severity of diarrhoea in survivors, and (4) duration of diarrhoea in survivors. After all, the success of antimicrobial therapy can vary with the route of administration and whether the antimicrobial is dissolved in milk, oral electrolyte solutions, or water (Thompson and Black, 1978; Palme *et al.*, 1983).

## 3.1 Study site

The experiment was conducted between March 2012 and February 2013 at calf Unit at Dairy Herd Section of the Agricultural Research Council (ARC)-Irene Institute (S: 28013'0" and E: 25055'0", altitude 1523 m) with the average annual temperature of (14 °C minimum and the maximum 24 °C) and the rainfall of (0mm in winter and 110 mm in summer), South Africa.

## 3.2 Animals and treatments

The use of animals conformed to the guidelines on the welfare and use of the animal in research was approved by Animal Ethics Committee of the Animal Production Institute at ARC.

Thirty two new born Holstein male and female calves averaging 36.2 ± 4.5kg birth weight from the ARC Animal Production Institute dairy herd were used. The calves were randomly allocated according to birth weight to four different dietary treatments, each having 8 replications. The treatments were Control with no additives (C); supplemented with 5g/d (Garlic powder (G); supplemented with 4 g/d Probiotic (P) and: supplemented with 5 g/d Garlic powder and 4 g/d Probiotic (GP). The probiotic compound contained *Lactobacillus bavaricus, L. casei, L. rhamnosus, L. coryniformis, L. curvatus, L. sake, Streptococcus species* and *Leuconostoc species* with the total viable count of 1.3 x $10^7$ cfu/g. The probiotic compound from ANB Store, Pretoria, South Africa and a local commercial Garlic powder from Delhi spice, Midland, South Africa were supplemented in the daily milk intake from day 4.

## 3.3 Feeding management

The calves were fed 4 l of colostrum per day (2 l in the morning and 2 l in the afternoon) for the first three days. From day 4, calves were allocated to treatments, and fed 2 l of milk (Table 4.1) in the morning and 2 l in the afternoon. The milk fed was increased to 3 l in the morning and 3 l in the afternoon from day 8 to 35. From day 36 to 42, calves were fed 4 l milk in the morning only. Starter (Table 4.1) feed and water was fed *ad libitum* from day 4 to day 42 daily. Milk samples from the main milk tank were analysed for fat, CP and milk lactose (Table 4.1).

## 3.4 Housing and management

The calves were moved to 6m x 2.5m individual pens immediately after birth. One third of each pen was roofed and the floor consisted of concrete, the remainder being soil surface. Every pen had a platted rubber matt and *Eragrostis curvular* grass hay on the concrete floor as bedding for the young calves. After birth calves' navel were disinfected with an iodine solution to prevent navel ill and other infections. No dehorning was done during the trial period to minimise stress. All sick animals were treated according to the diagnosis by the ARC's Veterinarian. The only illness found during the trial was diarrhoea. No antibiotics were administered and calves were fed electrolytes for diarrhoea treatment. Electrolytes were given twice a day (10H00 and 13H00) to calves with diarrhoea with a reduced milk feeding up to 50%. Calves were weaned on day 42 at which they were consuming approximately 1 kg or more of calf starter pellets per day.

## 3.5 Sample collection and analysis

### 3.5.1 Starter feed and milk

Starter samples collected from every bag opened for trial's animals were pooled to one sample for the analysis of crude protein (CP), fat, metabolisable energy (ME), calcium (Ca), phosphorus (P), non-structural carbohydrates, crude fibre and determination of moisture and DM. The DM contents of the starter was determined by oven drying at 60° C for 48 h. Dried starter was ground and analysed for crude protein according to AOAC (2000) procedure 968.06 and ether extract according to AOAC (2000) procedure 920.39. Calcium was determined according to Giron, (1973) using a Perkin Elmer Atomic Spectrophotometer. Phosphorus was assayed according to AOAC (2000) procedure 965.17. Crude fibre was assayed according to AOAC (2000) procedure 962.09.

Milk samples from the main milk tank were analysed for fat, crude protein and milk lactose. Metabolisable energy concentration of whole milk was calculated according to NRC (2001).

### 3.5.2 Serum Immunoglobulin (IgG)

Blood samples were collected on day 2, 12, 22, 32, 42 post-feeding from the jugular vein for determination of immunoglobulin G. Blood samples were collected in lithium / heparin vacutainer tubes, kept on ice and thereafter centrifuged in a refrigerated centrifuging machine at 2500 rpm for 20 min. After separation of serum and plasma, the obtained serum was stored at -20°C until analysis. The determination of levels of immunoglobulin G was done by bovine radial immune diffusion (RID) kit (The binding site Ltd. Birmingham, UK). The principle of the technique was derived from the work of Mancini *et al,* (1965).

### 3.5.3 Blood glucose

Blood glucose was measured using the needle glucose meter (Contour® TS glucose meter, Bayer, 400, Bessel, Switzerland). A drop of blood from the jugular vein was directed to the tip of the meter strip (Contour® TS) which was attached to the Glucose meter and the reading from the devise was recorded. The procedure was repeated twice for accuracy.

## 3.6 Measurements

### 3.6.1 Body weight

Calves were weighed at birth to determine birth weight or initial body weight (BW) and thereafter every week until 42 days when the trial ended. Animal were weighed before AM milk and starter feeding. Average daily gain was calculated from weekly weight gain.

### 3.6.2 Body structure

The following body stature measurements were made weekly when weighing the calves.

1. Body length (BL) - measured straight from the shoulder to the hip joint.
2. Heart girth (HG) - measured snug but not too tight just behind the fore legs and shoulder blade.

All the measurements were taken when the calves were standing comfortably on a clean, hard, level surface with their head upright and looking forward.

### 3.6.3. Water and starter feed intake

Daily individual calf starter and water intakes were calculated throughout the experiment from the difference of daily allocations and orts. Feed conversion ratio (FCR) was determined from feed intake measurement as kg DM consumed over kg weight gained (i.e. FCR = kg DMI/ kg weight gained).

### 3.6.4 Body temperature

Body temperature was measured daily between 8H00 and 9H00 to avoid sun heat using animal rectal thermometer. The procedure was repeated twice for accuracy.

### 3.6.5 Faecal scoring

Faecal score was subjectively measured every morning before feeding in order to assist in the evaluation of the health status of the calf as well as the treatment of diarrhoea. A faecal scoring from 1 to 4 point scale was recorded daily as described by Larson *et al.* (1977). Score was established as:

1. Being normal - firm but not hard
2. Being soft - does not hold firm
3. Being runny - spreads easily (beginning of diarrhoea)
4. Being devoid of solid matter - (severe diarrhoea)

## 3.7. Statistical analyses

All parameters measured were analysed by ANOVA using the MIXED model procedure of SAS (2009), and least squares means are reported. The statistical model included treatment (supplementation or not with Garlic, Probiotics and their combination) as the main effect. All parameters' observations were reduced to weekly except for IgG, body weight, HG and BL. Significance was declared at $P \leq 0.05$ and tendency to differ was consider if $0.005 \geq P \leq 0.10$. The linear model used is described in the following equation:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where $Y_{ij}$ = variable studied during the period,

$\mu$ = overall mean of the population,

$T_i$ = effect of the $i^{th}$ treatment and

$e_{ij}$ = error associated with each $Y_{ij}$

# CHAPTER 4: RESULTS

## 4.1 Experimental diet

The chemical composition of whole milk and starter pellet fed to calves during the experiment are shown in Table 4.1

**Table 4.1.** Milk composition ($g.l^{-1}$) and Starter feed composition ($g.kg^{-1}DM$) fed to the calves during the trial period.

| Nutrient | Milk ($g.l^{-1}$) | Calf starter feed ($g. kg^{-1}$ DM) |
|---|---|---|
| Dry matter | 127 | 912 |
| Moisture | 906 | 88 |
| Fat | 5.3 | 42.1 |
| Crude protein | 4.3 | 175 |
| Milk lactose | 6.1 | - |
| Crude Fibre | - | 122 |
| Non-structural carbohydrates | ND | 221 |
| Phosphorus | ND | 5.2 |
| Calcium | ND | 9.6 |
| Energy (MJ/Kg) | 0.23 | 15.8 |

ND = Not determined

## 4.2 Dry matter, water, milk Intake and feed conversion ratio

Results on milk, starter dry matter (DM), fat, crude protein (CP), metabolisable energy (ME), milk and water intake and feed conversion ratio are summarised in Table 4.2. The starter DMI of calves ranged from 0.22 to 0.27 kg $day^{-1}$ across the treatments. Calves fed GP did not differ ($P > 0.05$) with G and P, but tended to be higher than calves fed the C ($P = 0.055$) treatment. Calves in all treatments consumed equal amount of water and milk ($P > 0.05$). In feed conversion ratio With FCR, no deference was observed between groups. No differences ($P>0.05$) were observed between treatments on fat and CP intakes. The ME intake of calves on GP was higher ME intake ($P = 0.058$) than the C group though not significant.

Starter DMI was also evaluated against age of the calves Figure 4.1. Calves DMI increased with calf age from week 1 and the same in all groups. In the first two weeks, the DMI was low across all treatments and after, gradually increased until differences were observed only during week 6, where DMI was lower ($P \leq 0.05$) in control calves compared to the rest of treatments.

**Table 4.2**: Starter dry matter intake (DMI), milk, water intake, crude protein (CP), fat, ME, and feed conversion ratio (FCR) of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Treatments | | | | |
|---|---|---|---|---|---|
| | C | G | P | GP | SEM |
| Starter DMI (kg/day) | 0.22 | 0.23 | 0.25 | 0.27 | 0.021 |
| Milk intake (l/day) | 5.00 | 5.23 | 5.43 | 5.23 | 0.586 |
| Water intake (l/day) | 2.40 | 2.45 | 2.24 | 2.48 | 0.181 |
| CP intake (kg/day) | 1.27 | 1.28 | 1.28 | 1.28 | 0.004 |
| Fat (kg/day) | 1.54 | 1.54 | 1.54 | 1.54 | 0.001 |
| ME intake (MJ/day) | 95.0 | 95.2 | 95.6 | 95.9 | 0.328 |
| FCR | 0.52 | 0.48 | 0.32 | 0.27 | 0.113 |

C= control, G= Garlic, P = Probiotics, GP= Garlic + Probiotics, SEM = standard error of means, MJ = mega joules and l= litres

## 4.3 Growth performance

The results on initial and final BW, ADG, BL and HG are presented in Table 4.4. No differences were observed in the initial BW across the treatments. Meanwhile on the final BW, calves on GP and P treatments did not differ to one another but both tended to be higher than C ($P = 0.0577$) and G ($P = 0.0569$) respectively (Figure 4.2). Weight change was evaluated weekly and only in week 4 P group was higher than C statistically (Figure 4.2). No differences ($P > 0.05$) were observed between all treatments for ADG, BL and HG.

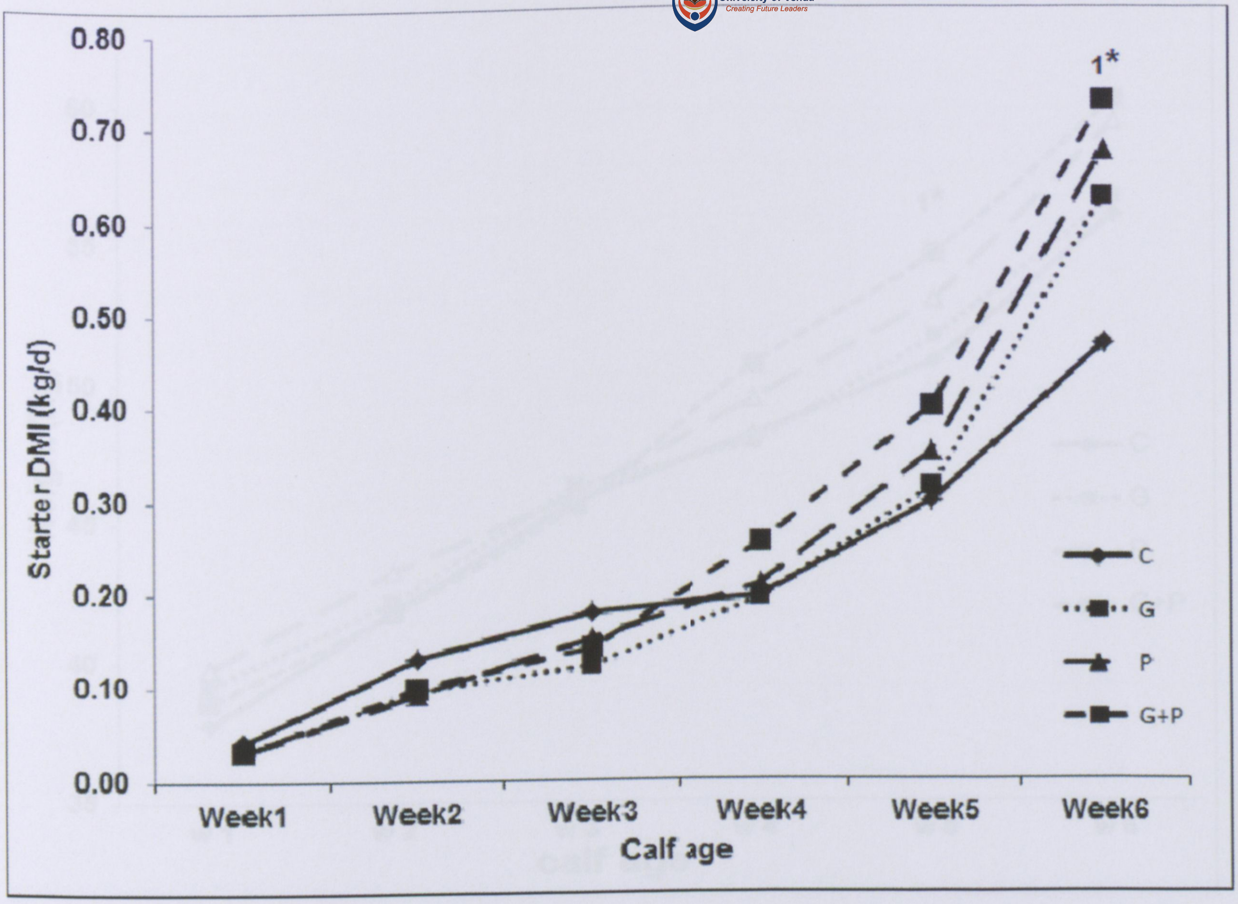


**Figure 4.1**: Change in starter DMI (kg/d) over time for control calves (C), fed Garlic (G), Probiotics (P) and their combination (GP). 1*: Control was lower than G, P and GP groups.

**Table 4.3**: Initial and final body weight (BW), average daily gains (ADG), heart girth and body length of Holstein calves as affected by Garlic and Probiotics addition.

| | Treatments | | | | |
|---|---|---|---|---|---|
| **Parameter** | **C** | **G** | **P** | **GP** | **SEM** |
| Initial BW (kg) | 35.6 | 37.1 | 37.4 | 36.8 | 1.82 |
| Final BW(kg) | 56.0 | 56.6 | 59.0 | 60.3 | 1.33 |
| ADG (kg) | 0.48 | 0.46 | 0.57 | 0.53 | 0.05 |
| Body length (cm) | 72.7 | 72.6 | 72.9 | 72.3 | 0.92 |
| Heart girth(cm) | 84.3 | 84.0 | 83.9 | 84.1 | 0.57 |

C= control, G = Garlic, P =Probiotics, GP = Garlic + Probiotics, SEM = Standard error of means.

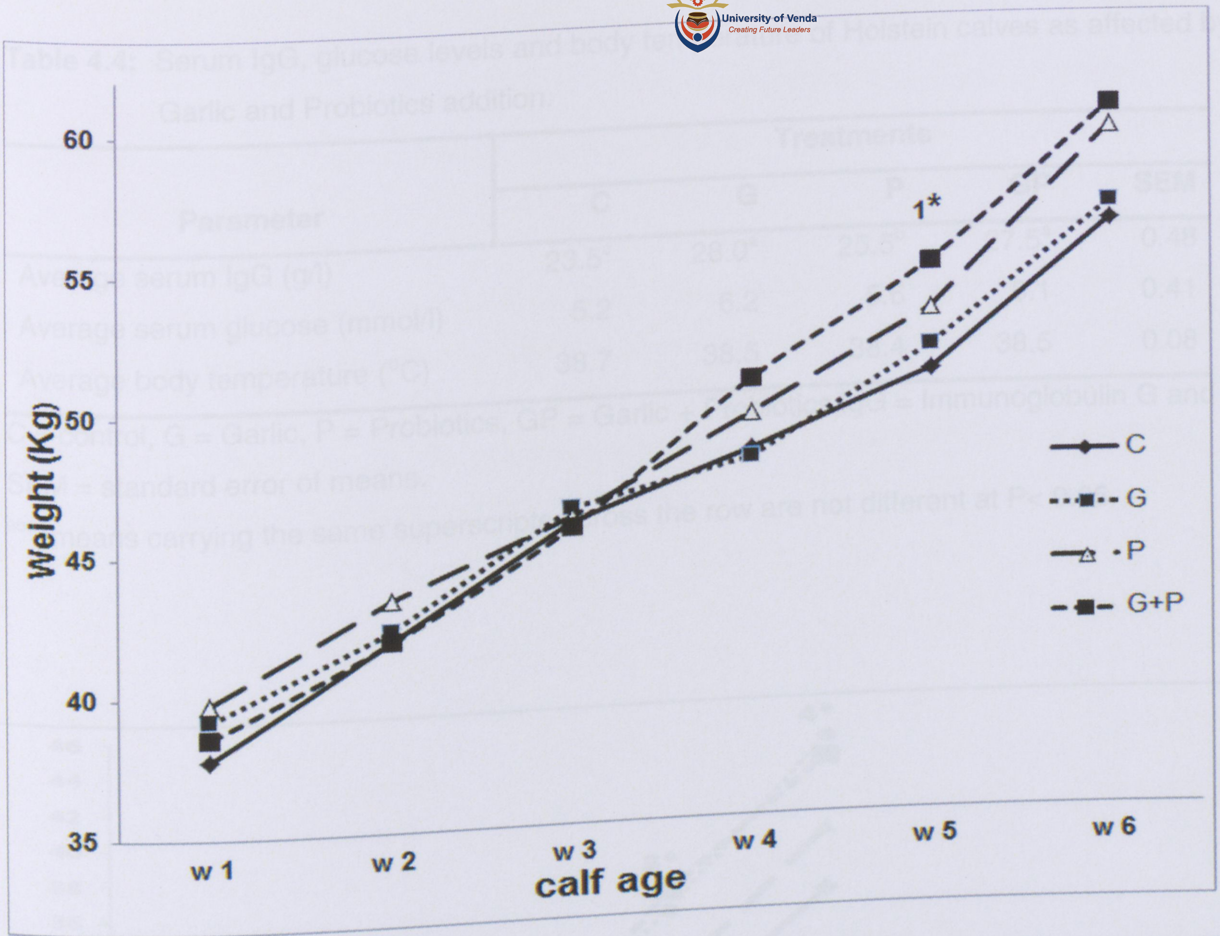


**Figure 4.2:** Change in average body weight of calves fed control (C), Garlic (G), Probiotics (P) and their combination (GP) over age in weeks (W). 1*: Difference between treatments; P and C.

## 4.4 Serum Immunoglobulin G (IgG) and Glucose, body temperature.

Results on serum IgG, and glucose levels and body temperature are presented in Table 4.4. Calves fed G and GP had similar serum IgG, and were both higher (P<0.05) compared to other treatments. No differences (P > 0.05) between treatments were observed on serum glucose content. Body temperatures across the treatments did not differ (P > 0.05). Serum IgG increased with calf age from day 12 in all the treatments as shown in Figure 4.2. No differences were observed across the treatments on day 12 (P > 0.05). From day 22 to 42, G and GP did not differ (P > 0.05) to one another but they were higher (P ≤ 0.05) than C and P. On day 42, G was higher (P = 0.026) than the rest of the treatments.

**Table 4.4:** Serum IgG, glucose levels and body temperature of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Treatments | | | | |
|---|---|---|---|---|---|
| | C | G | P | GP | SEM |
| Average serum IgG (g/l) | 23.5[c] | 28.0[a] | 25.5[b] | 27.5[a] | 0.48 |
| Average serum glucose (mmol/l) | 5.2 | 6.2 | 5.6 | 5.1 | 0.41 |
| Average body temperature ($^{o}$C) | 38.7 | 38.5 | 38.4 | 38.5 | 0.08 |

C = control, G = Garlic, P = Probiotics, GP = Garlic + Probiotics IgG = Immunoglobulin G and SEM = standard error of means.

[abc], means carrying the same superscripts across the row are not different at P< 0.05.

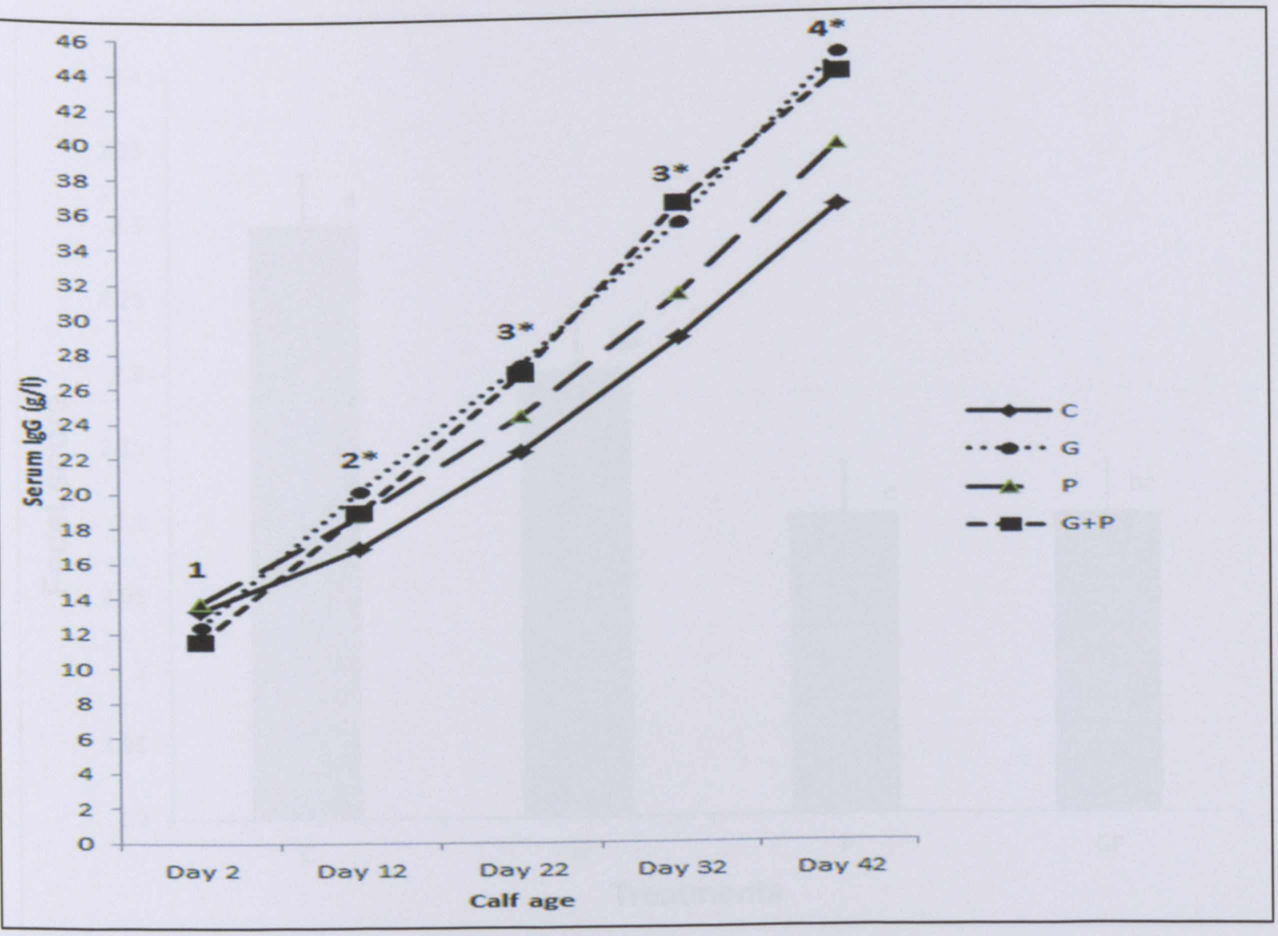


**Figure 4.2:** Average serum IgG of the calves fed control (C), Garlic (G), Probiotics (P) and their combination (GP) over 42 days. 1: No difference between treatments; 2*: Difference between C and G; 3*: Differences between C and G, and between C and G+P; 4*: Differences between C and other treatment, and between P and other treatments.

## 4.5 Incidence of Diarrhoea in the calves

Results on faecal score are shown in Figure 4.3. Calves in group C had a higher score ($P \leq 0.05$) when compared to P and GP. However, there were no differences between C and G, and between G and GP groups ($P > 0.05$). Results on average days of diarrhoea, diarrhoea frequency and number of days calves received electrolytes are presented in Table 4.5. No difference was observed on number of diarrhoea days across the groups. The diarrhoea frequency of C tended to be higher than G ($P = 0.054$) and P ($P = 0.054$) treatment but did not differ with GP ($P > 0.05$). No difference was observed, when evaluating the number of days calves received electrolytes across the treatments.

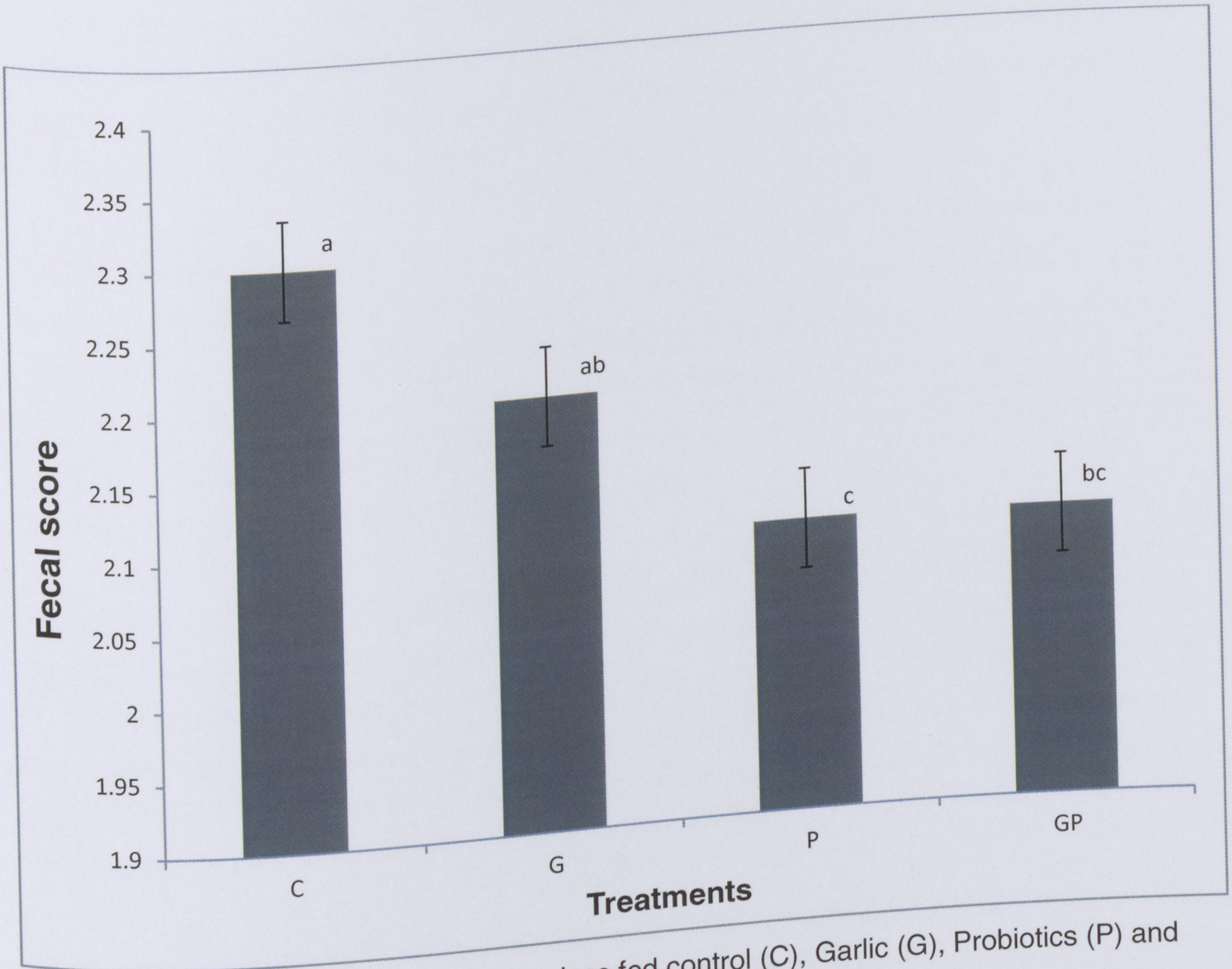


**Figure 4.3:** Average faecal score of the calves fed control (C), Garlic (G), Probiotics (P) and their combination (GP) over 42 days.

[abc], Treatments with the same superscripts do not differ significantly at $P < 0.05$.

**Table 4.5:** Diarrhoea incidence of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Treatments | | | | |
|---|---|---|---|---|---|
| | C | G | P | GP | SEM |
| Average diarrhoea days | 6.0 | 4.0 | 3.0 | 4.0 | 1.11 |
| Diarrhoea frequency | 2.0 | 1.1 | 1.1 | 1.3 | 0.30 |
| Days fed electrolytes | 4.0 | 3.0 | 2.0 | 3.0 | 0.83 |

C = control, G= Garlic, P = Probiotics, GP = Garlic + Probiotics, SEM = standard error of means.

# CHAPTER FIVE: DISCUSSION

## 5.1 Starter DM and water intake and FCR

Supplementation with G or P alone did not affect the average DMI. Previous studies have evaluated effects of extracts from Garlic and different results have been reported. Controversial reports of garlic and its extracts on DMI have been reported, Khalesizadeh *et al.* 2011 and Aiad *et al.* 2008 reported no effects, minimum effects by Benchaar *et al.* (2006) and 2007), Ahmed *et al.* (2009) while the significant results by Hassan and Abdel-Raheem, (2013). In the present study Garlic bulb powder was used and did not affect the average feed intake however at week 6 improved DMI significantly. The various results may be due to garlic and its extracts have a complex chemistry and their activity is affected by the different preparations and can yield unstable numbers and levels of thiosulphinates (Amagase *et al.,* 2001). In addition, Cardozo *et al.* (2004) postulated that variation in response with regard to the use plants secondary metabolites in livestock may be attributed to the various approaches (*in vivo vs. in vitro*) and the level of doses used.

The lack of effect of Probiotic observed on average DMI observed in the present study is in agreement with Gőrgülü *et al.* (2003) and Malik and Bandla, (2010). Krehbiel *et al.* (2003) emphasised that the general importance of DFM as Probiotics fed to young and stressed calves is to establish and maintain "normal" intestinal microorganisms rather than as a production (i.e., gain, intake and efficiency) stimulants. The increased DMI during the last week of the trial is in agreement with Laborde, (2008).

The significant 22.7% DMI over C group during week 6 by GP may be attributed to associative effect of both additives. The improved DMI with GP supplementation was observed from week 4, which suggests a time for adaptation of the ruminal microbes to the synergistic effects of the combined additives which also stimulated the intake of metabolisable energy. Additionally, the increased DMI on week 6 could be due to the rumen at that time is fully developed as postulated by Quigley at al., (2001).

Water plays a vital role in rumen digestion and microbial growth in young calves. All the treatments did not affect water intake because of the constant milk intake across the additives' treatment. In the present trial, water was *ad lib* available, and the measured amounts of water recorded were voluntary intakes. The tendency of higher water intake observed in group GP with higher DMI is consistent with previous research (Kertz *et al.,* 1984) which indicates a positive correlation between water intake and starter intake. High concentrates intake directly increase the number of the substrates in the rumen, stimulating the growth of microbes in number to be digested, which requires a wet environment (Quigley, 1997). Increased water intake with high DMI was also reported in growing buffalos by Soltan *et al.* (2009), in rat (Zafra *et al.,* 2003) and in heifers (Cardozo *et al.,* 2006).

Feed conversion ratio was not affected by treatment G supplementation. Other results concerning ineffectiveness of Garlic and its extracts on FCR were reported by Lovatto *et al.* (2005) in rat and Ahmed *at al.* (2009) in growing buffalos. Garlic supplementation showed the best feed conversion ratio than P and GP treatment. The efficacy of G increasing the FCR was also observed by Ahmed *et al.* (2009) suggesting that these results might be due to the effective to improve immunity and decrease debility incidence in calves. The Garlic's tendency to improve feed conversion ratio may be attributed to the activities of sulphur containing compounds contained by Garlic powder (Lawson, 1996). Rao *et al*, (2003) reported that Garlic powder's sulphinates were able to enhance the activity of pancreatic lipase and amylase, and this positive influence on the activity of enzymes may have a supplementary role in the overall digestive stimulant action, besides causing an enhancement of the titres of digestive enzymes in pancreatic tissue.

The results of Probiotics on FCR have been controversial. Some previous reports (Skrivanova and Machanova, 1990), Morrill *et al.* (1995) and Avila *et al.* (1995) indicated no change in calves' FCR. Meanwhile Feist *et al.* (1997) and Gilli *et al.* (1987) reported a great efficiency and performance in calves post supplementation of Probiotics to calves.

## 5.2 Growth Performance

Contradicting reports with respect to effect of Garlic and its extracts on body weight gain have been reported (Ahmed *et al.,* 2009; Aiad *et al.,* 2008). In the present study, Garlic powder did not affect the final body weight. This could be due to the unaffected DMI observed in Garlic supplemented calves and in addition this could be due to Garlic powder contain significantly lower amount of constituents such as alliin, though they are similar to those of raw Garlic (Amagase *et al.*, 2006).

The minimal effects of DFM on growth were well documented since the 70$^{th}$ century on animal performance in terms of growth (Morrill *et al.,* 1977; Ellinger *et al.*, 1978 and Abu-Tarboush, 1996). As shown in Table 4.2 the final BW for P (59.0 kg) group slightly increased the body weight which is in agreement with the results observed by Malik and Bandla, (2010) and Gőrgülü *et al.* (2003). When body weight change was evaluated weekly Probiotics increased the body weight significantly than control group on week 5 which might have contributed to the tendency in the final body weight (Figure 4.2). This was in agreement with Mosoni *et al.* (2007) who reported a two to four-fold increase in the copies of 16 genes of *Ruminococcus albus* and *R. flavefaciens* from sheep fed Probiotic yeasts. This effect on the cellulolytic population is believed to be the result of yeast scavenging ruminal oxygen which is detrimental to those populations. Yeasts as DFM are also reported to release vitamins and other growth factors (organic acids, B-vitamins and amino acids) which are essential for the growth of cellulolytic bacteria.

Combination of Garlic and Probiotics tended to increase final BW, which could be attributed to their stimulation to the increment of the gastric secretion such as enzymes production from the pancreas which is very important in young animals, where gut maturation can dictate digestive efficiency, improved secretion and increased digestion of protein and starch in the upper ileum, making more nutrients available for absorption (Rao *et al.*, 2003). Furthermore Yang *et al.* (2009) reported that the positively affected ammonia-nitrogen concentration with inhibited deamination and accumulation of N will result in increased flow of microbial N from the rumen to the duodenum. Therefore, increased bacterial protein synthesis in the rumen due to defaunation could benefit the host by supplying the additional amino acids absorption which is the muscle building block for growth. Moreover, improved efficiency of N metabolism in the rumen could reduce nitrogen losses in faeces and urine (McDonald *et al.*, 2002).

Although, combination of Garlic powder and Probiotic showed a tendency to increase final body weight compared to control group, generally the results of the present study revealed that all calves had less daily gain and feed intake than the breed standard for Holstein calves. The low feed intake and less BW gain throughout the trial could be attributed to birth weight of the calves used in this trial. The average BW (36.2 kg) of the calves in the present study was lower with 5-7kg than the breed standard of Holstein (42.76kg) reported by (Aksakal and Bayram, 2009). This could have contributed to tendency observed with G+P instead of significant improvement. It is well known that lower birth weight may result in decreased capability of adaptation to the solid feed intake and increase morbidity (Morril, 1991). DMI were also lower than the predicted or expected intake of the Holstein calves. In addition, the pre-weaned ruminant' ruminal size may also limit DMI and microbial protein synthesis this is because Quigley *at al.* (2001) revealed that rumen develop fully between day 30 and 60. It will make duodenal flow of amino acids from microbial protein synthesis to be inadequate for maximum growth (Ørskov and McDonald, 1979).

When replacement heifers are calved, they should not only have achieved a target body weight but also a target body size. It is therefore essential to monitor skeletal development. No study could be found that used Garlic powder on HG and BL, however, Hull *et al.* (2007) observed non-significance increase of the hip height from the Holstein calves fed mixture of extracts from Garlic, anise, cassia, rosemary, and thyme.

The results of the present study with regard to P supplementation in calves' diet are in consistent with those found by Laborde, (2008) on unchanged HG and BL. No study could be found evaluating the effects of Probiotics on HG and BL however, Lesmeister *et al.* (2004) reported increased hip height when 2% supplemental yeast cultures was added to a calf starter diet.

## 5.3 Serum IgG, glucose and Body temperature

Colostrum intake in neonatal calves is essential for passive immunity and influences metabolism, endocrine system and nutritional state as reviewed (Blum and Hammon 2000a and 2000b). To meet adequate passive immunity after colostrum ingestion, absorption of adequate amount of colostral immunoglobulin must occur until calf's own immunity becomes functional (Weaver *et al.,* 2000). Generally, it is well accepted that the greater the concentration of IgG in the blood circulation of calves within 24 to 48 hours after birth, the greater the protection against the array of pathogens to which the calf might be exposed. In the current study, observed serum IgG levels on day 2 across the treatments were above 10 g/l, the critical level for determining failure of passive transfer of immunity, clearly prohibiting the possibility of FTP (Welss *et al.*, 1992; Quigley *et al.*, 1998). In the present study calves were fed colostrum for the first three days using bottle system and their IgG level after 48 hours indicated no FTP, which contradicted with statement made by Roy, (1980), White and Andrews, (1986) and Rauprich *et al.* (2000) who concluded that bottle and bucket feeding methods of colostrum does not supply adequate amount of IgG due to the limited volume of colostrum fed to the calves.

The level of calves' serum IgG was evaluated over time until day 42. Similar to previous studies (Ishikawa and Kosishi 1982; Andrews *et al.,* 1992 and Ikeuchi *et al.*, 1997; Ahmed *et al.,* 2009), G increased average serum IgG. Average serum IgG in G supplementation surpassed the control with 19.4% and P with 17.1%. The dependence of IgG to Garlic addition could be attributed to therapeutic effects of Garlic's compounds (dithiins, ally sulphides and ajone) originating from thiosulphinates (Tsai *et al.,* 1985). Garlic's alliin have the antioxidant properties are able to modulate and regulate the early activation steps in the acquired immune system (Amagase *et al.*, 2001).

The aforementioned increase of IgG could also be credited to simulated B-lymphocytes. Consequently, the Ig's will elevate initiation of immune response through helper T-cell, cytotoxic T-cell and CD4 and CD8 T-cells. The T-Cells are the type of lymphocytes associated with the component of an immune response known as cell-mediated immunity (Zadik *et al.*, 1994).

Probiotics supplementation also increased serum IgG in agreement with Gőrgülü *et al.* (2003). In the current study, Probiotics stimulated 8.6 % increase of serum IgG, which was low compared to 36.6% recently reported by Al-Saiady, (2010) in Holstein calves. The latter observed increased IgG after using higher dose (1.85 x $10^7$ cfu) of Probiotics mixture compared to 1.3 x $10^7$ cfu used in the present study. Meanwhile, Riddell *at al.* (2010) used the lower dose ($10^9$ cfu $d^{-1}$ of Probiotics) and observed no effects on IgG throughout the trial period except little change on d 42 of the trial. It appears from these three studies that, the concentration of microbes dosed determine intensiveness of stimulation on immune cells and could affect level of circulating IgG. This is supported by the reported dependency of modulation of innate and adaptive immunity by Probiotics on the dose and the strain (Alberda *et al.*, 2007; Galdeano and Perdigon, 2004). Other reports indicated the ability of probiotic *Lactobacillus* species being effective in other specie's immunity, increasing IgG and IgA in human (Alberda *et al.* 2007), in chicken (Haghighi *et al.,* 2006) and in dogs (Bailon *et al.*, 2004).

No differences were observed with G, P and GP addition among the treatments on serum glucose level which was on the normal range (Quigley *et al.,* 2006; Quigley and Bernad, 1992). Aiad *et al.* (2008) and Meyer *et al.* (2009) reported no changes in blood glucose level after supplementation of Garlic juice on buffalos and mixture of essential oils in beef steers respectively. In a batch culture, Cardozo *et al.* (2005) reported an increased total VFA concentration and propionate proportion, decreased acetate proportion and acetate-to-propionate ratio with a Garlic oil supplementation, suggesting a shift in rumen microbial fermentation. Glucose production from propionate and valerate is of great importance at all times in ruminants and rate of gluconeogenesis increases after feeding (Armstrong, 1965; Leng, 1974). Increased propionate production in the rumen, leads to increased gluconeogenesis and decreased methane production (Nafikov and Beitz, 2007), sparing energy for growth.

Probiotics supplementation did not affect the level of serum glucose significantly which is in agreement with results of Al-Saiady (2010). The normal plasma glucose levels (Quigley *et al.*, 2006) across the treatments, throughout the trial period would suggest that calves were well adapted to feeding regime and could have a better ability to utilize glucose.

### 5.4 Diarrhoea incidents

Combination of other additives with bioactive compounds from plants may be the alternative to reduce the overuse of antibiotics and to yield positive effects on animal performance through the antimicrobial activity against pathogenic bacteria and stimulation of immune system (Small *et al.*, 1998). Diarrhoea is the most costly diseases in calf rearing, this is because the following pathogens: *Escherichia Coli*, *retrovirus*, *Cryptospodium* species and *Salmonella* species are resistance of to antibiotics (El-Mottaleb and Zaki, 2008). The overall faecal score of diarrhoea test of 2.1 observed in GP group was lower than the G (2.2).

In the current study, Garlic did not affect the faecal score however it tended to reduce diarrhoea frequency with about 45%. This is in agreement with Ahmed *et al, (*2009), who indicated a reduction of faecal coliform count. The lowered faecal coliform was also reported when using Garlic juice as a supplement for calves reported by Ghosh *et al.* (2010) and Soltan, (2009). The effect of Garlic could be attributed to combined antimicrobial activity of Garlic's sulphinates reported by El-Mottaleb and Zaki, (2000). The breakdown products of the sulphinates have the ability to cross cell membranes and combine with sulphur containing molecular groups in amino acids and proteins including bacterial enzymes, thus, interfering with some bacterial cell metabolism (Small *et al.,* 1947). The anti-bacterial properties of Garlic were also confirmed against *Escherichia coli* (Hughes and Lawson, 1991; Ahmed *et al.*, 2009) and *Salmonella species* (Ahmed *et al.*, 2009). In addition, laboratory studies also indicated that Garlic extract had significant inhibitory effect on coliform (Leuschner and Zamparini, 2002; Nikolic *et al.*, 2004).

A probiotic bacterium in the intestines prevents attachment of pathogenic bacteria by applying a barrier effect at the interface between the pathogen and intestinal epithelial tissue (Lema *et al.,* 2001). In the present study, the Probiotics used were *Lactobacillus* species*: (bavaricus, caseis sprhamnosus, coryniformis, curvatus* and *sake*), *Streptococcus* and *Leuconostoc* species which reduced faecal score in P and GP groups. Earlier studies reported reduced presence of pathogenic strains (Kuvda *et al.*, 1995; Lema *et al.*, 2001) and reduction of diarrhoea (Gőrgülü *et al.*, 2003; Hooper, 1989) in pre-weaned calves supplemented with *Streptococcus faecium*or mixed with *Lactobacillus species.* This combination of GP could be very beneficial to pre-weaned calves for diarrhoeal treatment because lactic acid bacteria (*lactobacillus* species as one of the probiotics) are the least sensitive microorganisms to the inhibitory effects of Garlic (Rees *et al*., 1993).

Electrolytes cost is the major components of calf rearing with regard to diarrhoea treatment. Higher amount of electrolytes feeding were used in the C group throughout the experimental period though it was not significant among the other treatment. This is due to the number of diarrheal days and diarrhoea frequency suffered by the C group. In the present study, the results showed supplementing G and P alone can be best for the reduced cost with regard to diarrhoea treatment in pre weaned calves. No such report has been traced out regarding electrolytes' cost in treatment of calves on diarrhoea supplemented with G, P, or GP.

## CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

Calf hood diseases are major impact on economic viability of cattle operation due to cost of calf losses and treatments and long term effects on performance. Low immunity as a result of failure passive transfer subject calves to various pathogens and result to fatal diseases. Adequate resistance to infection in the immediate post-parturient period is essential for health and survival of calf. As such many antibiotics drugs were/are used to control pathogenic agents. However, their use in animal production is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria. Therefore the search for alternatives, including yeast, organic acids, plant extracts (EO), Probiotics and antibodies, has therefore become necessary.

Essential oils since have been investigated in livestock industry either in health or nutritionally. Many of the benefits of the essential oils are due to improved ruminal functioning, enhanced cardiovascular functioning, antitumor and anticancer, antimicrobial as well as immunity modulation. These results in a growing calf body suffering from FTP, improve the immunity and ruminal function. Garlic, *Allium sativum* has many favourable experimental and clinical effects, which include the stimulation of the immune function, enhanced foreign compound detoxification, and the restoration of physical strength, and antimicrobial activity. Many Garlic studies on livestock nutrition are *in vitro* meanwhile less or no information is available on livestock health.

The current study investigated the effects of Probiotics, Garlic or their combination on calves' immunity, growth performance and incidence of diarrhoea. The thirty two experimental calves were fed either control diet, 5g of Garlic powder, 4g of Probiotics or combination of Garlic powder and Probiotics. Garlic, Probiotics and their combination improved DMI during the last week of the trial. Garlic and P alone reduced diarrhoeal incidence meanwhile G, P, and GP improved the serum IgG. Combination of Garlic powder and Probiotics or individually did not affect the average daily gain but tended to improve the final body weight supporting the hypothesis of shifting rumen microbial functioning to a better yielding of the end-products (VFA) for growth. If Garlic can stimulate human immunity, act against the diarrhoea causing bacteria *in vitro*, then that might happen in calves (*In vivo*). The hypothesis is supported by the results from the present study. Garlic increased the level of serum IgG from 12.4 to 44.8 g/l within 42days of Holstein calves, numerically reduced faecal score and diarrhoea days individually.

These results suggest that the antibacterial properties of both additives together with the antioxidant ability of Garlic have contributed to the better performance of calves fed GP as indicated by improved immunity, growth and reduced diarrhoea incidents in calves during pre-weaned period. Additional research studies on pre-weaned effects of Garlic powder and Probiotics mix should help improve our knowledge of the potential complementary health, nutrition and antimicrobial benefits of combined essential oils and Probiotics.

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# APPENDIX

**Appendix 1**: The effects of the fixed effects from 3 type tests for the starter DMI, milk, water, CP, fat, ME intake, and FCR of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Effect | DF | F-Value | P-Value |
|---|---|---|---|---|
| Starter DMI (kg/day) | | | | |
| | Treatment | 3 | 1.47 | 0.2253 |
| Milk intake (l/day) | | | | |
| | Treatment | 3 | 0.54 | 0.8616 |
| Water intake (l/day) | | | | |
| | Treatment | 3 | 0.40 | 0.7516 |
| CP intake (kg/day) | | | | |
| | Treatment | 3 | 1.46 | 0.2264 |
| Fat (kg/day) | | | | |
| | Treatment | 3 | 1.46 | 0.2264 |
| ME intake (MJ/day) | | | | |
| | Treatment | 3 | 1.46 | 0.2264 |
| FCR | | | | |
| | Treatment | 3 | 1.26 | 0.2903 |

DF = Degrees of freedom, DMI = Dry matter intake, CP = Crude protein, ME = Metabolisable energy, MJ = Mega joules and FCR = Feed conversion ratio.

**Appendix 2**: The effects of the fixed effects from 3 type tests for the initial and final BW, ADG, body length and heart girth of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Effect | DF | F-Value | P-Value |
|---|---|---|---|---|
| Initial BW (kg) | | | | |
| | Treatment | 3 | 0.31 | 0.8200 |
| Final BW (kg) | | | | |
| | Treatment | 3 | 1.35 | 0.2864 |
| ADG (kg) | | | | |
| | Treatment | 3 | 0.91 | 0.4373 |
| Body length (cm) | | | | |
| | Treatment | 3 | 0.08 | 0.9703 |
| Heart girth(cm) | | | | |
| | Treatment | 3 | 0.07 | 0.9764 |

DF = degree of freedom, BW = Body weight, ADG = Average daily gain, cm = centimetre

**Appendix 3**: The effects of the fixed effects from 3 type tests for average serum glucose, average serum glucose and Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Effect | DF | F-Value | P-Value |
|---|---|---|---|---|
| Average serum IgG (g/l) | | | | |
| | Treatment | 3 | 18.23 | <0.0001 |
| Average serum glucose (mmol/l) | | | | |
| | Treatment | 3 | 1.42 | 0.2390 |
| Average body temperature ($^0$C) | | | | |
| | Treatment | 3 | 2.19 | 0.0909 |

DF = Degree of freedom, IgG = immunoglobulin G.

**Appendix 4**: The effects of the fixed effects from 3 type tests for average diarrhoea days, diarrhoea frequency and days fed electrolytes of Holstein calves as affected by Garlic and Probiotics addition.

| Parameter | Effect | DF | F-Value | P-Value |
|---|---|---|---|---|
| Average diarrhoea days | | | | |
| | Treatment | 3 | 0.89 | 0.4598 |
| Diarrhoea frequency | | | | |
| | Treatment | 3 | 1.87 | 0.1582 |
| Days fed electrolytes | | | | |
| | Treatment | 3 | 0.49 | 0.6920 |
| Faecal score | | | | |
| | Treatment | 3 | 4.22 | 0.0066 |

DF= Degree of freedom.