EFFECT OF DIRECT-FED MICROBIAL ON THE IMMUNOLOGY, BLOOD PROFILE AND GROWTH PERFORMANCE OF SHEEP

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE (BIOTECHNOLOGY)

FACULTY OF BIOSCIENCES

COLLEGE OF SCIENCE

SEPTEMBER 2015

DECLARATION

I, Patience Awotwe- Mensa, hereby declare that this submission is my own work towards the M.Sc. degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree from the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Increasing public health concern on the effect of the use of antibiotics as growth promoters in the livestock production industry and it residual effect has caused the search for alternative growth promoting feed additive. An alternative feed additive that is gaining major attention is direct-fed microbial (DFM). This study was therefore aimed at assessing the effects of three different types of DFM namely RE3^{TM}, RE3^{TM} Plus (fermented products of RE3^{TM}) and *Paenibacillus polymyxa*-based DFM (PP) on the haematology, immunology, biochemical and pathogenic microbial counts as well as growth performance of sheep. A feeding trial which lasted for four months was conducted at the Ejura Sheep Breeding Station. Twenty four Ewes with their lambs of 2.5 ± 2kg average body weight were kept in individual pens and were randomly allotted four dietary treatments in a completely randomized block design with each treatment having six replicates. The dietary treatments which were T1 as the control, T2 – RE3^{TM}, T3 - RE3^{TM} Plus (fermented products of RE3^{TM}) and T4 - *Paenibacillus polymyxa*-based DFM were administered in two forms, diluted form(1.5 mL of DFM dissolved in 10 mL of water) and undiluted form (1.5 mL/ kg of feed). The diluted DFM (1.5 mL/day) was orally given to the lambs for a month during the suckling phase and the undiluted form of the DFM was mixed (1.5 mL/kg of feed) with the basal diet at feeding time during the creep and grower phase. Crude protein percentages in the diets were 16.1% and 18.6% for the pre-weaning and post-weaning phase respectively. The DFM supplementation did not significantly ($P>0.05$) affect feed intake and the growth performance among the lambs during the entire study. Haematological and biochemical parameters measured were not significantly affected by the DFM treatment. The immunological parameters measured responded to the DFM treatment offered, with lambs on treatments 3 and 4 recording the highest ($P=0.0122$) IgA levels than the control. The CD4 levels differed significantly among the treatments. The bacteria count values for treatment 1, 2 and 4 differed significantly from treatment 3 which recorded the highest ($P=0.0536$) *E. coli* levels in faeces. Though DFM treatments generally had no influence on growth performance, haematological and biochemical parameters measured, the treatments resulted in the differences in immunological values obtained, with the DFM containing *Paenibacillus polymyxa* numerically enhancing growth performances of the lambs.

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ACKNOWLEDGEMENT

I am most grateful to the Almighty God for his love, mercies and grace which saw me through this work. My heartfelt gratitude goes to my supervisors Dr. Christopher Antwi and Dr. F.K.N. Arthur for their selfless support in making this thesis a success. I also thank all my lecturers for their criticism and input that shaped this thesis.

The material and financial support from Basic Environmental Systems and Technology (BEST) Inc., Alberta, Canada is very much appreciated.

I also thank the management and staff of Ejura Sheep Breeding station for their immense support during the field trial especially Mr. Raphael Klido, Mr. John Nunoo and Mr. Robert Doudo for all the help they gave me.

Finally, I wish to thank my family especially my mother Mrs. Hannah Awotwe-Mensa, my lovely husband Mr. Isaac Amoah Kyemasi and my sweet daughter Miss Afua Adepa Nyarkoah Kyemasi for their prayers, emotional and financial support.

CHAPTER ONE

1.0 INTRODUCTION

The importance of small ruminant production in the tropics is well recognized (Williamson and Payne, 1978) because it forms an integral part of the livestock production industry and contributes greatly to agriculture's impact on the national gross domestic production (GDP). Small ruminants are reared mainly for four functions, namely, meat, milk, skin and wool, according to order of importance (Otchere, 1986). African sheep and goat make up about 0.0125 and 0.75% of the world total flock respectively (Wilson, 1982).

These reports indicated the importance of small ruminant production in the development of the livelihood of farmers but the various routine husbandry practices in the production mostly affect the output of these animals, thus making their importance less felt. Among the routine husbandry practices in ruminant livestock production, feeding is very important to the growth and productivity of the animals. The feeding cost and availability of the feed nutrients to the animals are important issues affecting ruminant production. The major focus of most ruminant nutrition research is improving feed utilization and not on feed additives such as antibiotics (Khaled and Baraka, 2011) which is used as both feed additive and as growth promoters for improving economic and effective animal production (Wierup, 2000). Antibiotics are mainly used for: therapeutic - treat sick animals; prophylactic - prevent infection in animals and as growth promoters to improve feed utilisation and productivity (Al-Saiady, 2010). Reports indicate that about 80% of antibiotics produced in United States of America (USA) are used in agriculture with a large portion for the non-therapeutic purpose of growth promotion

(Mellon *et al.,* 2001).

Antibiotic feed additive usage has been prohibited/restricted in most countries because of the concern that it may cause resistance to antibiotics for bacterial pathogens in human through consumption of animal products (Benko *et al.,* 2008). Antibiotic- resistant bacteria

have been found in animal food products (Chadwick *et al.,* 1996), in environments contaminated by animal waste and in farm workers where antibiotics are heavily used (Chee-Sanford *et al.,* 2001). A ban on antibiotics as feed additives in animal nutrition has been in place since 1986 in Sweden and since 1999 in Switzerland (Sarker *et al.,* 2010).

With the potential of a ban on antibiotic growth promoters in the United States (Patterson and Burkholder, 2003), potential alternatives to dietary antimicrobial agents that can improve and protect the health status, to guarantee animal performance and to increase nutrient availability to the animals are being explored. One alternative is DirectFed Microbial (DFM) which is defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). Direct Fed Microbial and probiotics are used interchangeably but according to FAOWHO (2001) probiotics are defined as live microorganisms which when administered in adequate amounts, confer a health benefit on the host; whiles DFM is defined as feed products that contain only live or naturally occurring microorganisms (Brashears *et al.,* 2005). Thus the U.S. Food and Drug Administration (U.S.FDA) has required feed producers to utilize the term DFM instead of probiotics (Miles and Bootwalla, 1991). Direct-Fed Microbial is composed of cultures of microorganism including bacteria and yeast. The colonization characteristics of DFM bacterial species can differ and different strains of the same species of DFM can have unique activity such as different sites of adhesion, specific immunological effects and fermentation characteristics (Isolauri *et al.,* 2004). According to Lema *et al.* (2001), addition of DFM to the ration of sheep decreases numbers of harmful microorganisms in the intestines, improves fattening performance and feed conversion rate. Direct-Fed Microbial has the potential to enhance nutrient synthesis, rumen microbes and their bio-availability which help to increase performance of farm animals (Sandine, 1979; Musa *et al.,* 2009). Chichlowski *et al.* (2007) reported that direct-

fed microbial increases metabolic efficiency via changes in intestinal physiology and metabolism. Several studies that attempted to define possible modes of action also examined the ability of DFM to favourably alter digestion in the rumen, through modulating ruminal acid production, promoting the establishment of desirable rumen microbial populations or enhancing ruminal fiber digestion (McAllister *et al.,* 2011). Bacterial DFM may also affect innate, humoral and cellular immune parameters as demonstrated by increased serum concentration of IgA, IgG and IgM and intestinal concentration of IgG and IgM in poultry (Haghighi *et al.,* 2006) and swine (Zhang *et al.,* 2008) respectively.

Paucity of information exists in ruminants, however an inflammatory response has been observed in steers fed on mixed DFM containing bacteria and yeast (Emmanuel *et al.,* 2007).

1.1 PROBLEM STATEMENT

Antibiotics are commonly used for treatment in animal production. In addition to being used to prevent infection, antibiotics are also used as growth promoters (Wierup, 2000). However, antibiotics have the potential of leaving residues in the meat or milk used as food and this is unacceptable (Al-Saiady, 2010). Antibiotic resistant (AR) bacteria in food animals threaten the efficacy of human antibiotics because if an AR bacteria or AR genes get incorporated into bacteria populations colonizing humans, the human bacteria might also develop resistance (Smith *et al.,* 2002). Aside the high cost of antibiotics, the effects from using/overusing antibiotics raised public concerns and alternative feed additives that can help improve the health status and performance of livestock were considered.

Among the available feed additives, DFM has attracted more attention because of its ability to impact some health benefits on it host through immunomodulation or production of

antimicrobial products, nutrient synthesis, improve digestibility of some dietary nutrients (Friend and Shalani, 1984), improve feed intake, weight gain and feed conversion ratio (FCR) in animals (Chiofalo *et al.,* 2004). The DFM organisms are usually non-pathogenic and they occur in nature (Danne *et al.,* 1999). Several works have been done on the effects of DFM on human, poultry and swine. Available literature, however, has little information on the effect of DFM on the growth performance and immune parameters of ruminant especially the small ruminants.

1.2 OBJECTIVES

1.2.1 MAIN OBJECTIVE

This study aimed at investigating the effect of Direct-Fed Microbial on intake, growth performance, haematology, immunology and faecal microbial load of lambs during both the pre-weaning and post weaning stages of production.

1.2.2 SPECIFIC OBJECTIVE

- To determine the effects of Direct-Fed Microbial on feed intake, efficiency of feed utilization and average daily weight gain during the pre-weaning and post weaning stages.
- To assess the effects of Direct-Fed Microbial on the faecal microbial counts.
- To evaluate the effects of Direct-Fed Microbial on the total serum protein, serum immunoglobulin A, M, CD 4, CD 3, white blood cells, packed cell volume of the blood and the level of haematocrit.

1.3 JUSTIFICATION

Antibiotics were used as growth promoters and for therapy. It promoted growth by enhancing the feed conversion efficiency and average daily weight gain of farm animals.

However, antibiotics usage as feed additive have raised concerns of bacterial resistance in human pathogens through the consumption of animal products, and this is a major public health concern worldwide (Benko *et al.,* 2008).

In view of this, DFM was used in this experiment as an alternative feed additive to antibiotics to eliminate the concerns of pathogen resistance and also to reduce the cost of feeding the lambs with high quality feed as it has the ability to improve feed conversion ratio (FCR) and promote growth.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Livestock feeding systems

Small ruminants production is well distributed worldwide and a major source of livelihood for small farmers and the landless in rural communities in Africa (Otchere, 1986). However, productivity from small ruminants is low generally due to inefficiencies in the nutritional management of the animals. The nutrition of ruminants is the most important factor affecting their performance and to improve it and ensure high productivity, factors including the availability of nutrients, type of feeding system and the level of feeding management cannot be over emphasized (Devendra, 1980).

To improve ruminants productivity through the development of feeding systems using tropical feed resources, an understanding of the nutritional characteristics of feed resources and the nutrient requirement of the animal based on the purpose and rate of productivity is required (Preston, 1986). Several feeding systems exist and in the developed countries feeding standards that interpret the chemical analyses of feed resources in terms of their nutritive values for a productive purpose have been developed. These feeding standards usually make use of conventional feed resources such as forages and concentrates (Giger-Reverdin *et al.,* 2003). Nutritive values in productivity predictions such that levels of production achieved when non- conventional feed resources are used are less than predicted (Preston, 1986). The use of these conventional feeding standards also leads to adaptation of feeding systems used in temperate countries which require unavailable feed resources or rejection of available feed resources which are inappropriate in terms of socio-economic values. Other limitations affecting feeding of ruminants in Africa are seasonal feed shortages, fragile ecologies and potential environmental degradation. To improve ruminant feeding systems in order to increase their productivity, new feeding system that

is socio-economically suitable and make use of locally available feed resources must be explored.

2.2 Conventional feeding practices

Conventionally, the feeding system used to raise ruminants to meet the high market value is feeding them with forages or pasture for most part of their lives and then placed in feedlots where they are fed with scientifically formulated rations made up of about 8085% grains or starch source, 10-15% forages/hay and the other 5% of protein-rich meal with some supplemental vitamins, minerals, ionophores, antibiotics and growth hormones (Mathews and Johnson, 2013). According to FAO (2012) and Giger-Reverdin *et al.* (2003), forages and concentrates form the major components of ruminants feed in the ruminant production industry. These feed components are used based on their nutritive values and utilization efficiencies of the animals. Ruminants have the ability to convert cellulose, a significant component of all plants into meat and the use of grain or starch source serve to provide the needed energy for the animals to obtain a good marketable gain (Mathews and Johnson, 2013). Even though the conventional feeding systems have the advantages of improving animal performance and productivity even in the dry seasons where grasses and other forage become scarce, landless communities and shortened period from birth to slaughter has its limitations.

Most of the grains used in animal feed are also used by man, making it expensive. The seasonal availability of green forages and poor quality ready-made concentrate ration are some of the limitations. The use of antibiotics and growth hormones is raising health concerns among consumers thus the need to find alternative feeding system.

2.3 Non- conventional feeding practices

Non – conventional feed resources are ingredients that are usually not common in market places and are not the traditional ingredients used for commercial feed production for animals (Devendra, 1988; Madu *et al.,* 2003). The use of feed resources like corn cobs, cocoa husks, sugar beets tops and leaves, wheat straw, peanut haulms etc. are usually considered non - conventional feed resources (Giger-Reverdin *et al.*, 2003). Usage of byproducts like oil cakes and milling by- products, bagasse and molasses are limited by their availability, high cost and alternative importance aside feeding of animals (Otchere, 1986) thus the need to use non- conventional feed resources. To reduce cost of using grains and other expensive concentrates, cultivation of pastures and other greens for ruminant production, factors limiting the use of non-conventional crop residues and agroindustrial by products which are readily available all year round can be considered. A common limiting factor to the use of these non- conventional feed resource known among farmers is digestibility but according to Preston and Leng (1984), the imbalance of nutrients at the level of both the rumen and whole animal is the major factor. In order to promote the efficient utilization of these non- conventional feedstuffs, strategies to maximize rumen function and balance end products of digestion must be developed (Preston, 2007). The conventional feeding system use feed additives such as antibiotics to increase feed and water intake and the digestive effectiveness of the animals. However concerns about antibiotic residues in animal products, anti-microbial resistance development and transfer of gene from animal to human microbiota make it unsafe for use (Castanon, 2007). Due to these concerns about antibiotic usage, alternative feed additives that can improve rumen function and feed efficiency is of interest to the livestock industry. Prebiotics, enzymes, organic acids (Acidifiers) and probiotics/direct fed microbial (DFM) are some of the alternatives to antibiotic.

The use of DFM (out of the list of feed additives) to replace antibiotics by most researchers is because of the DFM's ability to serve both therapeutic and growth promoting purposes (Martins *et al.,* 2005). DFM has the ability to prevent internal colonization of entero-pathogenic microorganisms, produce anti-microbial substances and enzymes (Liu *et al.,* 2007); stimulate intestinal immunity and also reduce stress in animals (Fuller, 1999). Several studies on the potential benefits of DFM have been done and some results reveal that efficiency of gain was slightly improved when a certain level of DFM was included in the diets (Abe *et al.,* 1995; Kyriakis *et al.,* 1999). Baker *et al.* (2013) reported a significant increase in litter weight gain in weaned piglets when sow was supplemented with DFM. Bonsu (2009) recorded higher broiler chicks and egg weights when birds feed were supplemented with DFM. However, Lantei (2008), Amoah (2010) among other researchers reported DFM supplementation had no significant effect on productivity and cost of production.

2.4 Feed Additives

Feed additives are non-nutritive products used in animal diets to improve production efficiency and performance (Jacela *et al.,* 2009). Lewis (2002) also defined feed additives as compounds that are added directly to a feed to improve flavour, odour and appearance, to preserve or extend it shelf life and enhance its natural properties. Feed ingredients of non-nutritive nature which stimulates growth or other types of performance or improve the efficiency of feed utilization and may be beneficial in a way to the health or metabolism of the animal are also termed feed additives (Kellems and Church, 2002). The ingredients used as feed additives, can be effective and help increase profitability of production if carefully chosen and properly used. Feed additives do not only promote feed efficiency and performance, but also improve intestinal health by controlling the growth of harmful gut bacterial (Jacela *et al.,* 2009). In the quest for high productivity from the limited feed

resources available to farmers and control the effects of diseases and stress especially on young animals, the use of feed additives in feeding systems has become very important. Feed additive usage has several benefits; they are able to elicit a response independent of the contributions to the animal nutrient requirements. According to Tisch (2006) feed additives have demonstrated their ability to increase growth rate, feed utilization efficiency and reduce mortality and morbidity especially in monogastrics. They have also reduced the risk of acidosis in feedlot cattle (Huffman *et al.,* 1992). Zimmerman (1986) gathered data from 239 separate experiments and recorded an average improvement response to the feed additives in starter pig's feed efficiency. Inclusion of feed additives in diet for five weeks of finishing pigs increased carcass characteristics without adversely affecting meat quality traits (Herr *et al.,* 2000). Notwithstanding all these benefits, feed additives add to the total cost of production and should be evaluated such that, the product would be able to pay for itself (Jacela *et al.*, 2009).

2.4.1 Types of Feed Additive

According to the European Food Safety Authority (EFSA, 2003), feed additives are grouped into:

i) A sensory additive: an additive that stimulates the appetite, improving the voluntary intake of a diet. E.g. Flavours and sweeteners.

ii) A nutritional additive: an additive that provides specific nutrients for an animal for optimal growth. E.g. vitamins and amino acids.

iii) A zootechnical additive: an additive that improves the nutritional value of a diet not by giving specific nutrients but by enabling the efficient use of the nutrients in the diets. E.g. enzymes.

iv) Coccidiostats and histomonostats control the health of poultry through direct effects.

v) Technological additive: an additive which influences the technological aspect of the feed. It does not directly manipulate the nutritional value of the feed but improve its handling or hygienic characteristics. For example preservatives, antioxidants, emulsifiers and acidity regulators. On the other hand, Jacela *et al.* (2009) grouped available additives into: Antimicrobials which include antibiotics, dewormers and chemotherapeutics, growthpromoting minerals, organic acids, enzymes, prebiotics, synbiotics and probiotics/direct fed microbial.

2.4.1.1 Antibiotics

Antibiotics are natural or synthetic compounds or medicine that kills or inhibits the growth of bacteria. According to US National Library of Medicine, antibiotics are powerful medicines that fight bacterial infections (www.nlm.nih.gov/medlineplus/ antibiotics.html). They are also known as antibacterial (against bacterial life). They either stop bacteria from reproducing or kill them (Nordqvist, 2013). Antibiotics (penicillin) were first discovered and developed prior to the Second World War and were used to cure diseases in humans and animals (Doyle, 2001). The major reason for the development of antibiotics was to treat infections caused by bacteria, fungal but not virus in human and animals. However, experiments in 1946, revealed that low sub-therapeutic levels of antibiotics in livestock feed or water could increase feed efficiency and growth in food animals. This led to the initiation of the addition of various antibiotics to livestock feed worldwide (Khachatourians, 1998). Many farms used antibiotics as growth promoters for improving economic and effective animal production (Wierup, 2000). Reports indicates that about 80% of antibiotics produced in USA are used in agriculture with a large portion being used for the non-therapeutic purpose of growth promotion (Mellon *et al*., 2001). The demand and usage of antibiotic increases especially during the pre-weaning and weaning/growing phase of production because according to literature available, more

response are elicited in young animals than finishing animal (Dritz *et al.,* 2002). Young calves suffered from diarrhoea as one of the commonest disease of calves, increasing the mortality of calves in the dairy farm. The use of antibiotics benefited the young calves in many ways such as decreased incidence of diarrhoea, lower calf mortality, and decreased protein requirements (Morrill *et al.,* 1977). Antibiotics also played major roles in the growth and development of livestock especially the poultry and swine industry for more than 50 years (Cromwell, 2002). Some of the mechanism by which antibiotics improve growth are; inhibition of subclinical pathogenic bacteria infections, alteration of microbial population activities which may prevent the loss of energy to microbial fermentation, inhibition of microbial growth, increasing nutrients available to the animal and increase uptake and utilization of nutrients through the intestinal wall (Anderson *et al.,* 1999; Gaskins *et al.,* 2002). Antibiotics also have the advantage of reducing excretion of nitrogen, phosphorus and manure by antibiotictreated animals and this may be due to the more efficient utilization of feed (Roth and Kirchgessner, 1993). Reduction in waste disposal from livestock operations may impact the environment and economy positively.

The use of antibiotic for growth promotion, treatment or control of animal diseases in agriculture, however generates reservoirs of antibiotic-resistant (AR) bacteria that contaminate animal food products and are also capable of transferring their resistance to pathogenic bacteria in both humans and animals through the consumption of animal products (Vondruskova *et al.,* 2010; Benko *et al.,* 2008; Van der Fels-Klerx *et al.,* 2011). Reports suggest that the overuse of antibiotics in animal husbandry may affect the antibiotic resistance of potential human pathogens) by exerting selective pressures which render antibiotics ineffective in controlling bacterial diseases (Amabile-Cuevas *et al.,* 1995). This is a major public health concern worldwide and as a result, many countries

have banned the inclusion of antibiotics in livestock feed as growth promoters (Sarker *et al.,* 2010).

In view of the ban on the use of antibiotics in livestock production, alternative additives that can produce similar results as the antibiotics without any drug residues or resistant pathogenic bacteria in animal products are being explored. The most widely researched alternatives include; organic acids (acidifiers), prebiotics (oligosaccharides), enzymes, growth-promoting minerals, synbiotics and direct-fed microbial /probiotics.

2.4.1.2 Acidifiers

Acidifiers are compounds with acidic properties which may be organic or inorganic. Organic acids contain one to seven carbon atoms widely distributed in plants and animals and are also produced during microbial fermentation (Doyle, 2001). Since these acids and their salts are easy to handle, they are used to acidify feed. Acidifiers have the ability to control bacterial growth in feed, improve nutrient digestibility, increase growth performance and control harmful bacteria in the gut (Jacela *et al.,* 2009). Organic acids and inorganic acids that have positive effect on growth performance include citric, formic, fumaric and propionic acids and phosphoric acid. Acidifiers seem to be more effective in young or newly weaned animals. Available data demonstrate that citric acid and formic acid improves feed conversion efficiency and growth performance better during growth of young animals than during the finishing phase of growth (Radcliffe *et al.,* 1998; Siljander-Rasi *et al.,* 1998). Age of animal and diets composition are some of the factors that affect the response of acidifiers; newly weaned animals on simple diets rather than complex diets containing milk products record greater responses. Some of the disadvantages of acidifiers are decreased palatability which may lead to feed refusal and the corrosive nature of acidic feed to cement and galvanized steel used in livestock housing (Partanen and Mroz, 1999).

2.4.1.3 Enzymes

Enzymes are organic catalysts which affect and speed up the rate of chemical reaction without being in the final products (McDonald *et al.,* 1992). They are biologically active proteins that break specific chemical bonds to release nutrients for further digestion and absorption (Thacker, 2013). They are usually substrates specific like a key for a particular lock. Enzymes used in the feed industry are usually produced by bacteria, fungus or yeast. Enzymes are used mainly to supplement animal's enzyme to improve the digestibility of feed ingredients (Okai and Boateng, 2007) and control the effect of anti-nutritional factors in some of the feed ingredients. Some of the enzymes used as feed additives are xylanase, β-glucanase, amylase, glucoamylase, phytase and α-glucosidase. Enzyme inclusion has been reported to improve nutrient digestion and absorption, hence improving growth rate for a range of diets (Partridge and Hazzledine, 1997). According to Harper (1997), the use of the enzyme phytase, decreased by 30% the amount of phosphate in swine manure. The efficiency of enzyme additives depends on factors such as age at weaning, components of the diet and enzyme source.

2.4.1.4 Growth-promoting minerals

Minerals such as zinc and copper and clay minerals have been used as feed additive especially in swine production. According to Cromwell (1991), copper as copper sulphate fed to very young piglets showed improvement in production similar to that obtained with antibiotics. The usage of zinc oxide decreased the usage of antibiotics in swine feed in Denmark. Zinc at a dose 3000 ppm improve performance as well as reduce the incidence and severity of diarrhoea in piglets (Holm, 1996). Copper or zinc can be used in the presence of antibiotics in feed.

Clay minerals as feed additive bind and immobilize toxic materials to reduce their biological availability and toxicity in the GIT of animals (Vondruskova *et al.,* 2010). Due

to clay's binding abilities, it is greatly used to improve performance when diets containing mycotoxins are fed farm animals (Schell *et al.,* 1993). It has also been proven that, clay can prevent diarrhea in weaned pigs (Trckova *et al.,* 2004). From available reports on the effects of minerals on livestock, they cannot be used to replace antibiotics as growth promoters.

2.4.1.5 Prebiotics (Oligosaccharides)

Prebiotics are defined as non-digestible feed ingredients which stimulate the growth/ activities of limited number of beneficial bacteria in the colon. Prebiotics are not living organisms but compounds that promote growth of gut bacteria, thereby improving the microbial profile in the gut. Prebiotics aim at stimulating the growth of a few potentially health promoting indigenous microorganisms that modulate the composition of the natural ecosystem (Choudhari *et al.,* 2008). Prebiotics usage as feed additive for livestock was discovered since 1980's and has been well documented by researchers (Patterson and Burkholder, 2003; Santos *et al.,* 2005).

Food ingredients used as prebiotics include some non-digestible carbohydrate (oligosaccharide and polysaccharides), peptides, proteins and lipids (both ester and ether). To be classified as a prebiotic, the food ingredient must be neither hydrolyzed nor absorbed in the upper part of GIT; a selective substrate for one or a limited number of beneficial commensal bacteria in the colon to be able to stimulate bacteria growth or become metabolically activated and alter the colonic micro-flora towards a healthier composition (Collins and Gibson, 1999). Prebiotics have been reported to affect livestock in ways such as stimulate the absorption of minerals to improve mineralization of bones; inhibit the colonization of pathogen and improve the utilization of feed ingredient, stimulate immunity and neutralizing toxins (Choudhari *et al.,* 2008).

2.4.1.6 Synbiotics

Synbiotics is the use of probiotics and prebiotics in combination which work together to promote healthy intestinal flora (Quigley, 2012). The potential synergy food containing both probiotics and prebiotics can offer is often referred to as synbiotics (Gibson and Roberfroid, 1995). In synbiotics, the prebiotics can serve as a source of energy for the growth and proliferation of the live microbes of the probiotics. There are a few reports on the effect of synbiotics on livestock. In animal models, the inclusion of resistant starches in the diet has been shown to increase the numbers of probiotics (Brown *et al.,* 1997) and gut microbial flora to improve the intestinal health of livestock. Though results of using synbiotics are promising, little information exist on its effect on ruminants and mechanism of action.

2.5 Direct-Fed Microbial (DFM)

2.5.1 Definition of Direct-Fed Microbial

Direct-Fed Microbial is defined, according to the Office of Regulatory Affairs of the US Food and Drug Administration (FDA) and the Association of the American Feed Control Officials (AAFCO), as feed product that contain only a source of live or naturally occurring microorganisms (Brashears *et al*., 2005). Probiotics are defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). Often DFM is used interchangeably with probiotics, however, according to FAO-WHO, probiotics is defined as live microorganisms which when administered in adequate amount, confer a health benefit on the host (FAO-WHO, 2001). Therefore U.S F.D.A has asked feed producers to use the term DFM instead of probiotics (Miles and Bootwalla, 1989) and has defined DFM as products that are purported to contain live (viable) microorganisms (bacteria and/or yeast) (Quigley, 2011).

DFM is an alternative introduced to effectively replace the use of antibiotics in animal production as concerns over antibiotic resistant pathogen in human increases.

2.5.2 Importance of DFM

Direct-fed Microbial is of high interest as an alternative to antibiotics because of it numerous benefits such as improvement of microbial ecosystem (Sandine, 1979; Musa *et al.,* 2009), nutrient synthesis and their bio-availability resulting in better growth performance in farm animals (Oyetayo and Oyetayo, 2005). The benefits of DFM are also based on the potential for a positive intestinal effect; establishment of desirable gastrointestinal microflora and prevention of pathogenic bacteria colonization of the gut. For ruminants, microbial cultures are used to reduce the use of antibiotics in newborn calves, to increase milk production in dairy cow, and to improve growth performance, feed efficiency and daily weight gain in cattle (Krehbiel *et al.,* 2003). DFM has the potential of maintaining appropriate ruminal pH (Umberger and Notter, 1989), improving fiber digestion and preventing ruminal acidosis in mature cattle (McAllister *et al.,* 2011). According to Tien *et al.* (2006), DFM has the beneficial effects of inducing an innate immune response and modulation of adaptive immunity but this usually depends on the interaction between DFM bacteria and intestinal epithelium. Among other benefits, DFM is not absorbed in the GI tract; it does not leave residues in tissues, cause no mutation of microorganisms but increase proliferation of microorganism in the digestive tract and compete with other pathogenic microorganism (Rolfe, 2000).

2.5.3 Types of DFM

Direct-fed microbial can be made up of one or several microorganisms including bacteria and yeast (Patterson and Burkholder, 2003) that are regarded generally as safe. Microbes that can be used as DFM should have different sites of adhesion, fermentation properties

and specific immunological effect (Isolauri *et al.,* 2004). The type of DFM is classified based mainly on it composition and the major types/classifications are;

1. Bacteria, mainly lactic acid bacteria (LAB) and other bacteria such as *Bacillus spp*., *Bifidobacterium* and *Paenebacillus polymyxa.*
2. Fungi or yeast.

According to Miron *et al.* (2001), rumen microbes can be explored to be used as DFM due to the large number of microbes it contains as well as crude enzyme extract which is the primary form in which *Aspergillus oryzae* and *Aspergillus niger* have been added to the diets of ruminants (McAllister *et al.,* 2011).

2.5.3.1 Lactic Acid-Producing Bacteria (LAB)

Most DFM used in ruminant production contain one or more lactic acid producing bacteria from various genera such as *Lactobacilli sp., Lactococcus sp., Streptococcus sp., Enterococcus sp.* and *Pediococcus sp.* (McAllister *et al*., 2011). LAB has been given to all classes of ruminant. They are desirable as DFM because aside being an industrial culture, they are environmentally robust and have the ability to alter or influence microbial communities. The lactic acid produced by LAB is an important antimicrobial compound that can disrupt the intracellular pH of bacterial competitors (Servin, 2004). Even though most species of LAB are facultative anaerobes, they can also produce hydrogen peroxide in the presence of oxygen, which has been shown to limit *Salmonella* activity *in vitro* (Pridmore *et al.,* 2008). Aside the numerous benefits/importance of LAB, in many commercial DFM, LAB are administered in combination with other bacteria or yeast to get a multi-factorial response to the use of these products (McAllister *et al.,* 2011).

Although *Bifidobacterium* spp. has been classified as a lactic acid producing bacteria, its use as DFM has primarily been in poultry (Flint and Garner, 2009). *Bacillus* sp. is another

bacteria specie used as DFM in poultry. Its ability to form thermotolerant and environmentally stable endospores has obvious advantages in ensuring their survival during feed pelleting and prolonged storage (McAllister *et al.,* 2011). In monogastrics, *Bifidobacterium* spp. colonize the intestinal tract shortly after birth and play a key role against enterovirulent microorganisms involved in diarrhoea (Liévin-Le Moal and Servin, 2006).

2.5.3.2 Paenebacillus polymyxa

Paenibacillus polymyxa is a non-pathogenic endospore-forming bacterium. It is a motile grampositive, rod-shaped bacterium and usually found in the environments such as in the soil and marine sediment (Zengguo *et al.,* 2007; Timmusk *et al.,* 2005). *Paenebacillus polymyxa* is able to move by it peritrichous flagella and it has the capabilities of nitrogen fixation, hormone production that promote plant growth, produce hydrolytic enzymes and produce antibiotics that fight against harmful plant and human microorganisms (Lal and Tabacchioni, 2009).

2.5.3.3 Yeast or Fungi

The type of yeast or fungi commonly used as DFM includes *Saccharomyces sp*. and *Trichlosporon sp.* The use of *Saccharomyces cerevisiae* as DFM has the ability to increase feed intake, rumen pH, volatile fatty acids (VFA) and organic matter digestibility, as well as decreasing rumen lactate concentration (Desnoyers *et al.,* 2009). *Saccharomyces cerevisiae* can metabolize lactic acid in the rumen and also cause shifts in rumen bacterial populations, such as an increase in the numbers of fibrolytic rumen bacteria. These shifts in bacterial populations according to Jouany *et al.* (1999) reflect the ability of yeast to utilize the trace amounts of oxygen present in the rumen, thereby creating an environment that is more conducive for the activity of anaerobic cellulolytic bacteria or since the yeast

culture itself contains micronutrients, it can simulate the growth of rumen microbial populations thereby altering rumen fermentation (Robinson and Erasmus, 2009).

2.5.4 The Role of DFM on Intake, Feed Efficiency and Growth of Ruminants The initial idea of introducing DFM to livestock was based primarily on its potential benefits on intestinal effects, including the creation of a desirable gut micro flora and/or prevention of the establishment of pathogenic organisms in the gut. However, reports indicate that DFM can increase daily weight gain and feed conversion efficiency in feedlot cattle, enhance milk production and improve health and performance of young calves (Krehbiel *et al.,* 2003). From the literature, the effect of DFM on performance of ruminants depends on the type of culture, strain, type of animal, age of animal and dose because many studies report no effect of yeast and yeast culture on growth performance (Morrill *et al.,* 1977; Ellinger *et al.,* 1978; Owen and Larson, 1984). Titi *et al.* (2008) also reported that adding DFM (yeast culture) had no effect on dry matter intake in lambs and kids supplemented with yeast culture in the diet.

Considering the effect of DFM on the rate of feed intake, feed conversion efficiency, average daily weight gain (ADG) and improved productivity, Krehbiel *et al.* (2003) indicated that for ruminants, DFM influence milk production, improve feed conversion efficiency and daily weight gain in cattle. Lee *et al.* (2000) reported that an anaerobic yeast DFM culture introduced into the rumen can improve nutrient utilization in sheep.

Several published articles indicate that adding direct-fed microbial to the feed of sheep increased body weight and average daily gain (Lubbadeh *et al.,* 1999; Chiofalo *et al.,* 2004). Also Bechman *et al.* (1977) and Christen *et al.* (1995) have noted improvement in BW gain when *Lactobacillus* product is added to milk or milk replacer fed to dairy

calves.

Research by Christen *et al.* (1995) also concluded that when calves were fed a *Lactobacillus* fermentation product there was a trend toward improved starter intake. Henderson *et al.* (1986) found that sheep fed with bacterial DFM inoculated silage, had improved intake of feed and increased ADG compared to the control. Adams *et al.* (2008) suggested a new direct-fed microbial (*Propionibacterium jensenii* 702) can improve bodyweight gain during both the milk feeding period and post-weaning period. Emanuelle *et al.* (1993) stated that, feed consumption, weight gain, and feed conversion rate of the animals are improved when fed DFM inoculated forage. Bechman *et al*. (1977) also noted improved feed efficiency when *L. acidophilus* was included in the diet of young dairy calves.

Other studies suggest that, treatment with DFM benefited calves receiving less than recommended amounts of colostrum to maintain a desirable balance of the microbial flora to stimulate rumen and/or intestine development and enhance BW gain in calves until weaning. DFM cultures are able to improve feed intake, feed conversion and improve performance. They have the ability to improve bacteria cellulolytic activities in rumen of lambs fed DFM diets (Wallace and Newbold, 1993). They also have a positive effect on ruminal pH, which helps improve fibre degradation and dry matter intake (Umberger and Notter, 1989).

2.5.5 The Role of DFM on the Haematology of Ruminants

Blood profile of animals is an important indicator of animal health. DFM has been found to improve the blood profile and induce health promoting activities such as reducing anti-nutritional factors in ruminants whose feed were supplemented (Belewu *et al.*,

2008). Sayed (2003) reported an increase in the haemoglobin (Hb) concentration, red blood cells (RBC) and packed cell volume (PCV) in kids and turkey fed with DFM supplementation. According to studies done by Aboderin and Oyetayo (2006), Hb, PCV, RBC, white blood cells (WBC), mean corpuscular volume (MCV), platelets and eosinophils in animals fed DFM supplemented diet were significantly affected.

Leucocytes were significantly higher in animals fed DFM than the control (Paryad and Mahmoudi, 2008). However, Rao (2007) reported no significant differences ($P>0.10$) in lymphocyte, neutrophils, monocytes, WBC count, RBC count, haematocrit and haemoglobin among treatments in pigs. Galip (2006) also stated that yeast DFM supplementation did not affect the haemoglobin of animals on DFM diet compared to the control. The effects of DFM on blood parameters depend largely on the effectiveness of the microbial activities, the type of microbe, animal and type of parameter being measured, nevertheless, DFM supplementation has some impact on the blood properties of livestock.

2.5.6 The Role of DFM on Blood Biochemistry of Ruminants

Total protein, albumin and globulin contents show the level of protein metabolism in the animal. Direct fed microbial has been found in several studies to affect blood biochemical parameters. In an experiment by Khaled and Baraka (2011), lambs on finishing diet fed TOMOKO® supplemented feed, showed a significant increase in serum total protein, globulin and urea nitrogen. In a study by Shareef and Al-Dabbagh (2009), it was revealed that the feeding of *Saccharomyces cerevisiae* at a rate of 1.5, 2 and 2.5 was responsible for a significant ($P<0.05$) increase in glucose and total serum protein levels in chicks. In contrast, Amoah (2010) recorded no effect of bacteria DFM on total protein, albumin and globulin of pigs.

2.5.7 The Role of DFM on the Immunology of Ruminants

Direct Fed Microbial was used to replace antibiotics because aside its ability to improve growth performance, it enhances immunity by promoting the production of antibodies, IgA and cytokines and colonize the intestines, increasing phagocytosis of pathogens (Higgins *et al.*, (2007).

According to Isolauri *et al.* (2001), the intestinal micro flora play an important role in host defence due to their ability to modulate both innate and acquired immunity at the local as well as systemic levels. Lactic acid bacteria have been found to induce protective immunity against pathogens and tumours and have the ability to increase the mucosal immune response (Goldin and Gorbach, 1980; Isolauri *et al.*, 1994; Majamaa *et al.*, 1995). Oral administration of lactobacilli led to improved innate immune responses (i.e., enhanced phagocytosis and natural killer cell activity), as well as elevate production of immunoglobulin IgA and reduce IgE production in both humans and animals (Erickson and Hubbard, 2000; Isolauri *et al.*, 2001). According to Lee *et al.* (2010), an increase in intra- epithelial lymphocytes (IEL) subpopulation T-cell markers in birds fed DFM compared to the control group has been observed. Dalloul *et al.* (2003) reported that, feeding chickens with a lactobacillus supplemented diet, demonstrated an increase in CD3, CD4, CD8 and TCR2+ IEL subpopulation in broilers. In a study on the importance of Bacillus-based direct-fed microbial supplementation on immune development of dairy calves, Novak *et al*. (2007) reported that, a *Bacillus*-based DFM fed at the start of scours has the potential to impact on innate and adaptive immune development in calves. Bacterial DFM can impact its host immune system through a series of mechanisms including up-regulation of cell-mediated immunity, enhance antibody production and epithelial barrier integrity, reduction of epithelial cell apoptosis, enhanced dendritic cell–T cell interactions, heightened T cell association with lymph nodes and greater Toll-like receptor signalling

and its ability to attach to and colonize the GIT is most likely important. The GIT does not only regulate the elective entry of nutrients but defend against pathogens, using specialized receptors and other mechanisms to fight against microbes and control and regulate the local immune response (Kogut *et al.,* 2013). In contrast, other bacteria such as *Bacillus subtilis* DFM had no impact on the expression of IFN-γ, IL-3 and IL-4 in chickens (Fujiwara *et al.,*

2009).

2.5.8 The Role of DFM on Pathogenic Microbes

The change in diet, environment and removal of lambs from ewe during weaning impose stress that can lead to an intestinal imbalance between beneficial and non-beneficial microflora which can increase the susceptibility of the neonate to post-weaning diarrhoea (Estrada *et al.,* 2001; Drew *et al.,* 2004).

The DFM *E. coli* strain Nissle 1917 produces microcins H47 and M (Patzer *et al.,* 2003), and has been reported to reduce neonatal diarrhoea in calves (von Buenau *et al.,* 2005), possibly by interfering with the invasion of epithelial cells by *Salmonella enterica* var. *typhimurium.* Recently, Tabe *et al.* (2008) reported that feedlot steers fed *L. acidophilus* (BT1386) did not affect shedding of *Salmonella sp.* in faeces, however, *E. coli* O157 shedding has reduced. Younts-Dahl *et al.* (2005) indicated a dose-dependent reduction in the shedding of *E. coli* O157 when levels of *L. acidophilus* NP51 in the diet were increased. Report by Ellinger *et al.* (1980) also indicated that feeding *L. acidophilus* to calves reduced the amount of faecal coliforms. Holstein calves fed with *L. acidophilus*

27SC had statistically higher colony counts in faeces than the calves fed a control diet (Abu-Tarboush *et al.,* 1996). Arthur *et al.* (2010) observed no significant effect of

Bacillus subtilis strain 166 on the faecal E. *coli* O157:H7 isolates in calves.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Ejura Sheep Breeding Station of the Animal Production Department, Ministry Of Food and Agriculture (MoFA) at Ejura in the Ejura-Sekyere Dumase District of the Ashanti Region, Ghana. Ejura lies within latitude 7º 23'N and longitude 1º 22'W, and in the transitional zone of the semi-deciduous forest and Guinea savanna zones of Ghana. It experience maximum and minimum temperatures of 30ºC and 21ºC respectively with relative humidity range of 55-90%. The research lasted four months from October 2012 to January 2013.

3.2 Experimental Animals, Design and Housing

A total of twenty four (24) 4- day old lambs with an average birth weight of (2.5 ± 0.2 kg) were randomly allotted to provide equal number of lambs and provided with feed with or without direct fed microbial. Each treatment had 6 replicates in a randomized complete block design.

The dams were drenched before placing them and their lambs in their individual pen. The lambs were housed in a cement-block, barn with concrete floors and corrugated aluminium-roofing sheets. Two barns were partitioned into twenty four individual pens with wood and wire mersh. Feeding and water troughs were provided in each pen.

3.3 Experimental diets

Four dietary treatments (T) designated as control – (distilled water T1); RE3™ (*Lactobacillus sp, Bacillus sp., Saccharomyces sp*. and fermentation products T2); RE3

Plus (fermentation products of RE3™ T3); and P3 (*Paenebacillus polymyxa*-based DFM T4) were used, with each treatment having six replications. The treatments were produced by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada. All the treatments except treatment two (T2) RE3™, were stored at 5^0C before use. The treatments were administered in two phase namely, the suckling and the weaned phase. The treatments applied in suckling phase were diluted at a ratio of 1.5 mL of treatment to 10 mL of distilled water which were administered orally to the lambs from 4th to the 32nd day of growth. During the weaned phase, the treatments (without dilution) were mixed with the feed at a ratio of 1.5 mL of treatment to 1 kg of feed.

3.4 Feeding

The study had three continuous feeding phases; the suckling phase, creep/pre-weaning phase and the grower/weaned phase. The suckling phase lasted four weeks and the lambs lived on the milk from their dams with the treatments being administered orally. The creep/ pre-weaning phase lasted for about 8 weeks. The lambs were introduced to a diet having crude protein (CP) % of 16.1 and a daily requirement of about 0.3 kg of feed per lamb (Table 3.1) with the treatments mixed with the feed at feeding time. The feed was given to the lambs around 9 am every day when the dams have been sent out to graze and removed around 5 pm before the dams are put in the pen to prevent the dams from eating the formulated feed. The weight of feed offered and feed refused were measured each day.

The last month of the study was the grower phase where the lambs had been separated from their dams. The CP% of the formulated diet was 18.6% according to Charray *et al*. (1992) and a daily requirement of about 0.5 kg of feed per lamb. The treatments were mixed with the feed and the inclusion rate was the same for both phases. The lambs were fed once daily around 9am to 8 am the next day and the leftovers were weighed. Fresh forage was used to supplement their diet during this phase.

Fresh water was provided *ad libitum*. Multivitamin was given to the lambs a day before the introduction to the formulated feed and every 3 weeks afterwards. Salt licks were also made available to the animals. Water troughs and feeding troughs were washed daily and every 2 weeks respectively and fresh water provided daily.

Table 3.1: Ration formula for young lambs

		Pre weaned lambs		Weaned lambs	
Lamb ration	CP (%)	Level (kg)	CP Supply	Level (kg)	CP Supply
Wheat bran	16	38	6.08	40	6.4
Maize	9	30	2.7	10	0.9
concentrate	30	9	2.7	7	2.1
CSM	22.9	20	4.58	40	9.2
DCP	0	1	0	1	0
Salt	0	1	0	1	0
Oyster shell	0	1	0	1	0
		100	16.1	100	18.6

Source: Charray et al. (1992). *CMS: Cotton Seed Meal, CP-crude protein, DCP-dicalcium phosphate*

3.5 Parameters Measured

3.5.1 Feed Intake

The feed offered and feed refused were weighed daily with a Camry 25 kg x 50 g scale (China). Daily feed intake for each lamb was determined by deducting the feed refused from feed offered. Weekly and total feed intake of each lamb was computed from the daily feed intake.

3.5.2 Weight Gains

The weight of each lamb was taken before the start of the experiment and was weighed weekly (Saturday morning) to obtain the weekly weight changes. Average daily gain was obtained by dividing the total weight gain of each lamb by the total experimental number of days of each lamb.

3.5.3 Feed Conversion Efficiency (FCE)

The feed conversion efficiency of each lamb was calculated as a ratio of total feed consumed to total weight gain by each lamb.

3.5.4 Faecal Microbial Counts

A sterilized swap was used to take faecal sample from the lamb's rectum for the microbial counts. The faecal sample was taken on day 28, 56 and 112 of the study and sent to the Komfo Anokye Teaching Hospital's microbiology laboratory for culturing and bacterial counts. Faecal samples collected were serially diluted and known quantities were inoculated into Plate Count Agar in petri dishes. These samples were then incubated at a temperature of 35°C for 24 hours after which colonies formed were countered with the aid of a colony counter.

3.6 Chemical Analysis

3.6.1 Blood Sample Collection

On day 1, 28, 56 and 112 blood samples from each experimental unit was taken. The blood samples were obtained by jugular vein puncture and two blood samples from each lamb were collected into vacutainer tubes containing clot activator gel for biochemistry and EDTA (K3) for haematology. The samples were transported in a cold environment to prevent denaturation of the blood before analysis. The blood in the tubes containing clot

activator gel was centrifuged at 2500 rpm for 3mins for serum separation. The serum was stored in micro tubes at -20°C until further analysis.

3.6.1.1 Haematology

The haematological analysis was carried out at the Regional Veterinary laboratory, Kumasi. Haematological parameters were determined using Sysmex automatic haematology analyser (USA). The parameters analyzed include red blood cell (RBC), haemoglobin (Hb); pack cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); neutrophils, eosinophils, monocytes, lymphocytes and basophils.

3.6.1.2 Biochemistry and Immunology

The immunological and biochemical studies were done at the Biochemistry and Microbiology Laboratory, Komfo Anokye Teaching Hospital. The blood serum was analyzed for total protein, albumin, globulin, IgA and IgM. IgA and IgM compositions were determined by the enzyme-linked-immunosorbent assay (ELISA) procedure as described by Granstrom *et al.* (1994). Whole blood sample was used to analyze the CD3 and CD4 levels of the lambs using the BD FACS Count System (Becton, Dickson and Company, San Jose, Belgium).

3.7 Statistical Analysis

The Generalized Linear Model (GLM) and the PROC MIXED procedure of Statistical Analytical System (SAS, 1998) were used to analyze all the experimental data. The differences between means were tested by Waller Duncan Multiple Range test in SAS. Values for which $p < 0.05$ was considered to be significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Feed Intake

The performance of lambs and their feed intake during the three phases of growth (i.e. suckling, creep and grower phases) are presented in the sections below.

4.1.1. Suckling Phase

The suckling phase spanned from day 4 to 32. The treatments T2 had significantly ($P<0.05$) higher average daily intake (ADI) than lambs on T3 and T1. From Table 4.1, Lambs on DFM treatments recorded higher ADI than control and this is might because DFM has the ability to stimulate early development of the rumen to improve intake. This is supported by Bechman *et al*. (1977), who reported that adding *Lactobacillus acidophilus* to milk or milk replacers for calves, recorded an increase in intake. A study by Quintero-Gonzalez *et al*. (2003) who added direct fed microbial (DFM) to the milk of young Holstein calves, however, observed no significant difference in milk intake regardless of the DFM type fed.

Table 4.1: Direct fed microbial effect on growth performance of suckling lambs

TRT	ADI	INIT (kg)	FWT (kg)	WG (kg)	ADG(kg)	FCR
1	0.06^{b}±0.02	2.78±0.39	5.23±0.80	2.45±0.82	0.09±0.03	0.75±0.40
2	0.11^{a}±0.05	3.30±0.30	6.08±1.25	2.78±1.40	0.10±0.05	1.23±0.36
3	0.08^{b}±0.02	3.64±0.38	6.18±1.33	2.54±1.15	0.09±0.04	1.00±0.30
4	0.10^{ab}±0.02	3.37±0.43	6.37±1.25	3.00±1.00	0.11±0.04	1.05±0.56
P value	0.04	0.52	1.91	1.91	0.07	0.70

Where TRT = treatment; ADI = Average daily intake INIT = Initial body weight; FWT = Final body weight; WG = Weight gain; ADG= Average Daily weight gain; FCR = feed conversion ratio; ^{ab}Means(±SD) with a common letter within treatments are not significantly different. ±SD= ± standard deviation.

4.1.2 Creep Phase

The creep phase started from day 33 to day 89, where the lambs were given a concentrate diet with the different treatments. Intake of concentrate diet by the lambs during the creep phase tended to be higher in lambs receiving DFM compared to the control though the difference was not significant (P>0.05) (Figure 4.1). Contrary to this result, Quintero-Gonzalez *et al*. (2003) reported significant difference in starter grain intake when DFM was added to diets fed the calves. Christen *et al.* (1995) also reported that when calves were fed *L. bacillus* fermentation products, there was a trend toward improved starter intake. The improvement in feed intake by treatment groups during the creep phase could be attributed to oral administration of DFM treatments to new-born lambs which might have helped the lambs establish and maintain a desirable balance of microbial flora to stimulate the early development of the rumen and intestine of the lambs (Nakanishi *et al*., 1993; Chaucheyras *et al*., 2000).

4.1.3 Grower Phase

The grower phase was between days 90 of growth to day 120. The average daily intake (ADI) values for growing lambs under T2, T3 and T4 were numerically higher compared to the control (T1) but the difference was not statistically significant. This was confirmed by Titi *et al*. (2008) who reported no significant impact of DFM supplementation on average feed intake in growing lambs and kids. Theodorou *et al.* (1990) reported that feeding anaerobic fungi, significantly increased feed intake in calves following weaning. The relatively higher ADI values observed in treated lambs may be as a result of DFM addition to the diet which according to Wallace and Newbold (1993) improves cellulolytic bacteria activities in the rumen and helps increase fiber degradation and dry matter intake (Umberger and Notter, 1989)

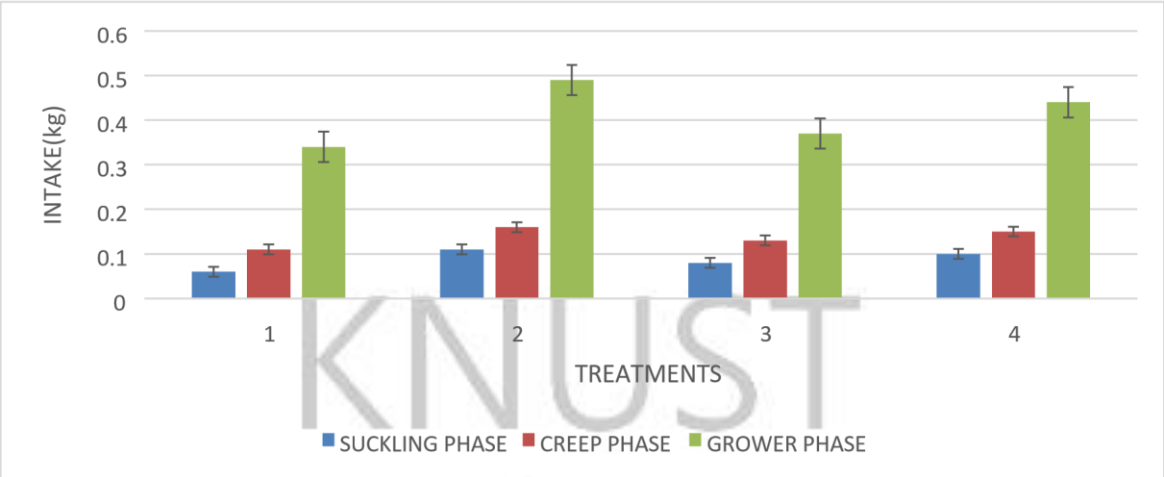

Figure 4.1: Effect of DFM treatments on feed intake in lambs for all the growth phases

4.1.4 Suckling to Grower phase

For the entire study period, average feed intake of the treatment group was not significantly different (P>0.05) from the control group (Table 4.2).This is supported by the report from Ghorbani *et al*. (2002) who fed high concentrate diet supplemented with DFM to feedlot cattle and observed no significant DFM influence on feed intake. Hernandez *et al*. (2009) also noticed no effect on feed intake of lambs fed diets containing DFM. Swinney-Floyd *et al*. (1999) fed *Propioni bacterium* and *Lactobacillus sp*. singly or combined and found that treatments did not influence feed intake in calves. In contrast, DFM has been reported to increase feed intake in lambs and calves in other studies (Desnoyers *et al*., 2009; Chiofalo *et al*., 2004; Antunovic *et al*., 2006). These contradictions in reports on the effect of DFM on feed intake may be due to differences in the type and quantity of microbes used, how viable the microbes were and the type and age of animals used (Krehbiel *et al*., 2003).

Table 4.2: Direct fed microbial effect on growth performance of sheep from suckling to grower

TRT	ADI	INIT (kg)	FWT (kg)	WG (kg)	ADG(kg)	FCR
1	0.51±0.21	2.78±0.39	11.7±1.62	8.87±1.9	0.08±0.02	6.33±2.03

2	0.76±0.26	3.30±0.28	14.1±3.1	10.8±3.13	0.1±0.03	7.74±0.57
3	0.58±0.17	3.64±0.38	12.7±2.32	9.06±2.27	0.08±0.08	7.14±0.61
4	0.69±0.22	3.37±0.43	14.5±3.96	11.2±3.73	0.1±0.03	7.32±1.86
P value	0.285	0.013	0.345	0.443	0.449	0.527

Where TRT = treatment; ADI = Average daily intake INIT = Initial body weight; FWT = Final body weight; WG = Weight gain; ADG= Average Daily weight gain; FCR = feed conversion ratio

4.2 Weight gain (WG)

4.2.1 Suckling phase

Treatment effect on average daily weight gain (ADG) in lambs under DFM treatments was not significant (P > 0.05) compared with the control lambs (Table 4.1). In contrast, an improvement in weight gain was seen in calves fed milk or milk replacers containing lactobacillus product compared to the control group (Christen *et al*., 1995). Abe *et al.* (1995) observed that oral administration of lactic acid bacteria DFM improved body weight gain. Pond and Goode (1985) fed lambs with DFM supplemented feed and observed a 24.7% increase in weight gain during the first two weeks of study. The improved weight gain in DFM treated lambs may be due to the increased number of cellulolytic bacteria and protozoa which create suitable condition for microbial growth in the rumen which might have led to increased consumption and improve efficiency of feed utilization in the DFM supplemented group (Antunovic *et al.*, 2006).

4.2.2 Creep phase

In the creep phase, lambs receiving the DFM treated diets had numerically a higher weight gain compared to the control lambs though the differences were not significant (P >0 .05) (Figure 4.2). The improved weight gain in the DFM lambs might be due to the fact that

DFM has the ability to improve rumen microbial ecology, protein synthesis and their availability to the animal, resulting in better weight gain (Oyetayo and Oyetayo, 2005). Dick *et al*. (2013) however, observed no significant difference (P>0.05) in weight gain and ADG of the calves fed bacteria DFM during the pre-weaning period. AbuTarboush *et al*. (1996) and Jost and Bracher (1999) reported similar results that, weight gain and average daily gain of calves given *lactobacillus* supplemented concentrated were not significantly affected. The creep/pre-weaning phase lasted almost 60days and this might be the reason why weight gain during this phase is higher than in the grower phase.

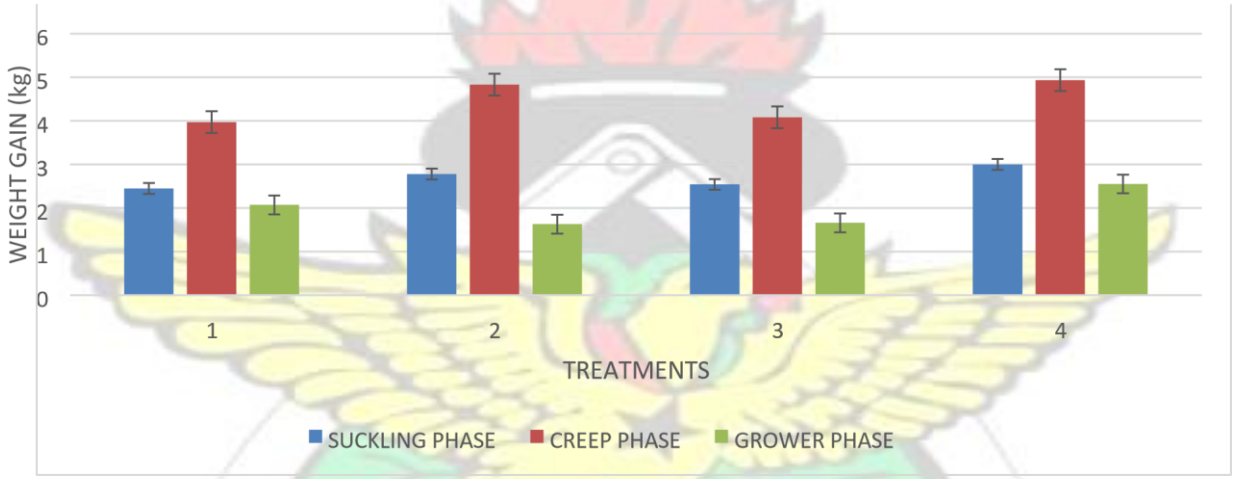

Figure 4.2: Effect of DFM treatments on weight gain in lambs for all the growth phases

4.2.3 Grower phase

At the end of the grower phase (Figure 4.2), the average final body weights and average daily gain obtained by the lambs on DFM treatments, were not significantly different from the control treatment. This might be because feed intake was not significant thus it corresponding effect on weight gain. However, a study by Haddad and Goussous (2005) and Jang *et al.* (2009) stated that DFM supplementation resulted in higher weight gain than

the control lambs. Improved weight gain in lambs fed diets containing DFM could be attributed to increased amino acids supply at post-ruminal level (Erasmus *et al.,* 1992).

4.2.4 Suckling – Grower phase

For the entire study period, animals on DFM treatments numerically had better mean final weight, weight gain and mean daily gain than animals on the control treatment (Table 4.2). There were no significant differences (P>0.05) in the mean final weight, weight gain and mean daily gain among all treatment groups. The results obtained in this study agrees with that of Elam *et al*. (2003) who observed higher ADG in steers fed DFM supplemented diets than control steers but no significant differences in the overall ADG or carcass-adjusted ADG. From Table 4.2 above lambs on DFM treatments had better feed intake and consequently a better weight gain. This corroborates report by Antunovic *et al*. (2006) suggesting that, the improved weight gain can be related to higher consumption and better efficiency of feed utilization in the DFM-supplemented lambs.

4.3 Feed Conversion Ratio (FCR)

4.3.1 Suckling Phase

Feed conversion Ratio (FCR) is defined as the total feed consumed divided by total weight gained by each lamb and this indicates how well the animal is able to convert feed efficiently into animal products such as live weight or milk production. The FCR among the lambs during the suckling phase did not significantly (P>0.05) differ (Figure 4.3). This is in agreement with report by Kiesling *et al.* (1982) who during a 28 day trial, observed no significant effect of

DFM on feed conversion ratio of cattle orally fed with *Lactobacillus* culture. Bechman *et al.* (1977), however, observed that suckling calves fed *Lactobacillus spp*. recorded

improved feed efficiency and this could be as a result of improved microbial ecosystem in the rumen (Musa *et al.*, 2009).

4.3.2 Creep Phase

Mean FCR values did not differ significantly (P>0.05) among all treatment groups. From Figure 4.3, control diet was more efficiently utilized than DFM diets. Contrary to this results, Antunovic *et al*. (2006) observed better feed conversion efficiency in lambs receiving DFM supplemented diets compared to the control but the difference was not significant. Abdelrahman and Hunaiti (2008) also stated that supplementation of animal diet with DFM improves feed efficiency. Whitley *et al*. (2009) in a DFM trial observed that goats fed supplemented diet recorded better FCR compared to the control. The improvement in feed efficiency upon feeding probiotics may be due to their positive effect on fiber degradation, nutrient digestibility and their bio-availability (Umberger and Notter 1989; Abd El-Ghani, 2004).

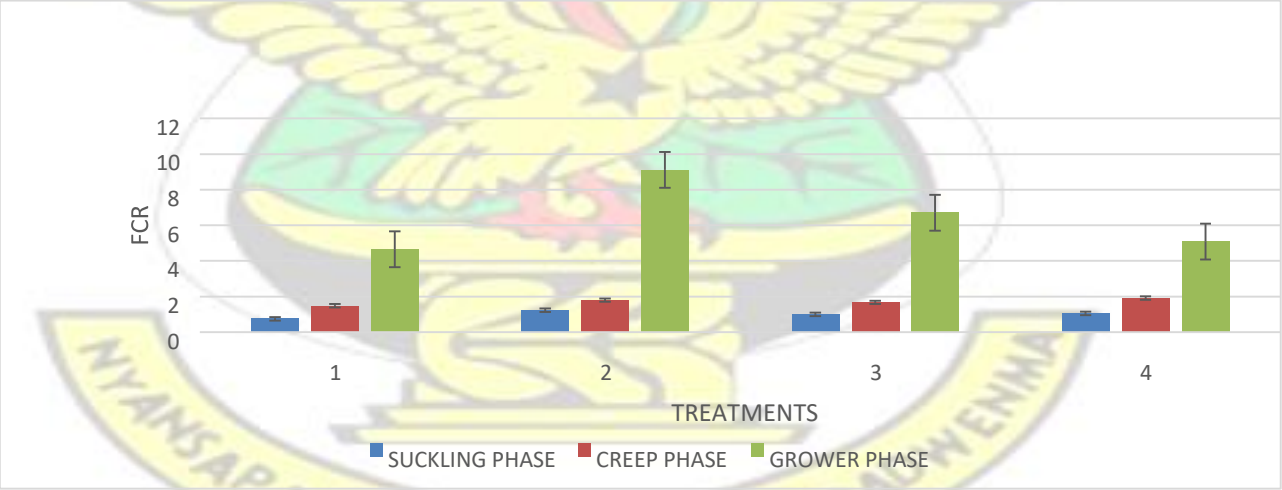

Figure 4.3: Effect of DFM treatments on feed conversion ratio in lambs for all the growth phases

4.3.3 Grower Phase

The feed efficiency during the grower phase differed significantly (P>0.05) comparing treatment 2 with treatments 4 and 1 (Figure 4.3). Treatment 3 was statistically similar to all the other treatments. However, the DFM treatments did not improve FCR since the control lambs had lower FCR than the treated lambs. These results are consistent with Swinney- Floyd *et al.* (1999), who gave *Propioni-bacterium* and *Lactobacillus spp.* singly or combined to calves and observed no influence of DFM on FCR. Baranowski *et al*. (2007) also observed no statistical influence of DFM on feed efficiency across the treatments in lambs given diet containing mineral bioplex and linseed during the grower phase. However, Robinson (2002) reported that DFM supplementation improved the feed conversion efficiency in ruminants. Increased feed conversion ratio was recorded in calves fed Probios® and *Streptpcoccus faecium*® (Strzetelski *et al*., 1998). The differences in results may be due to the type of microbes and quantity fed to the lambs that improved their microbial ecology and nutrients digestibility.

4.3.4 Suckling – Grower Phase

Statistical (P>0.05) difference did not exist in the FCR between the four dietary treatments (Table 4.1). In agreement with other reports, lambs fed TOMOKO® DFM showed no significant effect (P>0.05) on feed intake and feed efficiency during the study period (Khaled and Baraka, 2011). Keyser *et al*. (2007) and Peterson *et al*. (2007) also reported that, supplementing steer feed with yeast or bacteria DFM did not affect dry matter intake, average daily gain or feed efficiency between treated steer and control animals. Jenny *et al*. (1991) and Abu-Tarboush *et al*. (1996) reported that feeding young calves DFM did not alter their feed conversion efficiency. However, other reports by Malik and Bandla, (2010) and Krehbiel *et al.*(2003) indicates that DFM has significant influence on feed

conversion efficiency because DFM has the advantage of increasing cellulolytic bacteria activity to improve fibre degradation and feed conversion efficiency.

4.4 Blood Profile

4.4.1 Haematological Parameters

4.4.1.1 Effect of treatments on Haemoglobin and RBC

The effect of DFM treatments on haemoglobin and red blood cells (RBC) of the lambs during the entire study period are presented in the Figure 4.4 and Figure 4.5. The effect of DFM treatments on the haemoglobin (Hb) and red blood cells (RBC) concentration of the lambs were not significant (P>0.05). This result agrees with Aboderin and Oyetayo (2006) who reported that the haematological profiles of lambs remained unaffected at different levels of DFM compared to control diet. From the Figures, the Hb and RBC values during the pre-weaning phase were lower among all treatments and this could be due to the tick infestation which occurred during that period. All the lambs were treated with acaricide. Sampling period did not significantly (P>0.05) affect the Hb and RBC concentrations.

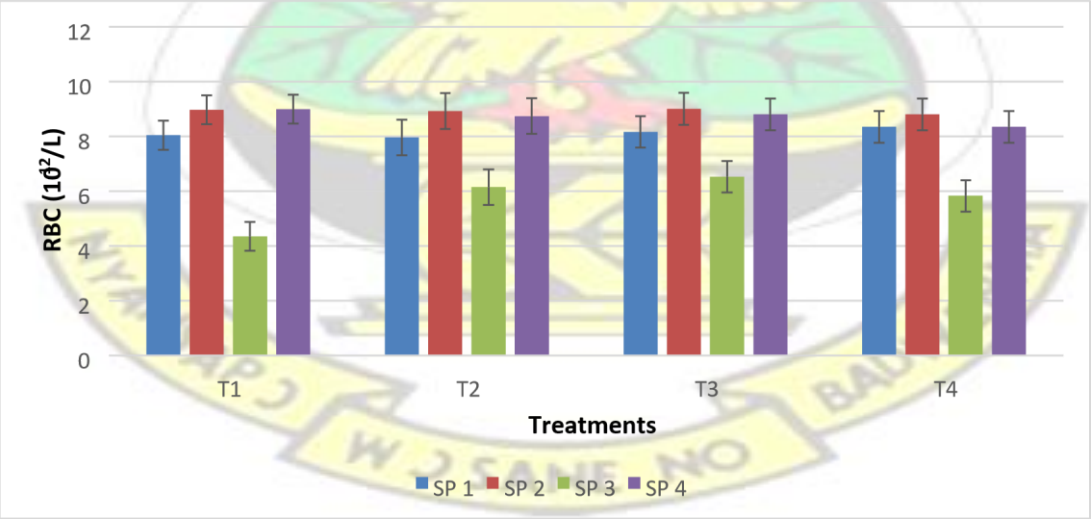

Figure 4. 4 Effects of Treatments and growth phases on RBC counts

Where RBC= red blood cell; T1=control, T2= RE3™ T3= fermentation products of RE3™, T4 = Paenibacillus polymyxa; SE = standard error. SP1= baseline, SP2= suckling phase, SP3= creep phase, SP4 =grower phase

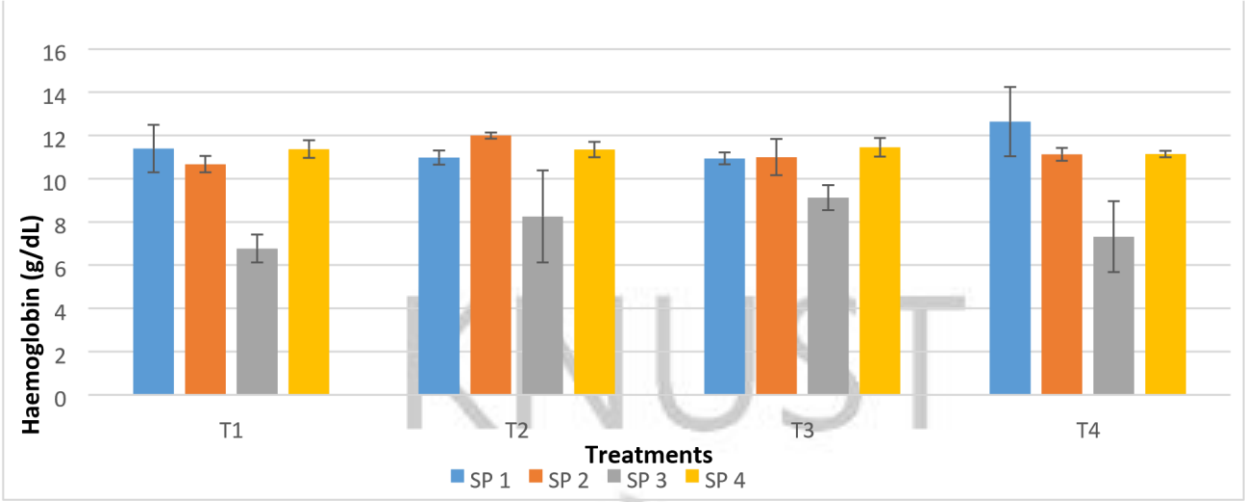

Figure 4.5: Effects of Treatments and growth phases on Haemoglobin levels

Where Hb = Haemoglobin, T1=control, T2= RE3™,T3= fermentation products of RE3™, T4 = Paenibacillus polymyxa; SE = standard error. SP1= baseline, SP2= suckling phase, SP3= creep phase, SP4 =grower phase

4.4.1.2 Effect of DFM Treatments on Red Blood Cell Indices

The packed cell volume (PCV), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) values of the lambs under all the treatments groups did not differ significantly (Figure 4.6). Numerical differences were observed but these differences did not follow any clear pattern. Aboderin and Oyetayo (2006) also observed that MCH, MCV and other haematological values remained unaffected at different levels of DFM supplementation compared to control diets.

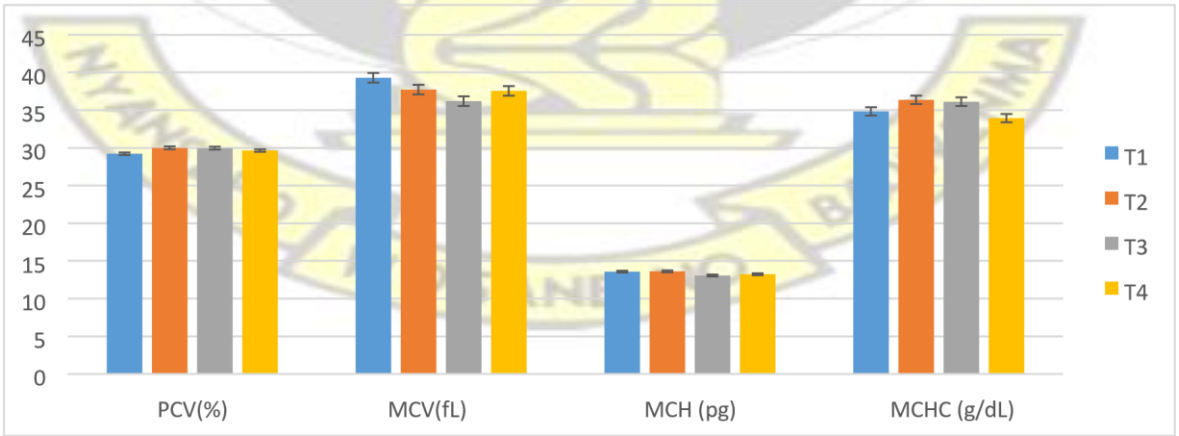

Figure 4.6: Effect of Direct Fed Microbial on MCV, MCH, PCV AND MCHC

values

Where T1=control, T2= RE3™,T3= fermentation products of RE3™, T4 = Paenibacillus polymyxa; SE = standard error PCV= pack cell volume; MCV = mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration.

4.4.1.3 Leucocyte Count

White blood cells (WBC) are major parts of the body's immune system and are very important in defending the body against infections. Within treatment, significant difference ($P<0.05$) was observed in monocytes levels during the sampling period 2 (SP2) (Table 4.3). Sampling period effect was observed in the eosinophils ($P=0.0197$) and lymphocytes ($P=0.0033$) levels in lamb under all the treatment groups. Higher eosinophils levels were observed during SP2 and this might be due to the tick infection which occurred during that period. More eosinophils are produced during parasitic infection (www.wikipedia.com). No significant ($P>0.05$) effect of DFM was observed in the overall leucocytes differential counts among all the treatment groups. This agrees with results of Galip (2006) who reported no significant differences in haematological values between lambs on DFM supplemented diets and control.

Table 4.3: Effect of Direct Fed Microbial on Leucocyte count for the study period.

Parameters		Treatments				P[1]
		T1	**T2**	**T3**	**T4**	
Leucocytes / Sampling Periods(SP)						
		9.17	8.49	11.14	10.34	0.0825
		15.67	19.24	18.94	15.54	0.2439
		10.67	8.99	11.94	9.94	0.7868
		11.83	12.24	14.00	11.94	0.5440
		47.00	46.44	44.87	47.67	0.8325
		47.00	42.69	43.27	45.67	0.0698
		46.50	47.94	43.27	47.87	0.0902
		46.94	45.69	43.80	47.07	0.1053
Basophils, %	SP1	0.17	0.25	0.20	0.001	0.2993
	SP2	0.001	0.25	0.001	0.20	0.7376
	SP3	0.17	0.25	0.20	0.001	0.8830
	Aveg	0.11	0.25	0.13	0.07	0.6381
Eosinophils, %	SP1					
	SP2					
	SP3					

	Aveg **Neutrophils, %** SP1					
	SP2					
	SP3					
	Aveg					
Monocytes, %	SP1	5.17	6.15	5.64	4.64	0.3652
	SP2	6.00[b]	8.66[a]	6.04[b]	6.64[b]	0.0177
	SP3	4.50	5.90	5.24	4.84	0.1997
	Aveg	5.22	6.90	5.64	5.37	0.0588
Lymphocytes, %	SP1	38.67	38.21	37.84	37.04	0.8742
	SP2	30.83	28.71	31.44	31.64	0.4633
	SP3	38.17	36.46	39.04	37.04	0.5551
	Aveg	35.89	34.46	36.10	35.24	0.7771

Means with a common letter within treatment are not significantly different T1= control, T2= RE3™, T3= fermentation products of RE3™, T4 = Paenibacillus polymyxa; SP= sampling periods

4.4.2 Serum Biochemistry

The mean total protein values increased significantly (P=0.0161) for lambs on T2 and T4 compared to the control (Table 4.4). Total protein and albumin levels among the lambs decreased with the sampling periods. Globulin levels tended towards significance during SP3. No significant differences (P>0.05) were observed in the serum albumin and globulin values among the treatments.

Table 4.4: Serum biochemical profile of lambs for the study period

Parameters	**Treatments**				**P[1]**
	T1	**T2**	**T3**	**T4**	

Biochemical Parameters/ Sampling Periods(SP)						
Albumin	SP1	43.63	45.72	39.94	46.95	0.5973
	SP2	30.64	33.13	31.45	32.74	0.5287
	SP3	33.70	40.54	37.73	37.50	0.2783
	SP4	29.44	31.88	31.88	31.43	0.5906
	Aveg	34.35	37.62	35.25	37.16	0.3074
Globulin	SP1	21.72	23.89	24.31	22.97	0.4427
	SP2	22.68	24.87	24.51	24.29	0.4410
	SP3	17.28	15.02	12.75	17.17	0.0907
	SP4	23.82	25.87	20.61	25.35	0.5629
	Aveg	21.38	22.42	20.55	22.45	0.4752
Total protein	SP1	65.33	69.67	64.50	70.16	0.2161
	SP2	53.3	58.04	56.16	57.28	0.1785
	SP3	50.98	55.64	50.72	54.92	0.1847
	SP4	53.25	57.04	52.74	57.04	0.2791
	Aveg	55.72^{b}	60.09^{a}	56.03^{b}	59.85^{a}	0.0161

Means with a common letter within treatment are not significantly different. Where T1 =control T2= RE3™ T3= fermentation products of RE3 ™, T4 = Paenibacillus polymyxa; sampling periods=SP,

4.5 Immunology

4.5.1 Immunoglobulin A (IgA) and Immunoglobulin M (IgM) Concentrations

Direct Fed Microbial was used to replace antibiotics because of its ability to enhance immunity by promoting the production of antibodies and cytokines and colonize the intestines, increasing phagocytosis of pathogens (Higgins *et al.*, 2007). IgA and IgM were

measured in this study because IgA is a surface antibody and predominant in the mucus and IgM is the first class antibody that appears initially when an organism is exposed to an antigen (primary infection).

The mean IgA values of lambs receiving DFM treatments tended towards significance (P=0.0631) with T3 lambs recording the highest IgA levels (1.25±0.07) compared to T1 (0.99±0.07), T2 (1.08±0.08) and T4 (1.18±0.07). The higher IgA levels in DFM treated lambs might be because feeding DFM elicits mucosal immunity and immunoglobulin A (IgA) is the dominant antibody of the mucus. Sampling period effects were also recorded with IgA levels increasing as they increased with age (Table 4.5).

Treatment effects on the mean IgM values recorded were statistically significant (P<0.05) among all the treatments (Table 4.5). Some sampling period effects were studied and the lambs recorded an increase in IgM levels with time. T2 and T3 lambs had the highest (1.79±0.16) and (1.64±0.14) IgM levels at the end of the study.

Table 4.5: Immunological profile of blood samples taken from lambs for the study period

Parameters		Treatments				P[1]
Immunological Paramet Periods(SP)						
CD3, cells/L	SP1	514.17	484.38	493.51	483.71	0.5277
	SP2	535.00	480.63	504.51	521.31	0.2516
	SP3	542.33	507.38	525.71	537.31	0.4591
	Aveg	530.5	490.8	507.9	514.11	0.5555
CD4, cells/L	SP1	386.0	296.53	299.29	345.89	0.0833

	SP2	382.17	312.78	320.69	339.29	0.1762
	SP3	407.17	327.03	334.49	353.09	0.1298
	Aveg	391.8	312.1	318.2	346.09	0.0316
IgA, 0.031-2.0ug/ml	SP1	0.45	0.60	0.72	0.47	0.4670
	SP2	0.95	0.80	1.20	1.22	0.4646
	SP3	1.25	1.42	1.46	1.41	0.2730
	SP4	1.36	1.49	1.62	1.63	0.1579
	Aveg	0.99	1.08	1.25	1.18	0.0631
T1		**T2**	**T3**	**T4**		

[a,b] Means in the same rows with different superscript(s) differ significantly (P<0.05), [1]P- probability IgA= immunoglobulin A; IgM= immunoglobulin M; CD3= cluster of differentiation 3; CD4= cluster of differentiation 4 Aveg = average values Where T1 =control T2= RE3™ T3= fermentation products of RE3™, T4 = Paenibacillus polymyxa; sampling periods=SP,

4.5.2 Effect of DFM Treatment on Cluster of differentiation 3 (CD3) and Cluster of differentiation 4 (CD4)

IgM, 0.031-2.0ug/ml	SP1	0.94^{b}	0.47^{a}	1.10^{b}	0.46^{a}	0.0263
	SP2	1.20	0.95	1.20	0.94	0.2469
	SP3	1.25	1.01	1.44	1.04	0.3384
	SP4	1.46	1.79	1.64	1.31	0.4775
	Aveg	1.21_{ab}	1.05_{ac}	1.35^{b}	0.94^{c}	0.0012

The major importance of the immune system is to recognize and eliminate foreign antigens, form immunologic memory and develop tolerance of self-antigens. No significant (P>0.05) difference in the levels CD3 of the lambs in all treatments was observed (Table 4.5). This result supports observation by Scharek *et al*. (2005) who reported that neither cellular nor humoral immunity was affected by the DFM treatment. This might be because CD3 is an antigen receptor, a molecule that helps to transmit a signal from the T- cell receptor (TCR) following its interaction with major histocompatibility complex (MHC) molecules thus might not be influenced significantly

by the DFM treatments. It remains present in all T- cell lymphomas and leukemia and is required for T-cell activation (Leong *et al*., 2003).

The CD4 levels on the other hand differed significantly among T2, T3 and control lambs. The control lambs recorded higher CD4 values (391.8±23.7) than the DFM treated lambs. The reason for this result might be because the lambs on the DFM treatments did not recognize the DFM as antigen that needs to be destroyed. There was no significant (P>0.05) sampling period effect among all the treatments groups.

A study on scouring dairy calves treated with a *Bacillus*-based DFM provided in an electrolyte treatment showed that DFM promoted the development of alpha-beta (CD4+and CD8+ subsets) and gamma-delta T lymphocytes (Novak *et al.,* 2012). This means that the mode of administering the treatments is also important in determining how it would affect the lambs' immunity.

4.6 Effect of Direct Fed Microbial on Microbial Count

A significant (P<0.05) increase in faecal shedding of *E. coli* was observed in lambs receiving T3 compared to T2, T4 and T1 respectively. There was no significant difference found in the microbial isolates of lambs under T1, T2 and T4. This result is supported by Arthur *et al*. (2010) who stated that, the prevalence of *E.coli* in either the hide or faeces was not significant among the treatment groups. Contrary to current results, calves fed DFM showed reduced faecal shedding of *E.coli* (Elam *et al.,* 2003; Peterson *et al.,* 2007).

Table 4.6 Direct Fed Microbial effect on faecal microbial count

	E coli counts (10^8org/ml)			
Treatments	E coli counts (10^8org/ml)	SP 1	SP 2	SP 3
T 1	26.02±2.6	25.50 ±4.9	25.38 ±3.5	27.17 ±3.5
T 2	28.34±3.7	31.50 ±8.2	25.74 ±4.3	27.90 ±4.3

T 3	31.96±2.7	32.49 ±4.3	31.03 ±3.8	32.35 ±3.8
T 4	26.14±2.4	22.82 ±3.5	26.69 ±3.5	28.92 ±3.5
P value	0.1658	0.7414	0.7414	0.7414

[abc]Means with common letters within treatments are not significantly different
Where T1= control, T2= RE3[TM], T3= RE3[TM] plus, T4= PP, SE= standard error, SP= sampling period

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The four months study conducted at the Ejura Sheep Breeding Station using twenty four lambs ascertained the effect of dietary supplementation of three different DFM products (RE3^{TM}, RE3 Plus and PP) on the growth performance, blood profile, immunity and pathogenic bacteria count in lambs. It was observed that the three different DFM products compared to the control did not significantly ($P>0.05$) affect growth performance, haematological variables, serum albumin and globulin, IgA, CD3 and pathogenic bacteria isolates. The addition of all the three different DFM products to creep feed and weaning diet did not also influence feed intake and weight gain in lambs. However, IgM, CD4 and total serum protein among the treatments differed significantly throughout the study. The lambs receiving DFM supplementation generally recorded numerically higher performance values especially lambs under treatment 4 (*Paenibacillus polymyxa based-DFM*) in respect to intake and weight gain.

The results of this study implies that, the DFM treatments have the potential of improving growth performance and lamb immunity. However, the mode of administration and inclusion rate can be altered to ascertain the optimum methods and levels of inclusion that would help improve our small ruminant industry.

5.2 Recommendations

It is recommended that further studies be done using these 3 DFM products without

dilution at the suckling since the dilution reduces the concentration of the DFM and if possible to the dams as well.

REFERENCES

Abdelrahman, M. M. and Hunaiti D. A. (2008). The effects of dietary yeast and protected methionine on performance and trace minerals status of growing Awassi lambs. *Livest. Science*; **115**: 235-241.

Abd El-Ghani, A.A. (2004). Influence of diet supplementation with yeast (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. *Small Rumin. Res.*; **52**: 223229.

Abe, F., Ishibashi, N. and Shimamura, S. (1995). Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *J. Diary Sci*.; **78**: 2838-2846.

Aboderin, F.I. and Oyetayo, V.O. (2006). Haematological studies of rats fed different doses of probiotic *Lactobacillus plantarum,* isolated from fermenting corn slurry. *Pakistan. J. Nutr*; **5**:102–105.

Abu-Tarboush, H.M., Al-saiady, M. Y. and Kier El-Din, A. H. (1996). Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young diary animals. *Feed Sci. Technology*; **57**:39-49.

Adams, M. C., Luo, J. Rayward, D. King, S., Gibson, R. and Moghaddam, S. (2008). Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. *Anim. Feed Sci. Technol*.; **145**:41-52.

Al-Saiady, M.Y. (2010). Effect of probiotic bacteria on immunoglobulin G concentration and other blood components of Newborn calves. *J. of Anim. and Vet. Adv*.; **9**(3):604-609.

Amabile-Cuevas, T., Cardenas-Garcia C.M. and Ludgar, M. (1995). Antibiotic resistance. *Anim Sci*.; **83**:320-329.

Amoah, K. O. (2010). Effects of RE-3, a direct-fed microbial (DFM) product on the growth performance, blood profile and carcass characteristics of pigs. MSc. Thesis, KNUST, Kumasi. p104

Anderson, D. B., McCracken, V. J., Aminov, R. I., Simpson, J.M., Mackie, R. I., Verstegen, M. W. A. and Gaskins, H.R. (1999). Gut microbiology and growth- promoting antibiotics in swine. *Pig News Inform.*; **20**(4):115N–122N.

Antunovic , Z., Speranda, M, Amidzic, D., Seric, V., Steiner, Z., DomaCinovic, N. and Boli, F. (2006). Probiotic application in lamb nutrition. *Krmiva*; **48**(4). 175-180.

Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Wells, S. D., Shackelfold, J. E., Wheeler T. L. and Koohmaraie, M. (2010). Evaluation of a direct-fed microbial product effect on the prevalence and load of Escherichia coli o157:H7 in feedlot cattle. *J. Food Prot.*; **73**:366-371.

Baker A.A, Davis E, Spencer J.D, Moser R, Rehberger T (2013). The effect of a Bacillusbased direct-fed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. *J. Anim. Sci*., **91**:3390-3399.

Baranowski, A., Gabryszuk, M., Jozwik, A., Bernatowicz, E. and Chylinski, W. (2007). Fattening performance, slaughter indicators meat chemical compositions in lambs fed the diet supplemented with linseed and mineral bioplex. *Anim. Sci. Papers and Rep.;* **25**(1):25-44.

Bechman, T. J., Chambers J. V. and Cunningham, M. D. (1977). Influence of *Lactobacillus acidophilus* on performance of young diary calves. *J. Diary Sci*.; **60**:74.

Belewu, M.A, Yahaya, A.A, and Adeyina A.O (2008). Study on some haematological parameters of goats fed aspergillus treated and untreated shea-butter cake. *Rev. J. Anim. Sci*.; **5**: 154-156.

Benko, R., Matuz, M. Viola, R., Doro P., Hadju, E. and Soos, G. (2008). Quantitative disparities in outpatient antibiotic exposure in a Hungarian county. *J. Antimicrib. Chemother*.; **62**(6):1448-1450.

Bonsu, F. (2009). The effects of DFM on health and growth performance of poultry in a hot humid environment. MSc thesis, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology. Kumasi, Ghana, **126** .

Brashears, M. M., Amezquita, A. and Jaroni, D. (2005). Lactic acid bacteria and their uses in animal feeding to improve food safety. *Adv. Food Nutr. Res*., **50**: 1-31

Brown I., Warhurst, M., Arcot, J., Playne, M., Illman, R.J. and Topping, D.L. (1997). Fecal numbers of bifidobacteria are higher in pigs fed *Bifidobacterium longum* with a high amylose cornstarch than with a low amylose cornstarch. *J. Nutr*., **127**: 1822-1827.

Castanon J. I. R. (2007). Review: history of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci*. **86**, 2466–2471 10.3382/ps.2007-00249

Cetin N., Guclu B. K. and Cetin E., (2005).The effects of probiotics and mannanoligo saccharide on some haematological and immunological parameters in Turkeys. *J. Vet. Med. A*.; **52**:263-267.

Charray, J., Humbert, J.M. and Levif, J. (1992) Manual of Sheep Production in the Humid Tropics of Africa. *CAB International Wallingford*, Oxon.

Chaucheyras. F., Fonly, G. and Durand, H. (2000). Effect of live yeast supplement on microbial growth of the rumen of newborn lambs. *Reprod. Nutr. Dev*.; **40**: 419 (abstract).

Chee-Sanford, J. C., Aminov, R. I., Krapac I. J., Garrigues-Jeanjean N. and Mackie R. I. (2001). *Appl. Environ. Microbiology*, **201**:1494-1502.

Chichlowski, M., Croom, J., McBride, B.W., Daniel, L., Davis, G., and Koci, M.D. (2007). Direct-Fed Microbial Primalac and Salinomycin modulate whole-body and integral oxygen consumption and intestinal mucosal cytokine production in the broiler chick. *Poult. Sci***; 86:**1100-1106.

Chiofalo, V., Liotta, L. and Chiofalo, B. (2004). Effects of the administration of lactobacilli on the body growth and on the metabolic profile growing Maltese goat kids. *Reprod*. *Nutr*. *Dev*.; **44**:449-457.

Choudhari, A., Shinde, S. and Ramteke, B. N. (2008). Prebiotics and probiotics as health promoter. *Vet. World*; **2**: 59-61.

Christen, S. D., Wardwell, K., Hill, T. M. and Roth, L. D. (1995). The effect of feeding direct fed microbials on the performance of pre-weaned Holstein calves. *J. Anim. Sci*.; **73**(Suppl. 1):249 -250

Collins, M. D., and Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr*.; **69** (Suppl. 1):1052S

Cromwell, G. L (2002). Why and how antibiotics are used in swine production. *Animal Biotechnology*; **13**:7-27.

Cromwell, G. L. (1991). Anitmicrobial Agents. In: Swine Nutrition. (Miller, E. R., D. Ullrey, E. and Lewis, A. J., eds.). Butterworth-Heinemann, Boston, p. 297-314.

Dalloul, R. A., Lillehoj H. S., Shellem, T. A. and Doerr, J. A. (2003). Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotics. *Poult*. *Sci*.; **82**:62-66.

Davis, P. (1976). Single cell protein. 2nd Ed. Academic press. *New York, USA.*

Desnoyers, M. S., Giger-Reverdin, Bertin, G., Duvaux-Ponter, C. and Sauvant, D. (2009). Meta-analysis of influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. *J. Dairy Sci*.; **92**: 1620-1632.

Danne, C., O'Mahony, L., Murphy, L., O'Halloran, S. O., Fecney, M., Flynn, S., Fitzgerald Daly, C., Krely, B., O'Sullivan, G., Shanahan, F., and Collins, J. K. (1999). Probiotcs; from myth to reality-Demonstration of functionality in animal model of disease and in human clinical trials. *Antonie Van Leeuwenhoek*; **76**:279-292.

Devendra, C. (1988). General approaches to animal nutrition research and their relevance to fish production in the Asian Region. In: DeSilva, S.S., (Ed.), *Fin fish Nutrition Research in Asia*. Heinemannn Asia Singapore, Singapore, p: 724.

Devendra, C, (1980). Milk production in goats compared to buffalo and cattle in the humid tropics. *Journal of Dairy Science*; **63**:1955-1767.

Dick, K. J., Duff, G.C., Pas, S. W., Limesand, Cuneo S. P., Knudson, D.K., McMurphy, C.P. , Hall, L.W , Bernal-Rigoli, J.C. and Marchello J. A. (2013). Effects of a direct-fed microbial on digestive-tract morphology of Holstein bull calves and performance and carcass characteristics of Holstein steers. *The Professional Animal Scientist*; **29**:107–115

Doyle, M.E. (2001). Alternatives to antibiotic use for growth promotion in animal husbandry. *Food Research Institute*, University of Wisconsin. Madison. pp1-17.

Drew, M. D., Syed, N. A., Goldade, B. G., Laarveld, B. and van Kessel, A. G. (2004). Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. *Poult*. *Sci*., **83**: 414–420 10.1093/ps/83.3.414

Dritz, S.S., Tokach, M.D., Goodband, R.D. and Nelssen, J.L. (2002). Effects of administration of antimicrobials in feed on growth rate and feed efficiency of pigs in multisite production systems. *JAVMA*, **220**:1690–1695

European Food Safety Authority (EFSA) (2003). Feed additives-breaking legislation in EU. *Veterinary Sci*. *Tomorrow*, **23**: 81-94.

Elam, N. A., Gleghorn, J. F., Rivera, J. D., Galyean, M. L., Defoor, P. J., Brashears, M. M. and Younts-Dahl, S. M. (2003). Effects of live cultures of *Lactobacillus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass and intestinal Characteristics and *Escherichia coli* strain O157 shedding of finishing beef steers. *J. Anim Sci*.; **81**:2686-2698.

Ellinger, D. K., Muller, L. D. and Glantz, P. J. (1980). Influence of feeding fermented colostrum and Lactobacillus acidophilus on fecal flora of dairy calves. *J. Dairy Sci.;* **63**:478-482.

Ellinger, D. K., Muller, L.D. and Glantz, P. J. (1978). Influence of feeding fermented colostrums and *Lactobacillus acidophilus* on fecal and selected blood parameters of young dairy calves. *J. Dairy Sci*.; **61**:126.

Emmanuel, D. G. V., Jafari, A., Beauchemin, K. A., Leedle, J. A. Z. and Ametaj, B. N. (2007). Feeding live cultures of Enterococcus faecium and Saccharomyces cerevisiae induces an inflammatory response in feedlot steers. *J. Anim. Sci.;* **85**: 233-239.

Emanuelle, S. M., Horon, G. M. J., Baldwin, J., Lee, D., Mahana, H. (1993). Effect of microbial inoculants on quality of alfalfa hay baled at high moisture and lamb performance. *J. Dairy Sci*.; **75**:3084-3090.

Erasmus, L.J., Botha, P.M., and Kistner, A. (1992). Effect of yeast culture supplement on production, rumen fermentation and duodenal nitrogen flow in dairy cows. *J. Dairy Sci*., **75**: 3056-3065.

Erickson, K. L. and Hubbard, N. E. (2000). Probiotic immunomodulation in health and disease. *Amer. Soc. Nutr. Sci*., pp403-490

Estrada, A., Drew, M. D. and Van Kessel, A. G. (2001). Effect of dietary supplementation of fructo-oligosaccharides and *Bifidobacterium longum* to early weaned pigs on performance and fecal bacterial populations. *Can. J. Anim. Sci*.; **81**:141–148.

FAO. (2012). *Crop residue based densified total mixed ration – A user-friendly approach to utilise food crop by-products for ruminant production*, by T.K. Walli, M.R. Garg & Harinder P.S. Makkar. FAO *Animal Production and Health Paper* No. **172**. *Rome, Italy.*

FAO/WHO. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a Joint FAO/WHO Expert Consultation

on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Córdoba, Argentina**,** pp1-33.

Flint, J.F. and Garner, M. R. (2009). Feeding beneficial bacteria: A natural solution for increasing efficiency and decreasing pathogens in animal agriculture. *J. Appl. Poult. Res*.; **18**: 367-378

Friend, B. A. and Shalani, K. M. (1984). Nutritional and therapeutic aspects of lactobacilli. *J. Appl*. *Nutr*; **36**:125-53.

Fujiwara, K, Yamazaki, M, Abe, H, Nakashima, K, Yakabe, Y, Otuska, M, Ohbayashi, Y, Kato, Y, Namai, K, Toyoda, A, Miyaguchi, Y, and Nakamura, Y, (2009). Effect of Bacillus subtilisvar. natto fermented soybean on growth performance, microbial activity in the cacca and cytokine gene expression of domestic meat type chickens. *J. Poult*.; *Sci*., **46**: 116-122.

Fuller, R. (1999). Probiotics for farm animals: In Probiotics A Critical Review. G.W. Tannocka (ed) *Horizon Scientific Press, Wymondham, England*. p15**.**

Fuller, R. (1989). Probiotics in man and animals. *J. Appl. Bacterriol*.; **66**:365-378.

Galip, N. (2006). Effect of supplemental yeast culture and sodium bicarbonate on ruminal fermentation and blood variables in rams. J. Anim. Phys. Anim. Nutri.; 90:446-452. Gaskins, H. R., Collier, C. T. and Anderson, D. B. (2002). Antibiotics as growth promotants: Mode of action. *Anim*. *Biotechnol*.; **13**: 29-42.

Ghorbani, G.R., Morgavi, D. P., Beauchemin, K. A., and Leedle, J.A.Z. (2002). Effects of bacterial direct fed microbials on ruminal fermentation, blood variables and the microbial population of feedlot cattle. *J. Anim Sci*.; **80**:1977-1985.

Gibson, G.R. and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr*.; **125**:1401-1412.

Giger-Reverdin, S., Morand-Fehr, P., and Tran, G. (2003). Literature survey of the influence of dietary fat composition on methane production in dairy cattle. *Livest. Prod. Sci.*; **82**: 73–79

Goldin, B. and Gorbach, M. (1980). Effect of Lactobacillus acidophilus dietary supplements on 1, 2 –di methylhydrazine di hydrochloride induced intestinal cancer in rats. *J. Natl. Cancer Inst.*; **64**: 263-265.

Granstrom, M., Holmet, T., Sjogrent, A. M., Ortqvists, A. and Kalin, M. (1994). The role of IgA determination by ELISA in the early serodiagnosis of *Mycoplasma pneumoniae* infection, in relation to IgG and p-capture IgM methods. *Journal of Medical Microbiology*, **40**: 288-292

Haddad, S. G. and Goussous, S. N. (2005). Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. *Animal Feed Sci. Tech.*; **118**:343-348.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Zhou, H., Sanei, B., Chambers, J.R. and Sharif, S. (2006). Probiotics stimulate production of natural antibodies in chickens. *Am. Soc. Microbiol.*; **13**:975-980.

Harper, A. F., Kornegay, E. T., and Schell, T. C. (1997). Phytase supplementation of lowphosphorus growing-finishing pigs' diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. *J. Anim.Sci.*; **75**: 3174-3186.

Henderson, A.R., Scale D.R., Anderson D.H. and Heron, A. (1986). The effect of formic acid and bacterial inoculants on the fermentation and nutritive value of perennial dry grass silages. *Proceedings of the Eurobac Conference, 12-16 Uppsala, Sweden*. pp93-98.

Hernandez, R., Gonzalez, S.S., Pinos-Rodrigues, J.M., Ortega, M.A., Hernandez, A., Bueno, G. and Cobos, M. (2009). Effect of yeast culture on nitrogen balance and digestion in lambs fed early, and mature orchard grass. *J. Appl. Anim. Res.*; **32**: 53-56.

Herr, C. T., Weber, T. E. and Richert, B.T. (2000). Effect of nutritional level while feeding Paylean TM to late-finishing swine. Available at: *www.purdue.edu/swine/ swineday 2000/htr.*

Higgins, S.E., Erf, G.F., Higgins, J.P., Henderson, S.N., Wolfenden, A.D., GaonaRamirez, G. and Hargis, B.M. (2007). Effects of Probiotics treatments in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella Enteridis* by sabdominal exudates cells. *Poultry Science*; **86**: 2315-2321.

Holm, A. (1996). Zinc oxide in treating *E. coli* diarrhea in pigs after weaning. *Compend. Contin Ed Practic Vet*.; **18**(1 Suppl S):S 26 ff.

Huffman, R. P., Karges, K. K., Klopfenstein, T. J., Stock, R. A., Britton, R. A., and Roth, L. D. (1992). The effect of *Lactobacillus acidophilus* on subacute ruminal acidosis. *J. Anim. Sci*.; **70**(Suppl. 1):87(Abstr.)

Isolauri, E., Salminen, S. and Ouwehand, A.C. (2004). Probiotics. *Best Pract. Res. Clin. Gastroenterol*; **18**:299-313.

Isolauri, E., Sutas Y., Kankaanpaa, P., Arvilommi, H. and Salminen, S. (2001). Probiotics: Effects on immunity. *Am. J. Clin. Nutr*., **73** (Suppl. 2) : 444- 450

Isolauri, E., Kaila, M., Mykkanen, H., Ling, W. and Salminen, S. (1994). Oral Bacterio therapy for viral gastroenteritis. *Dig. Dis. Sci*., **39:** 2595-2600**.**

Jacela, J.Y, DeRouchey, J.M, Tokach, M.D, Goodband, R.D, Nelssen, J. L, Renter, D.G and Dritz, S.S. (2009). Feed additives for swine: Fact sheets – acidifiers and antibiotics. *J Swine Health Prod*.; **17**(5):270–275.

Jang, D.Y., Oh, H., Kyong Piao, L., GuoChoi, H., BongYun, J., Hyeon, K. and Yong Y. (2009). Evaluation of probiotics as an alternative to antibiotics on growth performance, nutrient digestibility, occurrence of diarrhea and immune response in weaning pigs. *J. Anim. Sci. and Tech*.; **51** (1) 1-10 .

Jenny, B.F., Vandijk, H.J. and Collins, J.A. (1991). Performance and fecal flora for calves fed *Bacillus subtilis* concentration. *J. Dairy Sci*., **74**:1968-1973.

Jost, M. and Bracher, J.A. (1999). The effect of Sanobiotic "RS", an active Probiotic growth promoter, in reading piglets. Rev. *Sui d"Agric*.; **31**(1):43-48.

Jouany, J.P. Mathieu, F., Senaud, J., Bohatier, J., Bertin, G. and Mercier, G. (1999). Influence of protozoa and fungal activities on ruminal pH and redox potential. *S. Afr. J. Anim. Sci.*; **29**:65-66.

Keyser, S.A., McMeniman, J.P., Smith, D.R., MacDonald, J.C. and Galyean, M.L. (2007). Effects of *Saccharomyces cerevisiae* subspecies boulardiicncm i-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of new received beef heifers. *J. Anim. Sci*.; **85**:1264-1273.

Kellems, O.R and Church, D.C. (2002). Livestock Feeds and Feeding (5th ed.). *PrenticeHall, New Jersey;* **39**-248.

Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Can Med Assoc J*.; **159**:1129–1136.

Khaled, N. F. and Baraka, T. A. (2011). Influence of TOMOKO® (Direct-Fed Microbials) on productive performance, selected rumen and blood constituents in Barky finishing lambs. *J. Am Sci*; **7**(9): 564-570].

Kiesling, H.E., Lofgreen, G.P. and Thomas, J.D. (1982). A viable lactobacillus culture for feedlot cattle. *Proc. Western Sect. Am. Soc. M. Sci*.; **33**:53-56.

Krehbiel, C.R., Rust, S.R., Zhang, G. and Gililand, S.E. (2003). Bacterial direct-fed microbials in ruminant diets. Performance response and mode of action. *J. Anim. Sci*., **81**:120-32.

Kogut, M. H., Genovese, K. J., He, H., Swaggerty, C. L. and Jiang Y. (2013). Modulation of chicken intestinal immune gene expression by small cationic peptides as feed additives during the first week posthatch. *Clin. Vaccine Immunol*.; **20**:1440–1448 doi10.1128/CVI.00322-13

Kyriakis, S.C., Tsiloyiannis, V.K., Vlemmas, J., Sarris, K., Tsinas, A.C., Alexopoulos, C. and Jansegers, L. (1999). The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. *Res Vet Sci*., **67**(3):223–228.

Lal, S. and Tabacchioni, S. (2009). Ecology and biotechnology potential of *Paenibacillus polymyxa*; a mini review. *Indian Journal of Microbiology*, **49**(1):2-10.

Lantei, N. (2008). The effect of DFM and Mazorite on growth performance and carcass characteristics of growing pigs. B.Sc. dissertation, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, p56.

Lee, K.W., Lee, S.H., Lillehoj, H.S., Li, G.X., Jang, S.I., Babu, U.S., Park, M.S., Kim, D.K., Lillehoj, E.P., Neumann, T.G., Rehberger, T.G., and Siragusa, G.R. (2010). Effects of direct-fed microbials on growth performance gut morphometry and immune characteristics in broiler chickens. *Poul Sci;* **89**:203-216.

Lee, S.S., Hal, J.K., and Cheng, K.J. (2000). Influence of an anaerobic fungal culture administration on in vivo ruminal fermentation and nutrient digestion. *Animal feed science and Technology*; **88**:201-217.

Lema, M., Williams, L. and Rao, D.R. (2001). Reduction of fecal shedding of enterohemorrhagic Escherichia coli O157 H7 in lambs by feeding microbial feed supplement. *Small Rumin. Res*.; **39**:31-39.

Leong, A. S-Y, Cooper, K. and Leong, F. J. W-M. (2003). *Manual of Diagnostic Cytology* (2 ed.). *Greenwich Medical Media, Ltd*. **63**–64. ISBN 1-84110-100-1.

LillehoJ, H.S., Min, W. and Dallou, R.A. (2004). Recent progress on the cytokine regulation of intestinal immune responses to Eimiera. *Poult. Sci*; **83**:611-623.

Lewis, A.R. (2002). CRC Dictionary of Agricultural Sciences. *CRC Press. Boca Raton*, **630**.3 Ref Lew 012717. U.K. p 675.

Lievin-Le Moal, V. and Servin, A. L. (2006). The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial preptides, and microbiotaclin. *Microbiol. Rev*.; **19**:315-337

Liu, Z., Jiu, J., Liu, S., Fa, X., Li, F. and Du, Y. (2007) Blockage of tumor necrosis factor prevents intestinal mucosal inflammation through down- regulation of interleukin-23 secretion. *J Autoimmunl*; **29**: 187–194

Lubbadeh, W.M., Haddadin, M., Al-Tamini, A. and Robinson, R.K. (1999). Effects on cholesterol content of fresh lamb of supplementing the feed of Awassi ewes and lambs with Lactobacillus acidophilus. *Meat Sci*., **52**: 381-623.

Luckheeram, R.V., Zhou, R., Verma, A.D. and Xia, D. (2012). CD4+T Cells: Differentiation and Functions. *Clinical and Developmental Immunology*, Article ID 925135, **12** pages. http/dx/doi.org:10.1155/2012/925135

Madu, C.T., Sogbesan, O.A. and Ibiyo, L.M.O. (2003). Some Non- Conventional Fish Feed Resources in Nigeria. In: Eyo, A.A., (Ed.), Proceeding of the Joint Fisheries Society of Nigeria/National Institute for Freshwater Fisheries Research/FAO National Special Programme for Food Security National Workshop on Fish Feed Development and Feeding Practices in Aquaculture Held at National Institute for Freshwater Fisheries Research, 15th 19th Sept, *New Bussa*, p: 7382.

Majamaa, H., Isolauri, E., Salexin, M. and Vesikari, T. (1995). Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J. Pediatr. Gastroenterol. Nutr*.; **20**: 333-338

Martins, F.S., Nardi, R.M., Arantes, R.M., Rosa, C.A., Neves, M.J., and Nicoli, J.R. (2005). Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. *J. Gen. Appl. Microbiol.;* **51**: 83-92.

Mathews, K. H. Jr and Johnson, R. J. (2013). Alternative beef production systems: Issues and implications. *A report from the Economic Research Service. USDA LDPM* **281**-01. (available at www.ers.usda.gov) accessed on 21/6/15

McAllister, T.A., Beauchemin, K.A., Akzzeh, A.Y., Baah, J., Teather, R.M. and Stanford, K. (2011). Review. The use of direct fed microbials to mitigate pathogen and enhance production in cattle. *Can. J. of Anim. Sci*.; **91**(2): 193-211.

McDonald, R.D., Edwards R.A., and Greenhalgh, J.F.D. (1992). Animal Nutrition (4th Ed). *Longman, London and N.Y*. p 300

Mellon, M., Benbrook, C. and Benbrook, K. L. (2001). Hogging It: Estimates of Antimicrobial Abuse in Livestock (*Union of Concerned Scientists, Cambridge, MA*).

Miles, R. D., and Bootwalla S. M. (1991). Direct-fed microbials in avian. In Direct-Fed Microbials in Animal Production: A Review of Literature. *Nat. Feed Ingred. Assoc., West Des Moines, IA*. p 117

Malik, R. and Bandla, S. (2010). Effects of source and dose of probiotics and exogenous fibrolytic enzymes (EFE) on intake, feed efficiency and growth of male buffalo (bubalusbubalis) calves. *Trop. Anim. Health Prod*; **42**: 1263-1269.

Miron, J., Ben- Ghedalia, D. and Morrison, M. (2001). Invited review: adhesion mechanisms of rumen cellulolytic bacteria. *J. Dairy Sci*.; **84**: 1294-1309

Morrill, J. L., Dayton, A.D., and Mickelson, R. (1977). Cultured milk and antibiotics for young calves. *J. Dairy Sci*.; **60**:1105-1109.

Musa, H.H., We, S.L., Zhu, C.H, Seri, H.I. and Zhu, G.Q. (2009). The potential benefits of probiotics in animal production and health. *J. Anim. Vet Adv*.; **8**:313-321.

Nakanishi, Y., Arave, C. W. and Stewart, P. H. (1993). Effects of feeding Lactobacillus acidophilus yogurt on performance and behaviour of dairy calves. *J. Dairy Sci.;* **76**(Suppl. 1): 244.

Nordqvist, C. (2013). "What is bacteria? What are bacteria?." *Medical News Today*. Retrieved from http://www.medicalnewstoday.com/articles/157973.php. *Accessed 4th Nov. 2013*

Novak, K., Davis, E., Bos, K, Rehberger, T. and Kromm, C. (2007). Effect of oral administration of *Lactobacillus brevis* on turkey poult performance and immune development. *Poult. Sci*.; **86**(Suppl.) l: 248. (Abstr.)

Officer, D.I. (1995). Effect of multi-enzyme supplements on the growth performance of piglets during the pre- and post-weaning periods. *Anim Feed Sci Technol*.; **56**(1–2):55–65.

Okai, D. B and Boateng, M. (2007). Pig Nutrition Research in Ghana – Some Achievements, Prospects and Challenges. *Gh. J. Anim. Sci*.; **2-** 3: 19-25.

Otchere, A. O. (1986). Small Ruminant production In Tropical Africa. *FAO Animal Production and Health paper*; **58**: 203-210

Owen, F. G. and Larson, L. L. (1984). Effect of probiocin and starter preparations on calf performance. *J. Dairy Sci*.; **67**: 104-106

Oyetayo, V. O. and Oyetayo, F. L. (2005). Potential of probiotics as biotherapeutic agents targeting the innate immune system. *Afr. J. Biotech*., **4**(2): 123-127.

Partanen, K. H. and Mroz, Z. (1999). Organic acids for performance enhancement in pig diets. *Nutr Res Rev*.; **12**(1):117–145.

Partridge, G. and Hazzledine, M. (1997). The influence of feed enzymes on digestion disorders in swine. In: *Proceedings of the 28th Annual Meeting of the American Society of Swine Practitioners. Washington, USA*. pp183-193.

Paryad, A. and Mahmoudi, M. (2008). Effect of different levels of supplemental yeast (S. cerevisiae) on performance, blood constituents and carcass characteristics of broiler chicks. *African Journal of Agric. Research*.; **3**(12) 835-842.

Patterson, J.A and Burkholder, K.M. (2003). Prebiotic feed additives: rationale and use in pigs. Proceedings of the 9th International Symposium on Digestive Physiology in Pigs. *University of Alberta, Canada*; **9**: 319-331.

Patzer, S. I. Baquero, M. R., Bravo, D., Moreno, F. and Hantke, K. (2003). The colicin G. H and X determinants encode microcins M and H47, which might utilize the catecholate Siderohore receptors FepA, Cir, Fiu and Iron. *Microbiology*; **149**: 2557-2570

Peterson, R. E., Klopfenstein, T. J., Erickson, G. E., Folmer, J., Hinkley, T., Moxely, R. A. and Smith, D. R. (2007). Effects of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *J. Food Port*.; **70**:287-291.

Preston T. R. (2007). Better utilization of crop residues and by-products in animal feeding: research guidelines 2. A practical manual for research workers. FAO Animal production and health paper 50/2
http://www.fao.org/DOCREP/003/X6554E/X6554E00.htm#TOC (5TH Nov. 2014)

Preston T. R. and Leng R. A. (1984). Supplementation of diets based on fibrous residues and by -products. In: Sundstol F and Owen E (eds). Straw and other fibrous by products as feed. *Elsevier Press, Amsterdam, the Netherlands*. p. 373-413.

Pond, K. R. and Goode, L. (1985). The evaluation of Probios for weaned stressed lambs. *Microbial Genetics Division, Pioneer Hi-Bred International, Des Moines, Iowa.*

Pridmore, R. D. Pittet, A. C., Praplan, F. and Cavadini, C. (2008). Hydrogen peroxide production by Lactobacillus Johnson NCC 533 and its role in anti-Salmonella activity. *FEMS Microbial. Lett*; **283**: 210-215

Quigley, E.M. (2012). Prebiotics and probiotics: their role in the management of gastrointestinal disorders in adults. *Nutr Clin Pract*., **27**:195–200

Quintero-Gonzalez, C. I., Comerford, J. W., and Varga, G. A. (2003). Effects of directfed microbial on growth, health and blood parameters of young Holstein calves. *The Professional Animal Scientist*; **19**:211-220.

Radcliffe, J.S., Zhang, Z. and Kornegay, E.T. (1998). The effects of microbial phytase, citric acid, and their interaction in a corn soy bean meal-based diet for weanling pigs. *J Anim Sci.*; **7**:1880–1886

Rao, S. O. (2007). The effect of dietary supplementation of *Lactobacillus*-based probiotics on growth and gut environment of nursery pigs. M.Sc. thesis submitted to the Graduate School of Texas Tech University, *USA*; p 1-7.

Robinson, P. H. and Erasmus, L. J. (2009). Effects of analyzable diet components on responses of lactating dairy cows to *Saccharomyces cerevisiae* based yeast products: systematic review of the literature. *Anim. Feed Sci. Technology*; **149**: 185-198

Robinson, P.H. (2002). Yeast products for growing and lactating dairy cattle: Impact on rumen fermentation and performance. *Dairy Rev.*; **9**:1-4.

Rolfe, R. D. (2000). The role of probiotics cultures in the control of gastrointestinal health. *J. Nutr.*; **130**(2):396-402.

Roth, F.X. and Kirchgessner ,M. (1993). Influence of avilamycin and tylosin on retention and excretion of nitrogen in growing pigs. *J Animal Physiol Anim Nutr.*; **69**(4):175–185.

Sandine, W. E. (1979). Role of *Lactobacillus* in the intestinal tract. *J. Food Prot.*; **42**:259262.

Santos, A. A., Ferket, P. R., Grimes, J. L. and Santos, F. B. O. (2005). Reduction of intestinal Salmonella spp. colonization in turkeys by dietary wheat, triticale and enzyme supplementation. *Southern Poultry Science Society 25th Annual Meeting, Atlanta, GA.* p **132-**141.

Sarker, M. K., Lee, S. M., Kim, G. M., Choi, J. K. and Yang, C. J. (2010). Effects of different feed additives on growth performance and blood profiles of Korean Hanwoo calves. *Asian-Aust. J. Anim. Sci*., **23**:52-60.

Sayed, A.S. (2003). Studies on the influence of pronifer as a probiotics on the clinical, hematological and biochemical status of the goat`s kids. *Assiut. Vet. Med. J*.; **99**: 131-143.

Scharek, L., Guth, J., Reiter, K., Weyrauch, K. D., Taras, D., Schwerk, P., Schierack, P., Schmidt, M. F.G., Wieler, L. H. and Tedin, K. (2005). Influence of a probiotics *Enterococcus faecium* strain on development of the immune system of sows and piglets. *Vet. Immunol. Immunopathol*.; **105**:151-161.

Schell, T.C., Lindemann, M.D., Kornegay, E.T. and Blodgett, D.J. (1993). Effects of different types of clay for reducing the detrimental effects of aflatoxin-contained diets on performance and serum profiles of weanling pigs. *J. Anim Sci*.; **71**:1226–1231.

Schwab, C. G., Moore, J. J., Hoyt, P. M. and Prentice, J. L. (1980). Performance and fecal flora of calves fed nonviable *Lactobacillus bulgaricus* fermentation product. *J. Dairy Sci.*; **63**:1412.

Servin A. L., (2004). Antagonistic activities of Lactobacilli and Bifidobacteria against microbial pathogens. *FEMS Microbiol. Rev*., **28**:405-440.

Shareef, A. M. and Al-Dabbagh, A. S. A. (2009). Effects of probiotics (*Saccharomyces cerevisiae*) on performance of broiler Chicks. *Iraqi J. of Vet. Sci*.; **23**:23-29.

Siljander-Rasi, H., Alaviuhkola, T., and Suomi, K. (1998). Carbadox, formic acid and potato fibre as feed additives for growing pigs. *J Anim Feed Sci*., **7** (Suppl 1):205- 209.

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K. and Morris, G. J. Jr. (2002) .Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria *PNAS. vol*. **99** (9): 6434–6439

Strzetelski, J., Kowalczyk, J. and Krawczyk, K. (1998). Effect of various probiotics on calf performance. *J. Anim. Feed Sci*.; **7**(Suppl.1), 241-244

Swinney-Floyd, D., Gardner, B. A., Owens, F. N., Rehberger, T. and Parrot, T. (1999). Effect of inoculation with either *Propionibacterium strain* P-63 alone or combined with

Lactobacillus acidophilus strain LA53545 on performance of feedlot cattle. *J. Anim. Sci.;* **77**(1):77.

Tabe, E. S., Oloya, J., Doetkott, D. K., Bauer, M. L., Gibbs, P. S. and Khaitsa, M. L. (2008). Comparative effects of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and *Salmonella* in naturally infected feedlot cattle. *J. Food Prot*.; **71**:539-544.

Thacker P. A. (2013). Alternatives to antibiotics as growth promoters for use in swine production: a review *J. Anim Sci Biotechnol*., **4**(1): 35. doi: 10.1186/2049-1891-4-35

Theodorou, M. K., Beever, D. E., Haines, M. J. and Brooks, A. (1990). The effect of fungal probiotic on intake and performance of early weaned calves. *Anim. Prod*.; **53**:577583.

Tien, M. T., Girardin, S. E., Regnault, B., Bourhis, Le L., Dillies, M. A., Coppee, J. Y., Bourdet-Sicard, R., Sansonetti, P. J. and Pedron, T. (2006). Anti-inflammatory effect of *Lactobacillus casei* on *Shigella*-infected human intestinal epithelial cells. *J. Immunol.*; **176**:1228-1237.

Timmusk, S. N., Grantcharova, E., Gerhart, A. and Wagner H. (2005). *Paenibacillus polymyxa* Invades Plant Roots and Forms Biofilms. *Applied and Environmental Microbiology*; **71**(11):7292-7300.

Tisch, D. (2006). Animal feeds, feeding and nutrition, and ration evaluation. *Clifton Park, NY: Thomson Delmar Learning (SF95 .T527),* p 424.

Titi, H. H., Dmour, R. O., and Abdullah, O. (2008). Growth performance and carcass characteristics of Awassi lambs and Shami goat kid culture in their finishing diet. *J. Anim. Sci.*; **142**:1-2.

Trckova, M., Matlova, L., Dvorska, L. and Pavlik, I. (2004). Kaolin, bentonite, and zeolites as feed supplements for animals: health advantages and risks. A review. *Veterinarni Medicina-Czech*; **49**(10): 389-399.

Umberger, S. H. and Notter, D. R. (1989). Evaluation of Lactobacillus inoculants on feedlot lamb performance. *J. Anim. Sci*.; **8**: 40-54.

Van der Fels-Klerx, H.J., Puister-Jansen, L.F., Van Asseltl, E.D. and Burgers, S.L. (2011). Farm factors associated with the use of antibiotics in pig production. *J Anim Sci*., 89:1922–1929. doi: 10.2527/jas.2010-3046

VonBuenau, R., Jaekel, L., Schubotz, E., Schwarz, S., Stroff, T., and Krueger, M. (2005). *Escherichia coli strain nissle* 1917: Significant reduction of neonatal calf diarrhea. *J. Dairy Sci*.; **88**:317-323.

Vondruskova, H., Slamova, R., Trckova, M., Zraly, Z. and Pavlik, I. (2010). Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: A review. *Veterinarni Medicina*; **55** (5) 199–224

Wallace, R. J., and Newbold, C. J. (1993). Rumen fermentation and its manipulation. The development of yeast culture as feed additives. I Biotechnology in the Feed Industry, Lyons, T. P. (ed). *Alltech Technical Publications*, Kentucky, ; pp173-192.

Whitley, N. C., Cazac, D., Rude, B. J., Jackson-O'Brien, D. and Parveen, S. (2009). Use of commercial probiotics supplement in meat goat. *J. Anim. Sci*.; **87**:723-728.

Wierup, M. (2000). The control of microbial diseases in animals, alternatives to the use of antibiotics. *Int. J. Antimicrob. Agents*; **14**: 315-319.

Wilson, R.T. (1982). Small ruminant breed productivity in Africa. (Ed. R.M. Gaten by and J.C.M. Trail ILCA) Addis Ababa, Ethiopia

Younts-Dahl, S. M., Osborn, G. D., Galyean, M. L., Rivera, J. D., Loneragan, G. H., and Brashears M. M. (2005). Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbial. *J. Food Prot*.; **68**:6-10.

Zhang, W., Azevedo, M. S. P., Gonzalez, A. M., Saif, L. J., Van Nuyen, T., Wen, K., Yousef, A. E. and Yuan, L. (2008). Influence of probiotics Lactobacilli colonization on

neonatal B cell responses in a gnotobiotic pig modal of human rotavirus infection and disease. *Vet. Immunopathol*.; **122**:175-181.

Zimmerman, D.R. (1986). Role of sub-therapeutic antimicrobials in pig production. *J. Anim. Sci*.; **62**(Suppl. 3):6-17.

APPENDICES

APPENDIX 1: ANALYSIS OF VARIANCE (ANOVA) TABLES FOR GROWTH PERFORMANCE OF LAMBS (STARTER PHASE)

Variable: Initial weight

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	3. 596	1.199	0.94	0.443
Error	17	21. 676	1.275		
Corrected total	20	25. 272			

Variable: final weight gain

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	13.463	4.488	0.79	0.514
Error	17	96.130	5.655		
Corrected total	20	109.592			

Variable: weight gain

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	4.045	1.348	0.64	0.599
Error	17	35.762	2.104		
Corrected total	20	39.810			

Variable: average daily weight gain

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.001	0.0004	0.66	0.590
Error	17	0.011	0.001		
Corrected total	20	0.013			

Variable: average daily intake

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.008	0.003	0.66	0.590
Error	17	0.067	0.004		
Corrected total	20	0.075			

Variable: feed conversion ratio

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.590	0.197	0.43	0.733
Error	17	7.756	0.456		
Corrected total	20	8.346			

APPENDIX 2: ANALYSIS OF VARIANCE (ANOVA) TABLES FOR GROWTH PERFORMANCE OF LAMBS (GROWER PHASE)

Variable: Initial weight

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	25.971	8.657	1.46	0.262
Error	17	101.016	5.942		
Corrected total	20	126.987			

Variable: Final weight

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	29.570	9.857	1.18	0.345
Error	17	141.528	8.325		
Corrected total	20	171.098			

Variable: weight gains

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model 3	2.970	0.990	1.54	0.240	
Error	17	10.928	0.643		
Corrected total	20	13.898			

Variable: average daily weight gains

Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.004	0.001	1.54	0.240
Error	17	0.014	0.001		

Corrected total	20	0.018			

Variable: average daily intake					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.066	0.022	1.15	0.356
Error	17	0.324	0.019		
Corrected total	20	0.390			

Variable: feed conversion ratio					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	56.790	18.930	4.12	0.023
Error	17	78.192	4.600		
Corrected total	20	134.981			

APPENDIX 3: ANALYSIS OF VARIANCE (ANOVA) TABLES FOR GROWTH PERFORMANCE OF LAMBS (OVERALL PERFORMANCE)

Variable: Initial weight					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	2.159	0.720	4.87	0.013
Error	17	2.514	0.148		
Corrected total	20	4.672			

Variable: Final weight					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	29.570	9.857	1.18	0.345
Error	17	141.528	8.325		
Corrected total	20	171.098			

Variable: weight gain					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	22.771	7.590	0.94	0.443
Error	17	137.120	8.066		
Corrected total	20	159.890			

Variable: average daily weight g ain					
Source of Variation	d. f	s. s	m. s	f. value	F pr

Model	3	0.002	0.001	0.93	0.449
Error	17	0.011	0.001		
Corrected total	20	0.013			

Variable: average daily intake					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model	3	0.186	0.062	1.37	0.285
Error	17	0.770	0.045		
Corrected total	20	0.956			

Variable: feed conversion ratio					
Source of Variation	d. f	s. s	m. s	f. value	F pr
Model 3	5.473	1.824	0.77	0.527	
Error 17	40.280	2.370			
Corrected total 20	45.754				

APPENDIX 4: TABLES FROM THE MIXED PROCEDURE FOR BLOOD PROFILE

Varible : RBC				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	62.2	0.66	0.579
Month(Mth)	3	60.1	27.06	<0.0001
Trt*Mth	9	60.1	0.95	0.489

Varible : PCV				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	64	0.13	0.944
Month(Mth)	3	64	34.28	<0.0001
Trt*Mth	9	64	0.84	0.583

Varible : MCV				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	61.8	0.85	0.471
Month(Mth)	3	59.8	0.02	0.996
Trt*Mth	9	59.8	1.65	0.121

Varible : MCH				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	61.8	0.25	0.862
Month(Mth)	3	59.9	2.42	0.075
Trt*Mth	9	59.9	1.73	0.101

Varible : BASO				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	45.7	0.57	0.638

Month(Mth)	2	43.9	0.09	0.916
Trt*Mth	6	43.9	0.44	0.846

Varible : LYMPH				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	43.6	0.37	0.777
Month(Mth)	2	42.6	17.55	<0.0001
Trt*Mth	6	42.6	0.24	0.963

Varible : MONO				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	45.2	2.67	0.059
Month(Mth)	2	43.4	6.08	0.005
Trt*Mth	6	43.4	0.50	0.807

Varible : EOSIN				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	45.3	0.72	0.544
Month(Mth)	2	43.6	16.02	<0.0001
Trt*Mth	6	43.6	0.41	0.869

Varible : NEUT				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	44.1	2.17	0.105
Month(Mth)	2	43.2	1.19	0.313
Trt*Mth	6	43.2	0.58	0.744

Varible : TOTAL PROTEIN				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	59.8	3.72	0.016
Month(Mth)	3	58.2	29.06	<0.0001
Trt*Mth	9	58.2	0.14	0.998

Varible : GLOBULIN				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	61.7	0.84	0.475
Month(Mth)	3	59.4	17.61	<0.0001
Trt*Mth	9	59.4	0.83	0.596

Varible : ALBUMIN				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	60.5	1.23	0.307
Month(Mth)	3	58.9	19.51	<0.0001
Trt*Mth	9	58.9	0.47	0.891

Variable : IgM				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	64	5.94	0.001
Month(Mth)	3	64	20.57	<0.0001

Trt*Mth	9	64	1.27	0.302

Variable : IgA				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	61.8	2.56	0.063
Month(Mth)	3	59.1	36.85	<0.0001
Trt*Mth	9	59.1	0.51	0.861

Variable : CD3				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	43.9	0.70	0.556
Month(Mth)	2	42.2	1.13	0.333
Trt*Mth	6	42.2	0.08	0.998

Variable : CD4				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	42.8	3.23	0.032
Month(Mth)	2	40.4	0.49	0.618
Trt*Mth	6	40.4	0.05	0.999

APPENDIX 5: TABLES FROM THE MIXED PROCEDURE FOR MICROBIAL ISOLATES

Variable : E.COLI				
Source of variation	d.f	Den d.f	f. value	F pr
Treatment (Trt)	3	40.6	1.78	0.166
Month(Mth)	2	40.2	0.30	0.741
Trt*Mth	6	39.9	0.27	0.947