THE EFFECT OF DFM (RE-3, RE-3 PLUS AND P3) ON THE GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, MICROBIOLOGICAL AND HAEMATO-BIOCHEMICAL INDICES OF BROILER CHICKENS.

BY

GYAN ADJEI BOAMPONG KWASI (BSc. AGRICULTURE)

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DECLARATION

I, Gyan Adjei Boampong Kwasi, hereby declare that the work presented in this thesis is the result of my own effort and no such previous application for a degree in this University or elsewhere has the same work been presented.

All sources of information have been duly acknowledged by reference to authors.

Gyan Adjei Boampong Kwasi (PG7004112)	……..……	……..……
(Candidate's Name and ID)	Signature	Date

Certified by:

Professor Armstrong Donkoh	…….....	……..
(Supervisor's Name)	Signature	Date

Certified by:

Dr (Mrs) Victoria Attoh-Kotoku	……..…..	….……
(Head of Dept. Name)	Signature	Date

DEDICATION

This accomplishment is dedicated to my Uncle, Nana Apenteng Fosu Gyeabour II, Hansuahene and Banmuhene of Techiman Traditional Council.

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ABSTRACT

Two studies were conducted to determine the effect of DFM products (RE3, RE3+ and P3) on the growth performance, carcass characteristics, microbiological and haemato-biochemical indices of broiler chickens. Three hundred (300) unsexed day old Cobb commercial strain of broilers each were used for the two studies (experiment 1 with DFM in feed and experiment 2 with DFM in water). At 28 days of age, two hundred and forty birds each were randomly selected and divided into four groups, each group constituting a treatment with four replicates per treatment in a completely randomised design.

Basic diets were formulated for all the 4 experimental groups with treatment 1 devoid of the DFM supplement and three other diets containing DFM in feed or DFM in water each incorporated at levels of 1.5 ml in a kg feed and in a litre of water. The experimental diets and water were provided to the broiler chickens *ad-libitum* throughout the experiments. The control groups were given coccidiostat (Narcox-plus), each in experiment 1(DFM in feed) and in experiment 2 (DFM in water) while the probiotic groups were not given any medication.

Results of the first experiment indicated no significant ($P>0.05$) differences in feed intake, weight gain, feed conversion ratio and mortality. Haematological parameters were not significantly ($P > 0.05$) enhanced with the probiotics. However, significant ($P < 0.05$) differences existed in serum total protein, globulin and albumin levels among the treatment groups. Faecal enterococci were significantly ($P < 0.05$) lower in the probiotic administered groups than the control groups.

The results of experiment 2 showed that DFM administration in water produced significant ($P < 0.05$) effects on weight gain and feed conversion ratio of the broilers. Haematological parameters were not significantly ($P > 0.05$) influenced by DFM supplementation. However, significant ($P<0.05$) reduction in Low Density Lipoprotein (LDL) was recorded for broilers supplemented with the DFM. Faecal enterococci and salmonella were significantly ($P < 0.05$) lower in the probiotic supplemented groups than the control groups.

Based on the results of the study, both DFM in feed and in water for broiler chickens had beneficial effect on the health status, growth performance and even confer economic benefits.

Key Words: Broilers, DFM, Haematology, Microbiology, Performance.

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LIST OF ABBREVIATIONS	DESCRIPTION
AAFCO	Association of American Feed Control Officials
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AOS	Agaro-oligosaccharides
CDC	Center for Disease Control
CP	Crude Protein
CRD	Completely Randomised Design
DFM	Direct-fed Microbials
DM	Dry Matter
EFSA	European Food Safety Authority
FCE	Feed Conversion Efficiency
FDA	Food and Drug Administration
FOS	Fructo- oligosaccharides
Gh¢	Ghana Cedis
GOS	Galacto-oligosaccharides
HB	Haemoglobin
HCT	Haematocrit
HDL	High Density Lipoprotein
KNUST	Kwame Nkrumah University of Science and Technology
LDL	Low Density Lipoprotein
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
ME	Metabolisable Energy
MOS	Mannan-oligosaccharides
MPV	Mean Platelet Volume
NFE	Nitrogen Free Extract
NRC	National Research Council
PCT	Procalcitonin
PCV	Packed Cell Volume

RBC	Red Blood Cell
SED	Standard Error of Difference
TVC	Total Viable Count
WBC	White Blood Cell
WHO	World Health Organization
XOS	Xylo-oligosaccharides

CHAPTER ONE

1.0 INTRODUCTION

The increase in productivity of the poultry industry has been accompanied by various impacts, including emergence of a large variety of pathogens and bacterial resistance. These impacts are in part due to the indiscriminate use of chemotherapeutic agents as a result of management practices in rearing cycles (Kabir, 2009). Contemporary biosecurity threats arising from the increasing resistance of pathogens to antibiotics and the accumulation of antibiotic residues in animal products and the environment (Barton, 2000; Van den Bogaard and Stobberingh, 2000; McDermott *et al.*, 2002; Snel *et al*., 2002) elicit a call for a worldwide antibiotic growth promoter (AGP) ban. As a result, in the post-AGP era, it is extremely important for the highly intensive broiler production sector of the poultry industry to achieve performance optimization and minimization of economic losses while ensuring the safety of broiler meat via the control and elimination of food-borne pathogens.

It is becoming increasingly evident that to achieve the aims above and to significantly reduce the use of antibiotics, a combination of intervention strategies such as genetic selection of resistant animals, sanitation practices, elimination of pathogens from feed and water, vaccinations, and applications of suitable feed and water additives (Doyle and Erickson, 2006; Willis *et al.*, 2007) are required to promote intestinal health and product safety in broilers. Body weight gain, feed conversion and reduced mortality are characteristics of performance that ultimately dictate whether managerial and company practices will be altered for acceptance of a new way of managing poultry (Edens *et al.*, 1997b). One approach that is receiving attention is the use of probiotics.

Probiotics are live microbial dietary supplements that could possibly benefit the host by improving its intestinal microbial balance (Fuller, 1989; FAO/WHO, 2002).
In this context, the ability of probiotics to restore and maintain the digestive balance, which provides protection against pathogens or the effects of stress, offers great potential for broiler production.

Considerable attention has been paid to the potential of probiotics as a suitable alternative to antibiotics (Ghadban, 2002; Patterson and Burkholder, 2003). More recently, beneficial effects of probiotics on broiler i) performance (Jernigan *et al.*, 1985; Jin *et al.*, 1997; Zulkifli *et al*., 2000; Kabir *et al*., 2004; Kralik *et al*., 2004; Gil De Los Santos *et al*., 2005; Sun *et al*., 2005; Mountzouris *et al*., 2007; Willis *et al* .,2007; Rasteiro *et al*., 2007; Vicente *et al*., 2007; Apata, 2008); ii) nutrient digestibility (Apata, 2008; Li *et al*., 2008); iii) modulation of intestinal microflora (Koenen *et al*., 2004; Mountzouris *et al*., 2007; Teo and Tan, 2007; Yu *et al*., 2008); iv) pathogen inhibition (Rada *et al*., 1995; Jin *et al*., 1998; Line *et al*., 1998; Pascual *et al*., 1999; Kabir *et al*., 2005; Dalloul *et al*., 2005; Yaman *et al*., 2006; Higgins *et al*., 2008; Vicente *et al*., 2008; Mountzouris et al., 2007); v) immunomodulation and gut mucosal immunity (Jin *et al*., 1997; Salminen *et al*., 1998; McCracken and Gaskin, 1999; Matsuzaki *et al*., 2000; Zulkifli *et al*., 2000; Dalloul *et al*., 2003; Kabir *et al*., 2004; Koenen *et al*., 2004; Haghighi *et al*., 2005,2006; Khaksefidi and Ghoorchi 2006; Mathivanan and Kalaiarasi, 2007; Nayebpor *et al*., 2007; Apata *et al*., 2008; Farnell *et al*., 2006; Chichlowski *et al*., 2007; Teo and Tan, 2007; Gupta and Garg, 2009) and vi) ammonia gas emission in broiler house (Holland *et al*., 2002; Bansal *et al*., 2011) have been reported. Ammonia is considered the most harmful gas in broiler chicken housing as it irritates respiratory airways and predisposes chickens to respiratory infections, causes keratoconjunctivitis and reduces bacterial clearance

from lungs. The cost of probiotics is competitive with the use of antibiotic growth promoters making them just as attractive as the growth promoters (Fuller, 1989; Rolf, 2000; Sun, 2005).

However, probiotic beneficial effects have more often been demonstrated in model animals than by direct clinical evidences and depend largely on several factors such as microbial species composition (e.g., single or multi-strain) and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors (Rehman *et al*., 2007). Dose, timing and duration of the administration of probiotics may be a factor affecting efficacy: in acute infectious diarrhoea, higher dose of probiotic given for short period of time seems to be more effective than lower doses (Sazawal *et al.*, 2006). Dose of at least five billion colony forming units per day for at least 5 days is recommended (Gupta and Garg, 2009). This minimum dose takes into account the survival capacity of the ingested probiotics in the gastrointestinal tract, where they are in competition with the resident bacteria (Oelschlaeger, 2010).

The microecology of the intestinal tract is the determining factor in the viability of specific microorganisms. The production of lactic acid and hydrogen peroxide in addition to antibacterial substances such as bacteriocins, reuterin, nisin, or lactococcins all of which are known to have inhibitory effects on enterobacteriacea genera such as *E. coli* and *Salmonella* spp., and other bacteria such as *Staphylococci* spp., *Clostridium* spp., *Listeria* spp. both *in vitro* and *in vivo* (Maynell, 1963; Sarra *et al.*, 1992). In newly hatched chicks in commercial hatcheries, the volatile fatty acid concentration and pH are not sufficient to chemically suppress pathogens (Barnes *et al*., 1979, 1980a, b), and therefore, supplementation of probiotic microorganisms is critical to achieve the best results in poultry (Casas *et al*., 1993, 1998; Edens *et al*.,

1997a). Furthermore, some products must be provided constantly for the best results, and other products can be provided as a bolus at the time of placement for excellent but possibly transitory effects in the exclusion of certain pathogens.

The objective of this study was to evaluate the growth performance, microbiology, serum biochemistry and haematological indices of broiler birds supplemented with probiotics either through the feed or water.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. FEED ADDITIVES

Poultry feed formulations contain an array of substances known as "feed additives". These are non-nutritive in nature. Lewis (2002) defined feed additives as compounds that are added directly to a feed to improve flavour, odour and appearance, to preserve or extend its useful life and to enhance its natural properties. A feed additive was also defined by Hutjens (1991) as a group of feed ingredients that can cause a desired animal response in a non-nutrient role such as pH shift, growth, or metabolic modifier. To stimulate growth rate, feed conversion and health, an anti-microbial growth promoter or a natural additive can be added. Feed additives include enzymes, antibiotics, coccidiostats, antioxidants, pigments, antifungals, prebiotics, organic acids, botanicals, probiotics, etc. (Table 1).

2.1.1. Benefits of Feed Additives

Feed additives, like enzymes, and organic acids, can be used to enhance the nutrient availability of feed (Wenk, 2000). Some feed additives such as organic acids are also added to the diet of animals to modify its acidity so as to preserve and also enhance the utilization of the feed (Papatsiros *et al.*, 2012).

Other benefits of feed additives according to Pandey and Upadhyay (2012) include reduction in feed wastage through binding of powdered feed; improve acceptability of feed by enhancing texture, improving sweetness, improving odour, etc.; reducing toxicity by binding some of the toxins in feed and encouraging consumer acceptability of meat through colour modification.

2.1.2. Types of Feed Additives

Though several systems of categorization of feed additives exist, the European Food Safety Authority (EFSA, 2003), classified feed additives used in animal production into 5 distinct groups. These groups are:

i. Nutrient Additives- These are additives that are added to the diets of animals to supply some specific nutrients which may not be present or may not be in the required amounts. Nutrient additives may consist mainly of vitamin and trace mineral supplements which may be given to animals because they may not have access to their natural habitats where these nutrients may be in abundance. Furthermore, some essential amino acids may be supplied as additives in the diets of farm animals.

ii. Sensory Additives- These are additives that stimulate the animals' appetite and therefore improve the voluntary feed intake of the farm animals. Most of these additives improve the flavour of the feed or may take away some odours that reduce feed acceptance. Examples of sensory additives include sweeteners, and colouring and flavouring agents.

iii. Coccidiostats and Histomonostats- These are anti-protozoal agents that act on coccidia (parasites).

iv. Zootechnical Additives- The function of zootechnical additives is not to provide the animal with nutrients but rather to enhance the efficient use of the nutrients supplied in the diet. Most zootechnical feed additives such as enzymes may improve efficiency by degrading complex feed nutrients into forms which are readily absorbable or by stimulating the immune system of animals e.g. phytobiotics/phytogenics or by a combination of both mechanisms (probiotics). Aside their effects on the animal, some additives

in this group such as probiotics may also help reduce the harmful effects of environmental pollution that animal production may pose.

v. Technological Additives- This group of feed additives helps in the handling of feed. Technological feed additives used in animal production include acidifiers, preservatives, binders, anti-caking agents, coagulants, anti-oxidants and acidity regulators.

Kamra and Pathak (1996) earlier classified feed additives into the following groups:

i. Chemical compounds like arsenicals and copper sulphate
ii. Tranquilizers
iii. Surfactants
iv. Antioxidants
v. Antibiotics
vi. Hormones (natural, synthetic and hormone-like substances)
vii. Probiotics
viii. Miscellaneous substances like colouring and flavouring agents, etc.

A simple system of classifying feed additives according to Banerjee (1988) is where feed additives are grouped based on whether they supply animals with nutrients or not. Thus, this system groups feed additives used in animal production into nutritive and non-nutritive feed additives. Nutritive feed additives as the name implies are additives that supplies the animal with nutrients whilst non-nutritive feed additives consist of all other additives that do not supply the animal with nutrients but are required for the smooth growth of the animal. Several non-nutritive feed additives have come under serious scrutiny and according to Stephany (2010) and Vondruskova

et al. (2010), this has led to the ban on some of them, notably, antibiotics. Thus, the need arises to find suitable alternatives which are not harmful to the health of man and animals.

2.2. Antibiotics

Antibiotics are natural or synthetic compounds that are able to inhibit the growth of micro-organisms. Kellems and Church (2002) defined antibiotics as compounds produced by micro-organisms which have properties of inhibiting the growth or metabolism of other micro-organisms. According to Dibner and Richards (2005), antibiotics have been used in animal feed for over 50 years since its discovery not only as an anti-microbial agent, but also as a growth promoting agent and improvement in performance. Early indications of a beneficial effect on production efficiency in poultry were reported by Hutjens (1991).

Tetracyclines, penicillin, streptomycin and bactrican were the common additives in feed for livestock and poultry. Currently, chlortetracycline, procaine penicillin, oxytetracycline, tylosin, bacitracin, neomycin sulfate, streptomycin, erythromycin, linomycin, oleandomycin, virginamycin, and bambermycins antibiotics are used in livestock and poultry feed. In addition to these antibiotics, which are of microbial origin, there are other chemically synthesized antimicrobial agents that are also sometimes used in animal feeds. These include three major classes of compounds: arsenical, nito-furan, and sulfa compounds. Arsenical compounds include arsanilic acid, 3-nitro-4-hydroxy phenylarsonic acid, and sodium arsanilate; nitro-furan compounds include furazolidone and nitro-furazone; sulfamethazine, sulfathiazole, and sulfaquinoxaline. Other chemicals are also used as antiprotozoal agents to prevent coccidiosis and histomaniasis in chickens and turkeys. Antibiotics are used regularly

in animal feed at a rate of 2 to 50 grams per ton for improved performance in the animals.

Table 1: Non-nutritive feed additives commonly used in poultry feed formulations

Additive	Examples	Functions
Enzyme	Xylanases, ß-glucanases, phytase	To overcome the anti-nutritional effects of arabinoxylans (in wheat and triticale), ß-glucans (in barley) or phytate (in all plant feedstuffs);
		To improve the overall nutrient availability and feed value
Antibiotics	Avilamycin,virginiamycin, zinc bacitracin, avoparcin, tylosin, spiramycin	To control gram-positive, harmful bacterial species in the gut;
		To improve production efficiency; as a prophylactic measure against necrotic enteritis
Coccidiostats	Monensin, salinomycin, narasin	To prevent and control the clinical symptoms of coccidiosis
Pigments	Xanthophyll (natural and synthetic)	To increase yolk colour in eggs and to improve the skin colour and appearance of carcasses
Antioxidants	Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), ethoxyquin	To prevent auto-oxidation of fats and oils in the diet
Antifungals		To control mould growth in feed; to bind and mitigate the negative effects of mycotoxins
Direct-fed microbials	Probiotics	To provide beneficial species such as *lactobacilli* and *streptococci*
Prebiotics	Fructo oligosaccharides (FOS), mannan oligosaccharides (MOS)	To bind harmful bacteria
Organic acids	Propionic acid, diformate	To lower gut pH and prevent the growth of harmful bacteria

FAO (unpublished)

The reasons for the use of antibiotics include a more efficient conversion of feed to animal products, an increased growth rate and a lower mortality rate in general. The levels of antibiotics are often increased to 50-200 grams/ton or more when specific diseases are being targeted as when the spread of a particular disease is rampant. The levels are also increased in times of stress. This increased amount is often decreased when the threat of a disease is gone. Cromwell (1991) estimated that about three thousand tonnes of antibiotics were used in livestock feeds in the United States alone. The most current estimate is around eight thousand tonnes (Cromwell, 2002). It has been estimated that about ten thousand tonnes of antibiotics were used for livestock production and for companion animals, and nine percent of this (about 900 tonnes) was used for growth promotion purposes (Viola and DeVincent, 2006). Typically, they are administered to livestock through the feed, water or by injection.

2.2.1 Benefits of Antibiotic Use in Animal Feed

The benefits of antibiotics in animal feed include increasing efficiency and growth rate, treating clinically sick animals and preventing or reducing the incidence of infectious disease. Cervantes (2011) reported that many benefits come from using antibiotic feed additives (AFAs), such as: a) Prevention of subclinical diseases, like necrotic enteritis (NE), b) Reduction of human pathogens, by improving flock uniformity, enhancing intestinal strength, minimizing gastrointestinal ruptures during processing, and by reducing shedding of human pathogens, c) Improved animal welfare, d) Improving production efficiency, and e) Causing less contamination of the environment. By far the major use of antibiotics among these, however, is increased efficiency, i.e. a more efficient conversion of feed to animal products, and an improved growth rate. In chicken feed, for example, tetracycline and penicillin show

substantial improvement in egg production, feed efficiency and hatchability, but no significant effect on mortality. Chlorotetracycline, oxytetracyclin and penicillin also show an improved growth rate, but little effect on mortality. Antibiotics in animal feed, in general, are used regularly for increased efficiency and growth rate than to combat specific diseases.

2.2.2. Risks of Antibiotics in Animal Feed

Globally, the administration of antibiotics (excluding ionophores and non-human use antibiotics) via feed to groups of food producing animals for the purpose of performance or disease prevention has been a contentious and complex food safety and public health issue for over 40 years (Shryock, 2011). According to Witte (1997), these concerns may be due to emergence of multiple drug resistant bacteria when these antibiotics are used as supplement at sub-therapeutic levels in poultry feed. This resistance occurs after animals have been fed antibiotics over a period of time, they retain the strains of bacteria which are resistant to antibiotics.

These bacteria multiply in the animal. Through interaction, the resistant bacteria are transmitted to other animals, thus forming a colonization of antibiotic resistant bacteria. The bacteria flourish in the intestinal flora of the animal, as well as, in the muscle. Figure 1 highlights the complexity of the transmission routes to be considered in dealing with the spread of antibiotic resistance from animals to humans. These pathways need to be clearly understood if control of the spread of organisms is to be effectively managed.

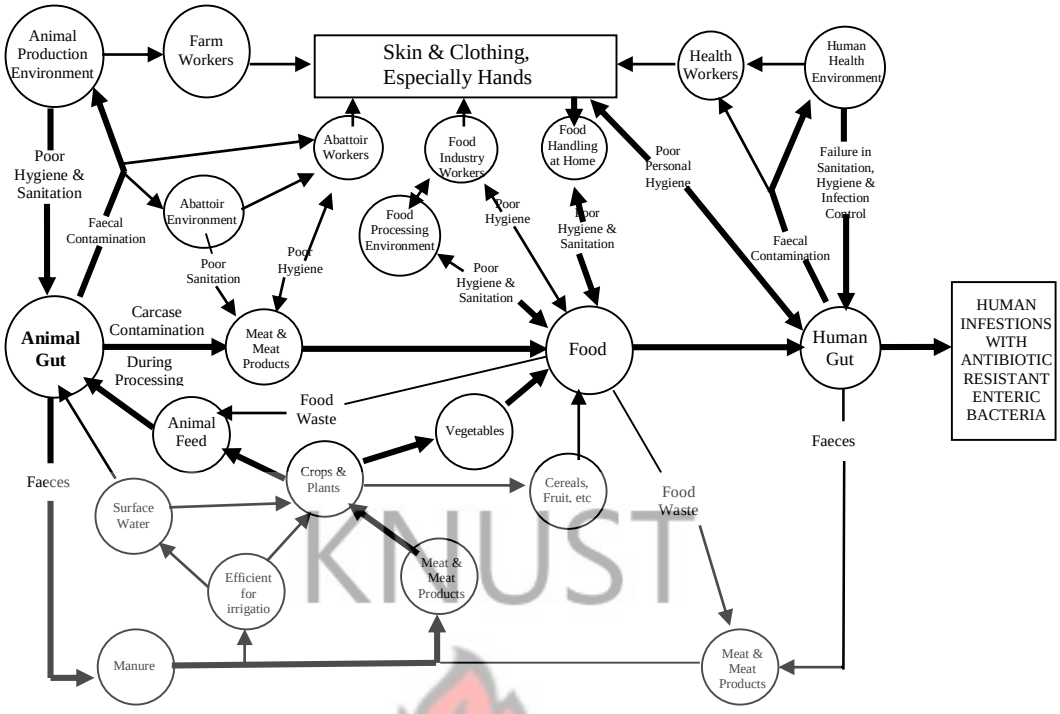


Figure 1: Possible pathways for the spread of enteric bacteria, including resistance strains, with the gastrointestinal tract as the main reservoir, between animals and humans (adapted from Witte, 1997)

2.2.3. Development of Resistance

The development of a drug resistance is not orchestrated specifically to counteract a drug. Rather, drug resistances arise because of spontaneous genetic mutations within a gene sequence. By chance, these mutations happen to produce some change in the cell that allows for drug resistance. These mutated bacteria then have a selective advantage over other non-resistant bacteria. The addition of antibiotics to the environment (the host organism) then selects for the resistant bacteria by killing off all of the non-resistant bacteria. This allows for the resistant cells to grow and divide, creating a large population of resistant bacteria. The larger population then increases the likelihood that plasmid transfer will occur to other non-resistant bacteria of various strains. This attained resistance has little effects on the host organism until plasmid/resistance transfer to a particularly virulent bacterium occurs. Then, the host

is susceptible to infection from this organism without the benefit of treatment with the antibiotic that the bacteria is now resistant to. There are three main ways in which genetic material (drug resistance genes) can be exchanged between bacteria. They are conjugation, transformation, or transduction; this is also known as horizontal gene transfer (Catry *et al*., 2003):

1. Conjugation – It is a direct cell-to-cell contact transmission. Catry *et al*. (2003) observed that, conjugation is the most important mechanism for horizontal gene transfer which involves the spread of mobile genetic elements such as plasmids. The plasmid containing cell generates a small tubule that connects the two cells (the sex pili). This tube then allows for the passage of DNA strands between the two cells. Newman and Scheuren-Portacarrero (2005) reported that conjugation is the major mechanism by which gram-negative bacteria transfer DNA and has been shown to occur between gram-negative and gram-positive bacteria. Plasmid transfer also occurs between pathogenic bacteria from different species of origin (porcine, bovine, fish etc) to humans. Schnappinger and Hillen (1996) stated that tetracyclines can promote the frequency of conjugation.

2. Transformation - the absorption of "naked", free-floating DNA by a cell. Upon the death of a bacterial cell, the components degrade, leaving the DNA and cell materials to disperse in the environment. If a cell with antibiotic resistance dies and breaks down, the resistance gene may be released into the environment and absorbed by another bacterial cell.

3. Transduction – This is the transportation of genetic material by a bacteriophage. When a bacteriophage infects and replicates in a cell, some new phages may be filled with cellular genetic material, rather than viral genetic material. In some cases, this

cellular material is a resistance gene. When the phage containing the resistance gene infects another cell, the infected cell then gains the bacterial resistance. According to Newman and Scheuren-Portocarrero (2005), transduction is transfer of DNA between two closely related bacteria.

2.2.4. Mechanisms of Resistance

There are several general methods through which a cell can become resistance to an antibiotic. These mechanisms are:

- Decreased cell permeability to the drug - the cell can change its membrane structure so that the drug cannot enter the cell and perform its function.
- Alter the drug binding/recognition site - by changing the structure of the membrane surface, the site which previously allowed the drug to bind to the cell can no longer do so.
- Chemical modification of the antibiotic - by cleaving a portion of the molecule or adding a substituent group, the properties of the active molecule in the antibiotic can be altered such that it is rendered harmless to the cell.
- Active transport - the transport of drug molecules out of the cell. In many cases, this is done via a drug/proton antiport system. With this mechanism, H^+ ions are pumped into the cell as drug molecules are pumped out.
- Enzyme or pathway alteration - the cell can change the pathway or enzyme used to carry out a cell process occurs. By doing this, the cell can bypass the enzyme that is affected and cause the drugs effects to have no bearing on the functioning of the cell.

2.3. Alternatives to Antibiotic Use

According to Plail (2006), the use of antibiotics to promote growth and control diseases in farm animals has been the usual practice for many decades among farmers. But due to the residual effect of antibiotics in animal products and the development of resistance to it by some bacteria, there has been decreasing acceptance of the additive in many countries across the world. As a result, it has become necessary to develop alternatives using botanicals, prebiotics or probiotics (Mathivanan and Edwin, 2012). Phytogenic feed additives (also called phytobiotics or botanicals) are defined as plant-derived compounds incorporated into diets to improve the productivity of animals through amelioration of feed properties, promotion of the animals' production performance, and improving the quality of food derived from those animals (Windisch *et al*., 2008) and prebiotics are polysaccharides and oligosaccharides which cannot be digested effectively by the animal, but are readily fermented by anaerobic, colonic bacteria that are regarded as beneficial (Zhang *et al*., 2003).

2.3.1 Direct-fed Microbials (DFM) (Probiotics)

Over the years, the word probiotic, has been used in several different ways. It was originally used to describe substances produced by one protozoan which is stimulated by another (Lilly and Stillwell, 1965), but it was later used to describe animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora (Parker, 1974). Crawford (1979) defined probiotics as "a culture of specific living micro-organisms (primarily *Lactobacillus spp.*) which implants in the animal to ensure the effective establishment of intestinal populations of both beneficial and pathogenic organisms". Fuller (1989) later gave a unique definition of probiotics as "a live microbial feed supplement which beneficially affects the host animal by

improving its intestinal microbial balance". The US National Food Ingredient Association presented, probiotic (direct fed microbial) as a source of live naturally occurring microorganisms and this includes bacteria, fungi and yeast (Miles and Bootwalla, 1991). According to the currently adopted definition by FAO/WHO, probiotics are: "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). More precisely, probiotics are live microorganisms of nonpathogenic and nontoxic in nature, which when administered through the digestive route, are favourable to the host's health (Guillot *et al.*, 1998).

Several varieties of DFM forms according to U.S. Food and Drug Administration, (1998), are available including powders, liquids, pastes, gels, boluses and capsules and may be administered through feed, top-dressed, given as a paste or mixed into the drinking water or milk replacer with handling instructions varying from single-dose to continuous feeding and it has been observed to contain desirable ingredients that may enhance the growth of desirable gastrointestinal microbes which help to establish a desirable balance of gastrointestinal organisms in the long run. The main advantage is that, it doesn't leave residues in animal products, in contrast to antibiotics which could have serious consequences such as drug resistance (Abe *et al.*, 1995).

2.3.2 The Development of Direct-fed Microbial

The idea that intestinal bacteria played a role in maintenance of health was originated by Metchnikoff (1907) when he studied "lactic acid bacteria" in fermented milk products and their use to increase longevity and maintenance of youthful vigour in humans. His landmark publication sparked researchers around the world, and by the

1930s, evidence was accumulating to show that normal intestinal microflora inhibited the growth of intestinal pathogens.

In 1940, penicillin was developed with the intention to suppress the interest in probiotics, but it was later realized that it rather indirectly increased the understanding of the benefit that might be derived from the gut microflora. Later it became clear that there were many species of lactic acid bacteria other than *L. acidophilus* present in the gut upon several years of research. As a result a variety of different species of the genera *Lactobacillus, Streptococcus* and *Bifidobacterium* were incorporated into probiotic preparations.

2.3.3. Efficacy of Probiotics

The use of probiotics in animal feeding could be enhanced by a preliminary *in vitro* screening: antimicrobial activity, survival in the GIT, adhesion studies and antibiotic susceptibility are among the main probiotic properties that should be analysed to assess functionality and safety. The advanced molecular methods, such as microarrays, will improve the detection of these multiple characteristics, also allowing the analysis of phenotypic and genetic properties useful for industrial production. Probiotic activity could be related to genera, species, or strains. An approach in probiotic application could be the use of mixtures of strains belonging to different genera or species (Timmerman *et al*., 2004). Dose, timing and duration of the administration of probiotics may be a factor affecting efficacy: in acute infectious diarrhoea, higher dose of probiotic given for short period of time seems to be more effective than lower doses (Sazawal *et al*., 2006); in atopic dermatitis, early treatment and long period of administration (2 years) induce better and long-lasting improvement in newborn than in children and/or short-course therapy with

Lactobacillus species (Rosenfeldt *et al*., 2003). Another determinant may be the age of the animals; during early life, colonization patterns are instable and newborn animals are then susceptible to environmental pathogens. Initial colonization is of great importance to the host because the bacteria can modulate expression of genes in epithelial cells thus creating a favourable habitat for themselves (Siggers *et al*., 2007).

2.3.4. Most Used Probiotic Genera

2.3.4.1. Lactobacillus. The genus Lactobacillus is a wide and heterogeneous taxonomic unit, comprising more than 100 different species, belonging to the group of Lactic Acid-producing Bacteria (LAB). Many of the species are significant constituents of the normal gut microbiota of humans and animals, and their occurrence and number are host dependent. Several species of the genus are intentionally introduced in the food chain, being involved in a range of food and feed fermentations, and applied as probiotics in humans and animals (Hammes and Hertel, 2007). However, some reports stated that these microorganisms might rarely be involved in human diseases, where *L. casei* and *L. rhamnosus* are the most common (Vesterlund *et al.*, 2007). No report can be found on safety concerns related to lactobacilli in animals. Due to the long history of safe use, a list of species has been proposed for QPS status (Table 2) (EFSA, 2007a).

2.3.4.2. Enterococcus. The genus Enterococcus belongs to the LAB group. Enterococci are found naturally in food products. These microorganisms are normal human and animal commensals. *E. faecium* and *E. faecalis* are the most common in the human gastrointestinal tract while in animals, *E. faecium* is prevalent (Fisher and Phillip, 2009). These microorganisms are used as starter cultures in food products,

such as cheese, as probiotic cultures for humans and animals and as silage additives (Foulquie *et al.,* 2006). Enterococci are sometime associated with human infections. The Enterococcus genus is of particular medical relevance because of increased incidence as a cause of disease in hospital-acquired (nosocomial) infections, and acquired antibiotic resistance towards the available antibiotic therapies. Several virulence factors have been described and the number of vancomycin-resistant enterococci is increasing (Leavis *et al.,* 2006). Strains belonging to *E. faecium* have a long history of apparent safe use in industrial and agricultural applications; however other species, such as *E. durans* and *E. hirae*, have been associated with infections in chickens (Chadfield *et al.,* 2005; Abe *et al.,* 2006). The use of enterococci as probiotics remains a controversial issue.While the probiotic benefits of some strains are well established, the emergence and the increased association of enterococci with human diseases and multiple antibiotic resistances have raised concern regarding their use as probiotics. The concern that antimicrobial resistance genes or genes encoding virulence factors could be transferred to other bacteria in the gastrointestinal tract contributes to this controversy (Kayser, 2003; Moreno *et al.,* 2006). Due to safety concerns, no members of the genus Enterococcus have been proposed for QPS status (EFSA, 2007a).

2.3.4.3. Bacillus. Bacillus species are Gram-positive, spores-forming microorganisms, commonly associated with soil, water and air. Bacillus species are normally allochthonous microbes to the intestinal tract as a result of an involuntary ingestion of contaminated feed. The use of viable spores of Bacillus as probiotic supplement raised a number of questions, including their safety: several Bacillus species used as animal feed supplements, probiotics, plant protection products or seed coating agents are also known as agents of food poisoning (Sanders *et al.,* 2003). The knowledge

gained from their use, as animal feed supplement, suggests that, for some species at least, their safety could be assured by the QPS approach (EFSA, 2007a) (Table 2). Since most Bacillus species potentially possess toxigenic traits, absence of toxigenic activity needs to be verified for qualification.

2.3.4.4. Saccharomyces. Saccharomyces is a genus of budding yeast. Yeasts are also part of the residual microbial system of the intestinal microbiota. *Saccharomyces cerevisiae* is widespread in nature and can be found in plants, fruit and soil. *S. cerevisiae* is included in foods and beverages for its key role in fermentation processes and in health foods. Strain known as *S. boulardii* was isolated from the skin of lychees grown in Indochina. This species does not have a taxonomic status and it is considered a biotype of *S. cerevisiae* (Van der Aa Kühle and Jespersen, 2003). *S. boulardii* is used as probiotic especially in ruminants and pig feeding.

2.3.4.5. Bifidobacterium. In the intestinal tracts of animals and humans, bifidobacteria are considered one of the key genera. Their presence in high numbers is associated with good health status of the host. There is a general belief that bifidobacteria are helpful in maintaining appropriate balance of the microbiota in the GIT, reducing the risk of pathogen infection. Several species are host specific (Biavati and Mattarelli, 2006). Bifidobacteria are very promising probiotics even if it is to be considered that probiotic properties are species and/or strain specific. They are frequently used in food and pharmaceutical preparations and their application in animal feeding is increasing. Due to the long history of safe use of bifidobacteria, many species are proposed for QPS status.

2.3.5 Undefined Microbial Preparations Used as Probiotics: Competitive Exclusion

Competitive exclusion (CE), also indicated as the "Nurmi concept", originate from the finding that newly hatched chicks could be protected against Salmonella colonization of the gut by dosing them with a suspension of gut content prepared from healthy adult chickens (Nurmi and Rantala, 1973). The introduction of CE bacteria from the gut content should occur early in life, such that the CE bacteria are preferentially established in the gastrointestinal system to become competitive or antagonistic to opportunistic pathogens. Because of the use of undefined preparations from the cecal or fecal material could result in the transmission of pathogens, regulatory restrictions for probiotic microorganisms (SCAN, 2000) made authorization difficult for this kind of products. However, CE products with defined and identified microorganisms have been developed and applied in animal breeding (Schneitz, 2005).

Table 2. Factors that Limit the Effectiveness of Probiotics in Poultry.

Stress factors affecting DFM performance	Causes of stress
Nutritional	Improper formulation of diet Poor quality protein and other nutrients Poor water quality Nutrient degradation (oxidized fats and vitamin) Molds and mycotoxins Other toxic substances
Environmental	Excessive cold Excessive heat High levels of chlorine or fluoride in drinking water Excessive humidity Ammonia Poor ventilation Wet litter Excessively dry litter Lack of maintenance of water supply lines and waterers Pathogenic microbes in overwhelming numbers
Physical and immunological	Poor chick quality Immunological diseases (infectious bursal disease, Mareks disease, all leukosis diseases including j-virus infections.
Managerial	Setting of dirty eggs Hatching too early Late removal from hatchery Poor beak trimming Too trimming Over-crowding Vaccinations and other injections and inoculations Poor dis-infection and sanitation programs Poor litter management Cannibalism lack of removal of moribund and dead birds Interrupted feed and water supply
Use of Antibiotics	Uncontrolled antibiotic use Antibiotic destruction of normal intestinal microbes Non-specific enteritis of viral origin (antibiotic are not indicated for use)
Lack of Association with Mother Hens	Hatchery-supplied that have never been on the ground with the mother hens require longer times for development of normal intestinal microbial populations. Lack of association with healthy adult chickens in a flock. Hatchery associated services of the chicks (under managerial)

Source: Edens *et al.* (2003).

2.3.6 Factors Affecting Probiotic Performance.

Use of probiotics for poultry production is not without certain risks and limitations. There are many stress factors in the environment of newly hatched poultry species that could in one way or the other reduce the effectiveness of the maternal antibody defense mechanism and normal colonization of the gut by beneficial microorganisms effectively allowing the colonization of pathogens during the early post-hatch stage. This seems to be somewhat ironic because there is evidence that probiotics can limit the consequences of exposure to stressors of many types (Edens *et al.,* 2003). Some of the stress factors and causes of the stress are listed in Table 1.

There are high probabilities that newly hatched chickens and turkeys will face a situation in commercial as well as in experimental settings that will alter the development of natural gut-associated beneficial microorganisms. The primary factor affecting this development can be the feed source and quality. Under-formulated diets result in nutritional stress and decrease the growth of beneficial organisms. Molds and mycotoxins further add to the problem of nutritional stress and can cause the loss of essential nutrients for the gut microbes. However, nutrient degradation may be the most important factor to affect the gut microbes. This can be caused by numerous factors such as oxidized dietary fat and lipid peroxidation, vitamins, amino acids and proteins also influence the populations of beneficial organisms in the gut, but in this era of concern about microbial contamination of feed, higher and higher pelleting temperatures in feed manufacturing causes the destruction of not only pathogenic but beneficial organisms as well (Edens *et al.,* 2003). The only probiotic organism that can tolerate relatively high temperatures associated with the pelleting of chicken and turkey feed are the spore-forming *Bacilli*. All other probiotic organisms will die as a

result of pelleting (Edens *et al.*, 2003). Therefore, most probiotics must be applied via drinking water or as a top dressing to pelleted feed.

Exposure of chickens and turkeys to extreme conditions in the environment can induce nonspecific stress responses leading to depressed immuno-responsiveness that will influence gut microbial populations. Unfortunately, the depression in the production of immunoglobulins, specifically IgA, tends to influence pathogen growth more than beneficial microbes. Many managerial stressors such as beak and claw trimming and other hatchery processes such as vaccinations and handling for sexing and high population densities after placement contribute to immuno-suppression in poultry. However, we always come back to antibiotic use/abuse in the poultry industry. Over use of antibiotics can have very negative effects in the young bird. In some commercial operations, it is common practice to add high levels of antibiotics to the first feed given to chickens and turkeys. Usually, this medicated feed can be available for as long as 10 days after placement. This medicated feed is replaced then with feed that does not contain antibiotics. Within a few days after the new feed has been provided, the chickens and turkey poults may begin to refuse feed and to develop signs of enteritis that is now frequently called "off-feed enteritis". When the intestinal tracts are analyzed for bacterial populations, there are usually low numbers of beneficial bacteria such as *Lactobacilli* and extraordinary numbers of potentially pathogenic *E. coli, Salmonella, Clostridium*, and others. Naturally, the producers revert to an antibiotic treatment, and sometimes they also think about the possibility of a probiotic. Unfortunately, there are a limited number of products that can be used along with certain antibiotics. Among the commonly used antibiotics, Bacitracin has been shown to have the least influence on *Lactobacilli* (Casas *et al.*, 1998). Therefore, producers of commercial poultry have created a situation that appears to be feeding

upon itself and continuing to grow. The end result of prolonged use of antibiotics is antibiotic resistant bacteria and inhibition of growth of beneficial bacteria in the intestinal tract of poultry and other livestock.

Nevertheless, this chain of events can be reduced by; (1) Reducing antibiotic use on a prophylactic basis, and (2) Developing a managerial plan that incorporates the use of probiotics into flock management programs.

Other factors that affect probiotic responses are as follows.

2.3.6.1 Method of production

Differences in presentations such as whether the probiotic is a powder or a liquid suspension are well noted by Fuller (1975) who indicated that, even if the two strains being used for production of the probiotic are identical, the way in which they are prepared can cause variation in the results they produce. Production methods have also been noted as one of the factors that can affect the viability of the probiotic as was stated by Gaggia *et al.* (2008). However, what is not so clear are the changes which may be induced by the way in which the probiotic organism is grown and harvested. For example, the carbohydrate source in the growth medium can affect the ability to adhere to the gut epithelium of chickens and the adhesion capacity also changes during the growth cycle (Fuller, 1975).

2.3.6.2 Method of administration

Although direct-fed microbial products may, in theory, improve gut microflora, research shows that the practical application on the farm can be more challenging since probiotic administration to the host animal occurs in a variety of ways and yet little is known about the minimum dose required for the probiotic effect. The amount

and interval between doses may also vary and may be given only once or periodically at daily or weekly intervals (Goldin and Gorbach, 1984). It therefore seems very likely that the effect obtained will be affected by which method was used during the administration such as the amount and frequency of dosing (Sazawal *et al.*, 2006).

2.3.6.3 Viability of the preparation

Studies have shown that if the viability of the preparation used was not properly examined before use, negative results may be obtained and this can be due to insufficient viable cells being present in the probiotic. In a survey of commercially available probiotic preparations, Gilliland (1981) found out that the viable count varied greatly after laboratory examination for total cell count and three of the fifteen preparations tested had no viable *lactobacilli* and sometimes *lactobacilli* other than the one listed on the label were present in the probiotic preparation.

2.3.6.4 Condition of the Host

Reports cited in literature for instance suggest that the earlier the probiotic is introduced in the host's life the more effective it becomes (Casas *et al*., 1993, 1998; Edens *et al*., 1997a). Other work also suggests that, the gut microflora become unstable during the early stages of the animal's life and organisms given probiotics by mouth are likely to find a niche which they can occupy. Pollman *et al*. (l980) for instance obtained a better probiotic response in starter than he did with growing-finishing pigs after feeding with probiotics. He obtained an improvement in average daily gain (11.0%) and feed conversion (1.5%) as compared to grower-finisher pigs when *Lactobacillus acidophilus* was incorporated in the diet of 7kg weanling pigs. Differences have also been observed by Pollman *et al.* (1980) in the response to fungal probiotics in lactating and non-lactating cows. While *lactobacillus* probiotics

showed effectiveness in calves, they were of limited use in adult ruminants where fungal probiotics were more effective.

2.3.6.5 Condition of Gut microflora

It is possible that an animal receiving a probiotic may not be able to subject itself to the effects that the probiotic reverse in its system such as an infectious disease, but it is less apparent when probiotics are used to stimulate the growth of farm animals. If, like antibiotics, probiotics stimulate growth by preventing a growth depressing organism present in the gut, then it will follow that if the organism is not present, no growth stimulation will occur. It may be that the conditions under which a probiotic will have its maximum effect are very strictly defined and that only if these conditions are met will it appear positive. These might have contributed to some of the inconsistencies that occur in results of works done with probiotics but none-the-less a close observation of other results leads one to conclude that with the right probiotic, using the right host, administered in the right way at the right time one can expect to obtain a beneficial effect. More knowledge of how probiotics work and the optimal methods for administration will enable users to select more active strains and administer them in a fashion that will make the results more consistent and predictable (Edens *et al.*, 2003).

2.3.7 Microorganisms Used in DFM

Several strains of bacteria, fungi or yeast have been used efficiently to produce different types of DFM. Various microorganisms that could be used as probiotics are isolated from gastrointestinal content, mouth and faeces of animals. The major microorganisms presently used as probiotics strains for animals are *Lactobacillus*,

Bifidobacterium, *Bacillus spp*, *Streptococcus* and *Saccharomyces cerevisiae* (Edens *et al*., 2003).

Table 3 shows a list of micro-organisms approved by the Food and Drugs Association (FDA, 1998) and American Association of Feed Control Officials (AAFCO, 1998) for use in DFM products. They are expected to possess qualities such as being non-pathogenic, gram-positive, acid resistant, strain specific, anti-*E. coli*, bile resistant, viable/stable, and must adhere to the intestinal mucosa and contain a minimum of 30 $\times 10^9$ colony forming unit per gram (Edens *et al*., 2003).

Most of the works on probiotics in the literature involved the use of either one (single) or two strains of beneficial bacteria (Rehman *et al*., 2007). Multiple-probiotic strains could increase the beneficial health effects compared with individual strains, because of their synergistic adhesion effects (Collado and Sanz, 2007; Timmerman *et al*., 2004; Williams *et al*., 2008). Bonsu (2009) observed significantly ($P<0.05$) higher weight gains when he fed a DFM product containing *Lactobacillussp, Bacillus sp* and *Saccharomyces cerevisiae* to broiler chicks and recorded higher egg weight in layers as well. Some experiments have however failed to show consistent and beneficial responses (Okai, 2008), who recorded no significant ($P>0.05$) effect on growth performance in the DFM-treated pigs and in laying hens (Day, 1987).

List of Micro-organisms Approved By the Food and Drugs Association (FDA,1998) and American Association of Feed Control Officials (AAFCO,1998) for Use in DFM Products.

Table 3: FDA and AAFCO Approved Microorganisms for use in DFM products

Aspergillus niger	*Bifidobacterium infantis*	*Lactobacillus reuteri*
Aspergillus oryzae	*Bifidobacterium longum*	*Leuconostoc mesenteroides*
Bacillus coagulans	*Bifidobacterium thermophilum*	*Pediococcus acidilactici*
Bacillus lentus	*Lactobacillus acidophilus*	*Pediococcus cerevisiae (damnosus)*
Bacillus licheniformis	*Lactobacillus brevis*	*Pediococcus pentosaceus*
Bacillus pumilus	*Lactobacillus bulgaricus*	*Propionibacterium freudenreichii*
Bacillus subtilis	*Lactobacillus casei*	*Propionibacterium shermanii*
Bacteriodes amylophilus	*Lactobacillus cellobiosus*	*Saccharomyces cerevisiae*
Bacteriodes capillosus	*Lactobacillus curvatus*	*Streptococcus cremoirs*
Bacteriodes ruminicola	*Lactobacillus delbrueckii*	*Streptococcus diacetilactis*
BactSeriodes suis	*Lactobacillus fermentum*	*Steptococcus faecium*
Bifidobacterium adolescentis	*Lactobacillus helveticus*	*Streptococcus intermedius*
Bifidobacterium animalis	*Lactobacillus lactis*	*Streptococcus lactis*
Bifidobacterium bifidum	*Lactobacillus plantarum*	*Streptococcus thermophiles*

Source: Alliance Animal Health: proven performance from innovative Nutrition®

Before health claims about the importance of DFM supplementation in diets could be made, it has become necessary to conduct an evaluation of the quality, safety and effectiveness of DFM using prescribed and standard guidelines as outlined by the FAO (2002) some of which are as follows:

i. A DFM must be alive when administered.

ii. A DFM must have undergone controlled evaluation to document health benefits in the target host.

iii. A DFM must be a taxonomically defined microbe or combination of microbes (genus, species and strain level).

iv. A DFM must be safe for its intended purpose.

2.3.7.0 RE3^{TM} as a DFM Product

RE3^{TM} is a DFM product produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada. RE3^{TM} is in the liquid form and is added to the diet, mixed thoroughly before being offered to the animals. The composition of RE3^{TM} is shown in Table 4.

Table 4: Composition of RE3^{TM}

Constituents	**Amount**
Water	99.9%
Microorganisms	
Lactobacillus sp.	1 x 10^8 CFU/g
Bacillus sp.	4 x 10^{12} CFU/g
Saccharomyces cerevisiae	11 x 10^5 CFU
Minerals	
Calcium	< 0.02 %
Sodium	< 0.02%
Potassium	< 0.005%
Magnesium	< 0.003%
Molybdenum	< 0.3ppm
Copper	< 0.3ppm
Iron	< 3ppm
Boron	< 3ppm
Zinc	< 2ppm

Source: Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada.

2.3.7.1 Bacterial Direct-fed Microbial

Basically, two groups of bacteria according to Fuller (1989) are used: The lactic acid bacteria group mainly *Lactobacillus spp.* and *Bacillus spp.*Among these bacteria, *Lactobacillus* (lactic acid bacteria) is the commonest in probiotics. Work done by Dalloul *et al*. (2003) shows that Probiotic supplementation of intestinal microflora in poultry, especially with *Lactobacillus* species, showed beneficial effects on resistance

to infectious agents such as *Escherichia coli, Salmonella spp.* and more recently, *Eimeria acervulina*. Pollmann *et al*. (1990) also confirmed an improvement in average daily gain (11.0%) and feed conversion (1.5%) when *Lactobacillus acidophilus* was included in the diet of 7 kg pigs. The genus *Bacillus* appears to be one type of probiotic commonly in use today even though *Lactobacillus* appears to be the commonest as indicated by Hong *et al*. (2005). Several *Bacilli spp*. have also been suggested to serve as a probiotic in broiler chickens. Barbosa *et al*. (2005) isolated several *Bacilli spp.* from the chicken gut and all strains examined demonstrated the ability to sporulate efficiently in the laboratory setting, to tolerate simulated gastrointestinal conditions and to exhibit antimicrobial activity against a broad spectrum of bacteria, including: *Clostridium perfringens, Listeria monocytogenes, and Staphylococcus aureus.*

2.3.7.2. Fungal/ Yeast Direct-fed Microbial

Probably the first microorganisms used as DFM feed additives for domestic livestock were yeasts according to Fuller (1989). *Saccharomyces cerevisiae* and *Aspergillus oryzae* happen to be some of the fungal sources. It is however clear from literature that, the most commonly used yeast probiotic in animal feeding is *Saccharomyces cerevisiae*. The word"*Saccharomyces*" is derived from Greek and it literally means "sugar mold" and "*cerevisiae*" comes from Latin and also means "of beer" and so in short the word *Saccharomyces cerevisiae* literally means ‘‘sugar mould of beer’’ (Day,1997). It is a species of budding yeast and it can be suggested as being the most useful yeast owing to its use since ancient times in baking and brewing (Santin *et al.,* 2001). Thayer and Jackson (1975) also suggested that most yeast cells used as DFM are produced through simple fermentation and culture methods.

The yeast, *Saccharomyces cerevisiae* according to Maurya *et al. (*1993), has shown promising effects on increasing the digestibility of feeds and the fibre fractions of feeds thereby increasing the availability of nutrients for animal productivity.

It was also mentioned in the reports of Matthew *et al.* (1998) that the supplementation of live yeast culture improves growth performance in weaning pigs. Similarly, studies with chickens, turkeys, and Bobwhite quail have showed improved body weight, egg production, and immune function (Parks *et al*., 2001).

Thayer and Jackson (1975) also reported an improvement in egg production, egg weight and egg specific gravity for turkey breeder hens fed diets containing low phosphorus level and live-yeast culture. Even though all these benefits are attributed to probiotics of fungal/yeast origin, some researchers have found inconsistencies in the effects of the use of live yeast cultures as feed additives in livestock production. For instance, Kornegay *et al.* (1995) reported that the addition of live yeast culture to the feed of swine could not show beneficial effect on the digestibility of nutrients.

2.4.1. Mechanism of Action of Probiotics

Much of the perception about the function of probiotic organisms in poultry is based upon work done in mammals, specifically humans (Kopp-Hoolihan, 2001), but the same principles might not always be the same in the avian species. Nevertheless, a delicate balance among microbes in the gastrointestinal tract of chickens provides the necessary protection that prevents invasion of a multitude of potential bacterial and protozoan pathogens that can disrupt the normal body functions of poultry. Animals and humans alike have developed an elaborate defense strategy whereby a symbiotic relationship has evolved in which commensial microorganisms actually protect and provide to the host certain benefits. Among these beneficial effects is modification of

the host immune system. In order for this mutual relationship to flourish, a complex physiological and host defense mechanism must be established. Once established, the microbiota of the gastrointestinal tract prevents colonization by other bacteria. The mechanisms used by one species of bacteria to exclude or reduce the growth of another species are varied, but Rolfe (1991) determined that there are at least four major mechanisms involved in the development of a microenvironment that favours beneficial microorganisms. Beneficial microorganisms possess certain favourable characteristics that allow for the expression of several mechanisms that prevent pathogens from colonizing the intestinal tract (Table 5). These mechanisms are listed as follows: (1) creation of a microecology that is hostile to other bacterial species, (2) elimination of available receptor sites, (3) production and secretion of antimicrobial metabolites, and (4) competition for essential nutrients.

Table 5. Desirable Characteristic and Functions of Probiotics Applied to Poultry and Livestock.

Desirable Probiotic Characteristics	Desirable Probiotic Functions
Host adapted	Exclude (prevent colonization) or kill pathogenic bacterial
Non-pathogenic	Stimulate the immune system
Tolerate processing and storage	Reduce inflammatory reactions
Resist gastric acid and bile salts	Enhance animal performance
Readily bind to epithelium and mucus	Decrease carcass contamination
Resistant viability in the gastrointestinal tract	Increase production of volatile fatty acids
Produce inhibitory substances against other bacterial	Increase vitamin B synthesis
Alter microbial activity	Improve nutrients absorption
Modulate immune responses	Decrease diarrhea
Actively competes for receptor sites	Competition of essential nutrients for bacterial growth. Creates a restrictive physiological environment. Stimulates peristalsis

Adapted from Simmering and Blaut (2001); Stavric & Kornegy (1995); Jenkins *et al.* (1999); Monsan and Paul (1995); Piva (1998).

2.4.1.1. Creating a Gut Microecology Favourable to Beneficial Microorganisms

The balance among the gut microflora and the host in both mammals and birds can be challenged on a daily basis because there are potential invasive microorganisms living in the common environments. Those potential invasive microorganisms can be commensial (they live in the intestinal tract but cause no problems when there is a normal balance among microbiological species) or nosocomial (opportunistic invaders living outside the body). The water we drink, the food we eat and the air we breathe have these potential invaders present and ready to challenge the symbiotic relationship between the host and the gut microbiota. Because of this constant state of siege, elaborate defense mechanisms have evolved to cope with the potential invaders (Table 5). All food, once ingested must be subjected to gastric pH in the range of 2.0 to 4.0, which can cause a 10 to 100 fold killing of bacteria in the food being digested in the upper part of the gastrointestinal tract. The microecology of the intestinal tract is the determining factor in the viability of specific microorganisms. The production of volatile fatty acids at a pH below 6.0 is known to decrease the populations of *Salmonella* and *Enterobacteriacea* (Maynell, 1963). Disruption of the normal intestinal microbial populations with antibiotics will abolish this protective mechanism because the concentrations of volatile fatty acids produced by the intestinal bacteria will decrease and gut pH will increase toward a more alkaline range. In newly hatched chicks in commercial hatcheries, the volatile fatty acid concentration and pH are not sufficient to chemically suppress pathogens (Barnes *et al*., 1979, 1980a, b), and therefore, supplementation of probiotic microorganisms will be very beneficial.

A good balance of beneficial microorganisms provided through supplemental probiotic bacteria prevents adaptation of ingested and transient pathogenic microbes. It is critical to apply probiotic products as early as possible to achieve the best results in poultry (Casas *et al*., 1993, 1998; Edens *et al*., 1997a). Furthermore, some products must be provided constantly for the best results, and some products can be provided as a bolus at the time of placement for excellent but possibly transitory effects in the exclusion of certain pathogens.

As soon as a chicken hatches into an environment that is heavily contaminated by bacteria, viruses, and protozoans, it must begin to develop protective gut microflora. The gastrointestinal tract of the chicken and turkey is practically void of beneficial bacteria at the time of hatching, and in some cases, a period of five to seven days after hatching is required to establish a healthy population of lactic acid bacteria in the gut. Because the lactic acid bacteria can survive and grow in both aerobic as well as anaerobic environments, they become the dominant bacteria throughout the gastrointestinal tract from the crop through the large intestine. Due to the abundance of substrates, the lactic acid bacteria thrive in the gut and produce lactic acid and hydrogen peroxide in addition to antibacterial substances such as bacteriocins, reuterin, nisin, or lactococcins all of which are known to have inhibitory effects on enterobacteriacea genera such as *E. coli* and *Salmonella* spp., and other bacteria such as *Staphylococci* spp., *Clostridium* spp., *Listeria* spp. both *in vitro* and *in vivo.*

Before the development of lactic acid bacterial populations in the gut, the newly hatched chicken begins to pick-up coliforms and streptococci from its environment. These bacteria can be beneficial or they can be pathogenic. Because there is a delay in the development of a population of beneficial bacteria, the potential for colonization

by pathogenic strains can be elevated, but usually, if the dam has done her job properly, maternal antibodies can help to prevent pathogen colonization. Nevertheless, under normal conditions, a three to five week period is required for development of a stable population of gut associated bacteria, and it is in the ceca where the greatest numbers reside (Sarra *et al*., 1992).

In the ceca, an anaerobic environment develops and favours the growth of organisms such as *Bifidobacterium* spp. and *Bacteriodes* spp. In this strictly anaerobic environment those named bacteria along with other lactic acid bacteria create a microecology that can be characterized by an acid pH resulting from the production of volatile fatty acids (acetic, butyric, propionic, and lactic acids) and antimicrobial substances that effectively exclude or kill many different pathogens.

2.4.1.2. Elimination of Available Receptor Sites

The adhesion of microorganisms to the gut epithelium is mediated through polysaccharide-containing components attached to the cell wall (Soerjadi *et al*., 1982). An acidic polysaccharide cell wall component mediates adherence of common bacteria to each other and to the intestinal epithelium preventing other bacteria from attaching to the epithelium, effectively blocking all receptor sites (Fuller, 1975). However, a multitude of other mechanisms also exist. Recently, it has been shown that it is possible for healthful organisms to express complex carbohydrates similar to the cell surface adhesions found on potential pathogens. Neeser *et al*. (2000) demonstrated that *Lactobacillus johnsonii* La1 had two major carbohydrate-binding specificities. These were the O-linked oligomannosides and the gangliotriosylceramide and gangliotetraosylceramide (asialo-GM1). Similar carbohydrate-binding specificities are known to be expressed on several

enteropathogens. Thus, *L. johnsonii* can inhibit the binding of the pathogens to the mucosal epithelial mannan receptors. Gusils *et al*. (2000) have shown that chicken *L. animalis* and *L. fermentum* utilize a lectin-like structure that has glucose/mannose as specific sugars of binding. Addition of mannose or sialic acid to culture media reduced adhesion of chicken *L. fermentum* to host specific epithelial cells. Chicken *L. fermentum* decreased adhesion to host-specific epithelial cells of *S. pullorum* by 77%, and *L. animalis* reduced adhesion by *S. pullorum, S. enteritidis*, and *S. gallinarum* by 90%, 88%, and 78%, respectively.

However, a report by Lee *et al*. (2000) suggested that even though probiotic bacteria such as *L. rhamnosus* GG and *L. casei* Shirota have similar carbohydrate-binding specificities compared with *E. coli*, they do not prevent binding of the pathogen to intestinal cells even if adequate probiotic cell numbers are present. If adequate numbers of probiotic bacteria are present, the probiotic bacteria appeared to inhibit *E. coli* adhesion to intestinal cells. The competition among probiotic and pathogenic bacteria is complex and very competitive. In the intestinal lumen, the *Lactobacilli* can be displaced by pathogens if the numbers of *Lactobacilli* decline. The mucus layer on the intestinal cells plays a significant role in the adhesion of probiotic and the pathogenic bacteria. Some probiotic bacteria have very high affinities for mucus binding sites and others have low affinity. Furthermore, pathogenic bacteria have variable affinities for binding sites on the mucus layer. If a probiotic bacterium has multiple binding sites in mucus and on the intestinal cell surface, its ability to exclude pathogens might be improved. Thus, it is important to provide the highest number of probiotic bacteria as possible and as soon as possible to achieve the best results in the control of pathogenic bacteria.

Competition for available binding sites on the intestinal mucosa is also influenced by the pH of the luminal contents. Fuller (1977, 1978) has demonstrated that an acid pH favours the survival of acid loving bacteria such as the *Lactobacilli*. Therefore, larger numbers of the Lactobacilli will bind to the intestinal mucosal epithelial cells and exclude pathogens such as *Salmonella* and *E. coli*. Furthermore, the composition of the medium in which the probiotic is growing will influence the adhesion of the organism to the mucosal epithelium and affect its resistance to acid (Fuller, 1975). The contents of the digestive tract are always moving. The transit of the intestinal contents is influenced by the microbes, both free and attached, that exist in the intestinal lumen, and the motility or peristalsis of the intestinal tract affects the ability of pathogens and probiotic bacteria to attach to the epithelial cells in the lumen (Savage, 1997). Many of the beneficial microbiota can stimulate lower gut motility via production of short chain fatty acids and decreasing pH (Ohashi *et al*., 2002). The involvement of mucus in the ability of microbe to attach to the underlying epithelial cells is influenced by the carbohydrate and protein content of the mucin (Mikelsaar *et al*., 1987). It is apparent that *Lactobacilli* require the mucin for their attachment, and if the mucin content decreases, the beneficial *Lactobacilli* numbers also decrease (Mikelsaar *et al*., 1987). However, some pathogens have evolved to take advantage of this response in the gut and even increase the rate of mucin degradation (Mikelsaar *et al*., 1987). Additionally, the beneficial *Lactobacilli* also metabolize both protein and sugar content of the mucin and use it for energy and growth.

There has been a significant amount of speculation regarding modulation of mucosal immunity in animals given probiotic microorganisms. The influence of probiotic microorganisms has been reviewed extensively (Marteau and Rambaud, 1993; McCracken and Gaskins, 1999; Perdigón *et al*., 1995). Because the gastrointestinal

tract contains the majority of all of the immuno-competent cells in humans and other animals, local stimulation of gut associated lymphoid tissues can provoke a generalized systemic response (McCracken and Gaskins, 1999). Sanders (1999) has summarized numerous immuno-modulator events in human and animal models given probiotics. Probiotic bacteria are capable of enhancing both specific and nonspecific immune responses by activating macrophages, increasing cytokine production by intraepithelial lymphocytes (IEL), and increasing levels of immunoglobulins especially IgA. The immunoglobulin IgA is the most active in the gut and inhibits bacterial colonization via agglutination and direct binding to attachment sites. Cross *et al*. (2002) have shown enhanced production of Th1 and Th2 cytokines in ovalbumin primed mice fed *L. rhamnosus* HNOO1 bacteria. In rats, *L. casei* has been shown to induce mucosal IgA levels thereby improving the surface epithelial immunological barrier (Malin *et al*., 1996). However, it has been shown that all probiotic organisms do not act to induce the same immunological functions in the gastrointestinal tract and that proper strain selection or probiotic product with the desirable probiotic strains will affect the outcome of treatment (Maassen *et al*., 1998).

The poultry literature concerning these processes is very meager. Casas *et al*. (1998) reported that turkey poults given *L. reuteri* had enhanced humoral antibody levels against *S. typhimurium,* and this appeared to be highly correlated with increased numbers of ileum IEL $CD4^+$ (helper) T-cells that function to expand the humoral immune response. On the other hand, the number of ileum IEL $CD8^+$ (cytotoxic) T-cells were not different in *L. reuteri*-fed poults. The ileum $CD4^+/CD8^+$ ratio in *L. reuteri*-fed poults increased from 2 to 3.5, but in the duodenum, where few to no *L. reuteri* reside, the $CD4^+/CD8^+$ ratio was not affected. Dalloul *et al*. (2003) report that a *Lactobacillus*-based probiotic treatment given to chickens challenged with *Eimeria*

acervulina sporulated oocysts resulted in larger numbers of IEL $CD3^+$, $CD4^+$, $CD8^+$, and a b TCR than those on a control diet. Probiotic-fed chickens also shed fewer oocysts than controls.

Laying hens given probiotics have given variable results. Balevi *et al*. (2001) reported that probiotic treatment had no significant influence on peripheral immune response. Panda *et al*. (2003) reported that 64 weeks old Leghorn hens, given probiotic therapy, had increased cutaneous basophilic hypersensitivity responses against phytohemagglutinin and had higher antibody titers against sheep red blood cells. Casas *et al*. (1998) actually observed a decreased cutaneous basophilic hypersensitivity to phytohemagglutinin antigen, but attributed the smaller response to intensive recruitment of T-cells to the ileum in *L. reuteri*-fed broilers.

2.4.1.3. Production and Secretion of Antimicrobial Metabolites

Many of the probiotic organisms that produce antimicrobial substances often times will have an advantage over organisms that grow and compete vigorously for intestinal sites for colonization. Antimicrobial substances produced and secreted by natural inhabitants of the intestinal tract can either kill or inhibit growth of pathogens (Rolfe, 1991). Generally, most bacteria produce agents that either kill or inhibit related species or even different strains of the same species of bacteria (Iglewski and Gerhardt, 1978). These products include the short chain volatile fatty (lactic, propionic, butyric, and acetic acids), hydrogen peroxide, and diacetyl and each has a different mode of action.

Additionally, there are metabolic products frequently classified as bacteriocins to distinguish them from antibiotics. Bacteriocins are produced by a large variety of

organisms and the bacteriocins are frequently mediated through plasmids (Mishra and Lambert, 1996). Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. It is assumed that some of the bacteria in the intestinal tract produce bacteriocins as a means to achieve a competitive advantage, and bacteriocin-producing bacteria might be a desirable part of competitive exclusion preparations (Joerger, 2003). In this capacity, the acid-loving *Lactobacilli* have shown that as a group, they produce significant amounts of bacterial growth inhibitory substances such as nisin and reuterin. Nisin is generally recognized as safe. Its mode of action is as a targeted membrane-permeabilizing peptide that induces pore formation in bacteria (Breukink *et al*., 2003). Reuterin, a product of glycerol metabolism that is secreted by *L. reuteri*, has broad-spectrum killing abilities in the intestinal tract of chickens (Dobrogosz *et al*., 1989; Talarico *et al.*, 1988; Talarico and Dobrogosz, 1989, 1990). *Bacillus subtilis* now used as an oral probiotic organism has a wide range of antimicrobial activities associated with a serine protease called subtilisin. It has been demonstrated that *Bacillis subtilis* facilitates the growth of another probiotic organism, *L. reuteri*, through production of catalase and subtilisin (Hosoi *et al*., 2001). Colicin is produced by *E. coli* to enhance their competitiveness in the gut of animals. Colicins are plasmid-encoded polypeptide toxins produced by and active against *E. coli* and closely related bacteria. The channel-forming colicins are transmembrane proteins that depolarize the cytoplasmic membrane, leading to dissipation of cellular energy (Parker *et al*., 1992; Braun *et al*., 1994).

2.4.1.4. Competition for Essential Nutrients

Competition for available nutrients as a means to control intestinal bacterial populations is probably not the most effective means for Competitive Exclusion (CE).

Rolfe (1991) indicated that there were many environmental factors that come into play that either enhances availability of nutrient from the diet of the host or through manipulation of dietary ingredients that enhances the growth of certain microbial populations which may result in exclusion of other bacterial species. A normal balance of bacteria in the gastrointestinal tract is capable of utilizing all of the potential carbon sources in the environment (Freter *et al*., 1983). It has been shown that by manipulating the lactose concentration in the diets of chicks and poults, one can selectively provide an advantage for the enhancement of *L. reuteri* (Casas *et al*., 1993, 1998). Behling and Wong (1994) gave day old chickens an *E. coli* (O75:H10) with 2.5% dietary lactose and found that there was significant protection against *S. enteritidis*. Using this method of deduction, provision of certain types of feed ingredients may also enhance the presence of certain other types of gut microflora. Oyofo *et al*. (1989) studied *in vitro* the effect of mannose on the colonization of *S. typhimurium* in chickens. They incubated intestinal sections, isolated from one-day-old chickens, with either radiolabeled-*S. typhimurium* strains ST-10 and ST-11 (mannose-sensitive), or strains Thax-1 and Thax-12 (non-yeast-agglutinating strains), or with only phosphate buffered saline in the presence of D-mannose, arabinose, methyl-a-D-mannoside, or galactose. The incubation of intestinal sections with bacteria and mannose resulted in a significant reduction of *S. typhimurium* adherence. This same group of investigators also confirmed this result *in vivo* (Oyofo *et al*., 1989). When they gave mannose orally to chickens and subsequently challenged the chickens with *S. typhimurium*, they reported that mannose inhibited *S. typhimurium* colonization to the intestine. In a different study, Oyofo *et al*. (1989c) tested other carbohydrates such as dextrose, sucrose, and maltose with little if any inhibition of colonization.

Since bacteria use lectins on their cell surface to bind to mannan on the intestinal epithelial cells to initiate attachment and colonization, it has been suggested that mannanoligosaccharide (MOS), a yeast cell wall derivative, might inhibit the colonization of bacteria to the intestine by binding to bacterial mannan-binding lectin. Spring *et al*. (2000) report that MOS (BioMos, Alltech, Inc., Nicholasville, KY USA) acts to bind and remove pathogens from the broiler chicken intestinal tract and stimulate the immune system. Swanson *et al.* (2002) investigated whether supplemental BioMos influenced microbial populations in dogs. Dogs treated with BioMos were shown to have a higher number of *Lactobacilli* that produce lactic acid as their major end product during fermentation of carbohydrates. Not only does BioMos inhibit the attachment of some enteropathogenic bacteria to the intestinal epithelium, but it also alters the numbers of the broiler chicken intestinal microflora (Spring *et al*., 2000). Fernandez *et al*. (2002) investigated the effect of BioMos on the number of microflora in chickens and showed that there was increased numbers of *Eubacterium spp*. and *Enterococcus spp*. while the number of *Bacteroides spp*. were found to be decreased. The increased number of these bacteria probably indirectly inhibited the colonization of pathogenic bacteria by preventing their attachment to the gastrointestinal epithelial cells. In a study in young turkeys fed BioMos, Bradley *et al*. (1995) observed improved body weight and altered ileum morphology. In the ileum, the crypt depth was less and the number of goblet cells per mm of villus was increased significantly. Edens *et al*. (1997a) reported an increase in goblet cell numbers and mucus secretion in the intestine of chickens challenged with *S. typhimurium*, but this condition was corrected by the application of a probiotic.

A recent study in mice has shown that *Saccharomyces cerevisiae* var. *boulardii* stimulated secretory IgA production (Rodrigues *et al*., 2001). *Saccharomyces*

cerevisiae NCYC 1026 is the basis for BioMos. BioMos also has been reported to exert an immuno-stimulatory characteristic. The levels of IgG in serum and IgA in bile and cecum were elevated in turkeys and rats, respectively, fed with BioMos compared to control (Kudoh *et al*., 1999). In addition, pigs fed BioMos had an increased number of blood lymphocytes (Spring and Privulescu, 1998). The elevated levels of IgA may be associated with increased rate of bacterial clearance via antibody-mediated phagocytosis.

2.4.1.5 Performance of Poultry Given Probiotics, Prebiotics and Synbiotics.

Body weight gain, feed conversion and reduced mortality are characteristics of performance that ultimately dictate whether managerial and company practices will be altered for acceptance of a new way of managing poultry. Mead (2000) described field experiences with competitive exclusion usage for control of Salmonella in poultry and clearly states that it is possible to control pathogen infection without sub-therapeutic antibiotic application, which was incompatible with probiotics. In field trials with market turkeys, *Lactobacillus reuteri* improved weight gain at 120 days of age by 4.8% (Casas *et al*., 1998). In ovo *Lactobacillus reuteri*-treated broiler chickens given a *S. typhymurium* challenge, body weights were improved by 206 g at 40 days of age and mortality was reduced by 32% (Edens *et al*., 1997a). Lan *et al*. (2003) reported that broiler chickens given *Lactobacillus agilis* JCM 1048 and *Lactobacillus salavarius* subsp. *salicinius* JCM 1230 significantly increased weight gain by 10.7%. Use of *Bacillus subtilis* (Calsporin; Calpis Corporation, Tokyo, Japan) did not improve body weight (Calsporin 2416 g *vs*. control 2407 g) at 42 days of age but feed conversion was improved (Calsporin 1.741 vs. control 1.773) (Edens, unpublished), but Fritts *et al*. (2000) have shown that Calsporin will improve broiler body weight

gain and fed conversion. There is only one report on a probiotic product based upon the presence of *Bacillus subtilis* in Calsporin that demonstrates the effectiveness of *Bacillus subtilis* in significantly reducing carcass contamination from enteric bacteria that have the potential to become human pathogens (Fritts *et al*., 2000). However, there are earlier reports indicating that *Bacillus subtilis* can effectively reduce the numbers of potential pathogens in faeces from broiler chickens (Maruta *et al.,* 1996a) and from swine (Maruta *et al*., 1996b).

Laying hens have needs that differ from broilers. Among the problems the laying hen encounters is *S. enteritidis* that contaminates eggs. As indicated already, it is possible to use probiotic bacteria to reduce or eliminate the *S. enteritidis* problem. However, there are other benefits to the egg producer. Pedroso *et al*. (1999) have reported that the use of probiotics (*Bacillus subtilis*) improved feed conversion and egg shell thickness. Improvement of these two factors alone will result in significantly improved profit margins for the egg producer.

The adaptation to the post hatching period and the increased stressors, deriving from practices used in modern broiler production, e.g. feed changes or imbalances, transportation, processing at the hatchery and high stocking densities (Pinchasov and Noy, 1993), may weaken immune functions and thus predispose broilers to colonization of the gastrointestinal tract by bacterial pathogens, posing a threat to birds health and food safety. Among pathogens, *Salmonella spp.* has been the most studied because of its ability to infect chickens and hens increasing the risk of contamination through the food chain (Humphrey, 2006). In the last years, application studies have been extended to other bacteria such as *Campylobacter jejuni and Clostridium perfringens,* which could be both considered an emerging and increasing

threat for poultry and human health (Humphrey *et al*., 2007; Van Immerseel *et al*., 2004).

Probiotics could be a possible strategy to control pathogen shedding and thus maintain a healthy indigenous gut microbiota.

The application of probiotics in poultry is strictly associated with the concept of competitive exclusion (CE). Since the first applications on new hatched chicks, several experiments with undefined and defined probiotic cultures have been developed and successfully applied to control and reduce Salmonella colonization. Moreover, it has been shown experimentally that the CE treatment also protect chicks against *C. jejuni, Listeria monocytogenes, pathogenic E. coli, Yersinia enterocolitica and C. perfrigens* (Nisbet, 2002; Schneitz, 2005).

A variety of well-characterized probiotic strains have been selected to evaluate modulation of the avian gut microbiota and protection against a variety of pathogens; in particular, there has been a recent increase in the investigation of the effect of feeding *Lactobacillus spp*. to broilers. Studies have focused on strains previously selected in vitro for adhesion properties and antimicrobial activity (Patterson and Burkholder, 2003).

Higgins *et al*. (2008) showed that Lactobacillus-based probiotic cultures significantly reduced Salmonella enteritidis recovery in challenged neonatal broiler chicks. Furthermore, administration by vent application, compared to traditional application by drinking water, resulted in significant reduction of *S. enteritidis* one hour following oral challenge. In a previous trial, the same probiotic cultures affected the

concentration of *S. enteritidis*, both in cecal tonsils and in cecal content, whereas no relevant results were obtained towards *S. typhimurium* (Higgins *et al.*, 2007).

No differences in cecal and colonic counts were observed testing the efficacy of *L. johnsonii* F19185 in reducing the colonization and shedding of *S. enteriditis* in newly hatched chicks; nevertheless, the colonization of *E. coli* O78K80 and *Clostridium perfringens* were compromised significantly (La Ragione *et al*., 2004). Lactobacilli were also successful in decreasing mortality due to necrotic enteritis from 60% to 30% in a challenge trial, when they were given orally at day 1 of life (Hofacre *et al*., 2003).

To date, few studies evidenced a possible role of probiotics in preventing the shedding of *C. jejuni* at the level of primary production, although *in vitro* studies reported a strong antimicrobial activity of several species of Lactobacillus towards this pathogen (Chaveerach *et al.*, 2004; Fooks and Gibson, 2002). Willis and Reid (2008) showed that *C. jejuni* presence was lower in broiler chickens fed with a standard diet supplemented with a minimum presence of 108 cfu/g of *L. acidophilus, L. casei, Bifidobacterium thermophilus,* and *E. faecium.*

With regard to probiotic microorganisms, other than *Lactobacillus spp*., Vila *et al.* (2009) reported a reduction of *S. enteritidis* colonization and invasion by continuously feeding spores of the probiotic strain *B. cereus var. toyoi*, both in broiler chickens and white leghorn chickens.

In a study conducted by La Ragione and Woodward (2003), 1-day-old and 20-day-old specific pathogen free chicks were dosed with a suspension of *B. subtilis* spores prior

to challenge with *S. enteritidis* and *C. perfringens*; the treatment suppressed completely the persistence and colonization of both pathogens.

Studies testing the use and efficacy of *Bifidobacterium spp.*, following pathogen challenge, have not yet been described. Mainly, authors have focused on the beneficial impact on the gut microbiota and growth performance (Estrada *et al.,* 2001; Jung *et al.*, 2008).

The use of bifidobacteria in poultry feeding is, to our knowledge, less common in comparison to lactobacilli administration.

Along with the control of food-borne pathogens in the avian gut, selected probiotic cultures, mainly *Lactobacillus spp.*, may also potentially increase performance parameters; among poultry farmers, objectives such as increasing growth rate, improving feed conversion and meat quality are undoubtedly of primary importance. Kalavathy *et al.* (2003) found that a supplementation of twelve Lactobacillus strains in broiler diets improved the bodyweight gain, feed conversion rate and was effective in reducing abdominal fat deposition.

Mountzouris *et al.* (2007) investigated the efficacy of selected probiotic bacteria, isolated from the gut of healthy chickens (*Lactobacillus reuteri, L. salivarius, Enterococcus faecium, Bifidobacterium animalis and Pediococcus acidilactici*) on body weight, feed intake and feed conversion ratio of broiler chickens; overall the probiotic formula added to water and feed displayed a growth promoting effect that was comparable to avilamycin treatment. In addition, the probiotic cultures modulated the composition and the enzymatic activities of the cecal microflora, resulting in a significant probiotic effect.

The available body of literature offers a variety of conflicting results concerning the efficacy of probiotics for increasing growth performance in broilers; inconsistent results have been also reported from other authors (Estrada *et al.*, 2001; O'Dea *et al*., 2006) showing a confusing state of the art. Timmerman *et al.* (2006) underlined the importance of way and timing in the administration as main factors affecting the efficacy of the probiotic preparations. Administration via the feed, compared to administration in the drinking water, resulted in a higher increase of average daily gain; moreover the supplementation of probiotics during early life is of great importance to the host because the bacteria can modulate expression of genes in intestinal epithelial cells, thus creating a favourable habitat for themselves.

Eggs production has been also investigated in relation to probiotic application; Davis and Anderson (2002) reported that a mixed cultures of *Lactobacillus acidophilus, L. casei, Bifidobacterium thermophilus* and *Enterococcus faecium*, improved egg size and lowered feed cost in laying hens. Moreover, probiotics increase egg production and quality (Kurtoglu *et al.*, 2004; Panda *et al.*, 2008).

The prebiotic approach has not a long history of use in broiler chickens (Yang *et al.*, 2009). However, application studies have been increasing in the last years to assess their effect on gut health, performance, and reduction of pathogen shedding. Xu *et al.* (2003) found a dose-dependent effect of fructooligosaccharides (FOS) on average daily gain; whereas Juskiewicz *et al.* (2006) reported no impact on the performance or productivity of turkeys after feeding for eight weeks with different amounts of FOS.

By feeding chicory fructans to broilers, Jin *et al.*, (2008) showed an improvement in weight gain, feed conversion, carcass weight and serum cholesterol decrease; additionally, the supplementation of fructans resulted in increase of lactobacilli counts

in the gastrointestinal tract and Campylobacter and Salmonella decrease (Jin *et al.*, 2008). Kleessen *et al.* (2003) described decreased *C. perfringens* number and a reduction in bacterial endotoxin levels by adding 0.5% of fructan-rich Jerusalem artichokes syrup in broilers drinking water.

No weight gain was observed in turkeys fed two different concentration of inulin and mannanoligosaccharides (MOS) (Stanczuk *et al.*, 2005), whereas Sims *et al.* (2004), feeding turkeys a standard diet supplemented with MOS, reported an improvement on live weight.

Yeast cell wall containing MOS reduced intestinal Salmonella concentrations by 26% in broiler chicks compared with chicks fed an unsupplemented diet (Spring *et al.*, 2000). Thitaram *et al.* (2005), with different amounts of isomaltooligosaccharide (IMO), showed a significant 2-log reduction in the level of inoculated *S. enterica* serovar typhimurium present in the ceca of young broiler chickens. Feed consumption, feed conversion and feed efficiency were not significantly changed compared to the control; however, the IMO containing diets significantly increased the number of the intestinal bifidobacteria. Feeding young chicks with five different oligosaccharides (inulinoligofructose, mannanoligosaccharide, short-chain fructooligosaccharide, and transgalactooligosaccharide), no significant responses in weight gain for any of the oligosaccharides fed have been registered. Moreover the study outlined that a high dosage of prebiotics can have negative effects on the gut system and retards the growth rate of birds (Biggs *et al.*, 2007).

Likewise, a recent study reported no effects in body weight, feed intake and feed conversion ratio in broiler chickens fed with a standard diet and GOS at two different

concentrations; however the study clearly showed a significant increase in the intestinal bifidobacteria population (Jung *et al.,* 2008).

Mainly, prebiotics seem to selectively enhance lactobacilli and bifidobacteria populations and reduce colonization by pathogenic bacteria (Baurhoo *et al.,* 2009; Biggs and Parsons, 2008).

Results on animal performance, either with a probiotic or a prebiotic treatment, are often contradictory and mostly affected by the microorganisms or compound chosen, the dietary supplementation level, and duration of use. In many cases, the environmental and the stress status of the animals are not reported or considered, as the experimental settings are often too far from farm conditions.

Recent development and applications of synbiotic products have focused on the assessment of beneficial effects in poultry health and production; however, information available to date is scarce. Mohnl *et al.* (2007) found that a synbiotic product had a comparable potential to improve broiler performance as avilamycin treatment. A *Lactobacillus spp*.-based probiotic product, in combination with dietary lactose, was successfully assessed, improving body weight and feed conversion in Salmonella-challenged turkeys (Vicente *et al.*, 2007). Li *et al.* (2008), adding FOS and *B. subtilis* to the diet, observed that average daily gain and feed conversion ratio were improved; diarrhoea and mortality rate were reduced compared to aureomycin treatment.

A considerable increase in the bifidobacteria, lactobacilli and total anaerobes populations has been shown when feeding a diet containing a combination of a

galactooligosaccharide and Bifidobacterium lactis but no effect on body weight, feed intake and feed conversion was observed (Jung *et al.,* 2008).

Awad *et al.* (2009) investigated the effect of a dietary treatment with a synbiotic product (a combination of *E. faecium*, a prebiotic derived from chicory, and immune modulating substances derived from sea algae) on broiler chickens. Body weight, average daily weight gain, carcass yield percentage, and feed conversion rate were significantly increased compared with the control, whereas no increase in organ weight was found, with exception for the small intestine; a significant increase in the villus height in both duodenum and ileum was also observed.

Overall, all the authors agreed that a synbiotic product displayed a greater effect than individual preparations (Awad *et al.*, 2009; Jung *et al.*, 2008; Revolledo *et al.*, 2009; Vandeplas *et al.*, 2009). This coupling could represent an important and synergistic strategy to improve gut health of chickens from the first days of life and control pathogen release in the environment, decreasing the risk of foodborne infections in humans. Thus, future research and applications in field trials are necessary to look for new combinations with the aim to produce standard safe compositions at a high functional level.

Starvric and Kornegay (1995); Jin *et al.* (1998); Zulkifii *et al.* (2000); Simmering and Blaut (2001); Kabir *et al.* (2005); and Apata (2008); summarized the beneficial effects of probiotics in poultry as follows;

- Enhance growth performance
- Modify intestinal microbiota
- Improve nutrient digestibility
- Stimulate immune system

- Lower serum cholesterol
- Reduce inflammatory reactions
- Decrease carcass contamination
- Prevent pathogen colonization
- Increase feed efficiency
- Improve carcass yield and sensory characteristics

2.5.1 Effects of DFM on the Gastrointestinal Microflora

Several different microorganisms coexist in the gastrointestinal tract most of which are bacterial population (Gaggia *et al.*, 2008) which allow the digestion of compounds, such as cellulose, that require specific sets of enzymes. The bacteria are able to benefit from this habitat by making use of the energy provided by ingested food and the stable synergistic habitat as reported by Gaggia *et al.* (2008).

The DFM enhances the balance between beneficial and pathogenic bacteria within this microflora in a normally functioning gastro- intestinal tract (without any intestinal disorders) since any disorder or stress could impact feed intake, nutrient conversion and survival rate. In addition to the beneficial effect of DFM on access to nutrients, it also improves the action of bacteria on intestinal physiology, morphology, mucus secretion, metabolism and immune functions (Shirkey *et al*., 2006) thereby stabilizing the digestive microflora and for them to compete with pathogenic microflora.

2.5.2 Effects of DFM on Nutrient Synthesis and Digestibility

The intestine is an organ that has the function of maximizing nutrient uptake and to minimize antigenic disturbance while tolerating the presence of indigenous micro-biota and other antigens introduced by the presence of feed within the intestinal tract.

Direct-fed microbial help to enhance nutrient utilization, synthesis, and digestibility and production performance by reducing the competitions that exist between the host and its enteric pathogenic microflora as related by Santos *et al.* (2005). However, apart from nutrient synthesis, probiotics may improve the digestibility of some dietary nutrients such as carbohydrates, fats and proteins (Friend and Shahani, 1984) by increasing the activities of enzymes such as lactase, lipase and peptidase respectively. Other reports however show no effect on digestibility of Dry Matter (DM), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and amino acid when pigs were fed probiotics containing *Lactobacillus* or *Bacillus* cultures (Kornegay *et al*., 1995).

2.5.3 Effects of DFM on Growth Performance

The number of eggs produced in layer poultry production is one of the key indicators affecting the profitability of layer production even though egg weight and size are equally important. Improvement in egg numbers and feed to gain ratio will result in improved profitability due to greater output and reduction in overhead feed costs.

Probiotics become beneficial to the host animal by increasing competition for adhesion receptors and nutrients with the pathogenic bacteria in the gut besides producing antibacterial substances which help in controlling the pathogenic gut microflora (Fuller, 1989). However some other factors can make the effects of probiotics more complicated these include the environmental conditions of the research site, handling of the animals, genetic background of the animals, different stress factors, composition of gut microflora in the animals and chances for cross-contamination (Jonsson and Conway, 1992).

Types of microorganisms (bacteria and fungi) and carriers in probiotics can also cause modifications in gut microorganism populations and as a result intestinal health modifications (Hill *et al.*, 1986).

2.5.4 Effects of DFM on the Immune System

Kabir *et al.* (2004) evaluated the dynamics of probiotics on immune response of broilers and they reported significantly higher antibody production ($P<0.01$) in experimental birds as compared to control ones. They also demonstrated that the differences in the weight of spleen and bursa of probiotics and conventional fed broilers could be attributed to different level of antibody production in response to SRBC. Similarly, Khaksefidi and Ghoorchi (2006) reported that the antibody titer in the 50 mg/kg probiotic supplemented group was significantly higher at 5 and 10 days of postimmunization (PI) compared to control, when SRBC was injected at 7 and 14 days of age. In addition, Haghighi *et al.* (2005) demonstrated that administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. On the other hand, Dalloul *et al.* (2005) examined the effects of feeding a *Lactobacillus*-based probiotic on the intestinal immune responses of broiler chickens over the course of an *E. acervulina* infection and they demonstrated that the probiotic continued to afford some measure of protection through immune modulation despite a fairly overwhelming dose of *E. acervulina*. They also suggested a positive impact of the probiotic in stimulating some of the early immune responses against *E. acervulina*, as characterized by early IFN-γ and IL-2 secretions, resulting in improved local immune defenses against coccidiosis. Brisbin *et al.* (2008) investigated spatial and temporal expression of immune system genes in chicken cecal tonsil and spleen mononuclear cells in response to structural constituents of *L. acidophilus* and they

found that cecal tonsil cells responded more rapidly than spleen cells to the bacterial stimuli, with the most potent stimulus for cecal tonsil cells being DNA and for splenocytes being the bacterial cell wall components. They also discovered that in both splenocytes and cecal tonsil cells, STAT2 and STAT4 genes were highly induced and the expression of STAT2, STAT4, IL-18, MyD88, IFN-alpha, and IFN-gamma genes were up-regulated in cecal tonsil cells after treatment with *L. acidophilus* DNA. Simultaneously, several investigators demonstrated the potential effect of probiotic on immunomodulation (Matsuzaki and Chin, 2000; Zulkifli *et al*., 2000; Dalloul *et al.*, 2003; Koenen *et al.*, 2004; Haghighi *et al.*, 2005; Mathivanan *et al.*, 2007; Nayebpor *et al.*, 2007; Apata, 2008). On the other hand, Midilli *et al.* (2008) showed the ineffectiveness of additive supplementation of probiotics on systemic IgG.

2.5.6 Haematological Data and their Relevance in Animal Studies

An analysis of haematological parameters of chickens is vital for the diagnosis of various pathological and metabolic disorders. Blood analysis is performed as a diagnostic tool to assess the health status of humans or animals. Any haematological changes observed through the analysis are used to determine the body status or health condition, metabolic profile, production patterns and to assess the impact of environmental, nutritional and pathological stresses on the animal. Haematological parameters provide valuable information on the immune status of animals (Kral and Suchy, 2000) as well as serve as indicators of physiological state of birds (Castagliulo *et al.*, 1996; Sarker *et al.*, 1995; Chowdhury *et al.*, 2005). Such information, apart from being useful for diagnostic and management purposes, could equally be incorporated into breeding programmes. Conducting haematological studies helps to

determine the normal physiological values (Table 6) under local conditions for proper management, feeding, breeding, prevention and treatment of diseases.

Studies of haematological parameters in birds show that they are influenced by some factors such as age, sex, season and nutrition. Oyewale and Ajibade (1990) and Pavlak *et al*. (2005) observed that the PCV and Hb values tend to be higher in males than in females in turkeys and pigeons. Packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC) have been reported to increase with age in chickens (Islam *et al*., 2004). Table 6 shows normal values of haematological parameters of chickens.

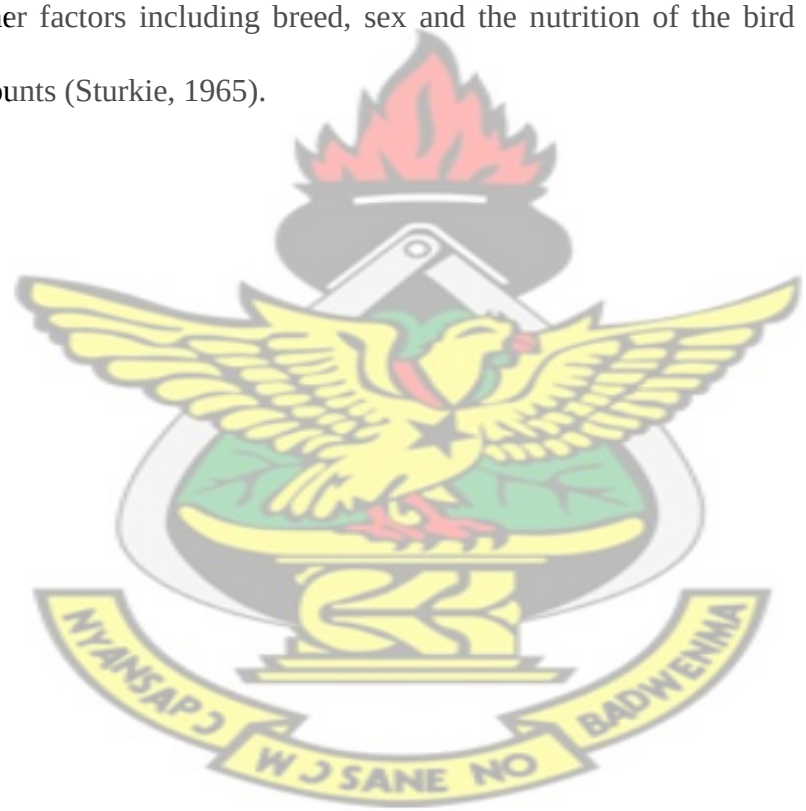
Table 6: Normal Blood Values for the Chicken (*Gallus gallus domesticus*)

ERYTHROCYTIC SERIES			LEUKOCYTIC SERIES		
Parameters	Range	Mean	Parameters	Range	Mean
Erythrocytes ($x10^6/\mu l$)	2.5-3.5	3.0	Leukocytes /µl	12,000-30,000	12,000
Haemoglobin (g/dl)	7.0-13.0	9.0	Heterophil (band)	Rare	-
PCV (%)	22.0-35.0	30.0	Heterophil (mature)	3,000-6,000	4,500
MCV (fl)	90.0-140.0	115.0	Lymphocyte	7,000-17,500	14,000
MCH (pg)	33.0-47.0	41.0	Monocyte	150 - 2,000	1,300
MCHC (%)	26.0-35.0	29.0	Eosinophil	0-1,000	400
Reticulocytes (%)	0-0.6	0.0	Basophil	Rare	-
ESR (mm)*	3.0-12.0	7.0			
RBC size (µm)	7.0x12.0		% distribution		
Other data			Parameters	Range	Mean
Thrombocytes ($x10^3/\mu l$)	20.0-40.0	30.0	Heterophil	150-400	28.0
Icterus index (units)	2-5	2	Lymphocyte	45.0-70.0	60.0
Plasma proteins (g/dl)	4.0-5.5	4.5	Monocyte	5.0-10.0	8.0
Fibrinogen (g/dl)	0.1-0.4	0.2	Eosinophil	1.5-6.0	4.0
Erythrocytes life span (days)	20-35 days		Basophil	Rare	-

ESR determined after 1 hour at 45* angle

Source: Jain (1993)

Jain (1993) reported that many factors influence the composition of blood drawn from animals, namely, time of day, genetic factors (breed or strain), age, sex, nutrition, environmental conditions, physiological status, capillary or heart blood, anaesthesia and type of anaesthetics and the animal's state of excitement. Similar reports have been provided by Sanni *et al*. (2000) and Piccione *et al*. (2001, 2005) that **haematological parameters** are also influenced by diurnal fluctuations or changes in daily physical and metabolic activities. The mean haematological values of RBC, Hb and erythrocyte sedimentation rate (ESR) of birds vary among the various species and that other factors including breed, sex and the nutrition of the bird also affects the RBC counts (Sturkie, 1965).



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Duration of the Project

Two studies were conducted at the Poultry Section of the Department of Animal Science of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi for a total period of 8 weeks each to determine the effect of DFM in broiler production. The first experiment using the DFM in feed was carried out from February to April, 2013. Mean annual rainfall of 1100 mm and mean monthly temperature of 34.0^{o} C were recorded during this period. The second experiment using DFM in water was also carried out from July to August, 2013 with mean annual rainfall of 1600 mm and mean monthly temperature of 33.33^{o} C (Ghana Agro-Meteorological Station, 2013).

3.2 Experimental animals and design of experiment

Three hundred (300) unsexed day old Cobb commercial strains of broiler chickens were used for each study. The chicks were obtained from Akate Farms and reared in a separate brooder house for the first 28 days (0 - 4 weeks). One hundred (100) watt electric bulbs were used to provide continuous light and heat during the brooding stage. The diets offered in the first experiment contained 22.20% CP with a metabolizable energy (M.E) of 2884 kcal kg^{-1} while that of the second experiment contained 22.8% CP with metabolizable energy (M.E) of 2833 kcal kg^{-1}. The diets were fed to the bird's *ad-libitum*. In experiment 1, the control diet (T1) did not contain any DFM, whereas T2 (DFM-1) contained 1.5ml Rumen enhancer -3 (RE-3), T3(DFM-2) contained 1.5ml Fermented product of rumen enhancer -3(RE-3+) and T4(P-3) also contained DFM that had a combination of 1ml rumen enhancer and

0.5ml *P. polymyxa* to form 1.5 ml P-3. Each of these DFM products was incorporated in a kilogram of feed.

In experiment 2, the control diet (T1) did not contain any DFM, whereas T2 (DFM-1) contained 1.5ml Rumen enhancer -3 (RE-3), T3(DFM-2) contained 1.5ml Fermented product of rumen enhancer -3(RE-3+) and T4(P-3) also contained DFM that had a combination of 1ml rumen enhancer and 0.5ml *P. polymyxa* to form 1.5ml P-3. Each of these DFM products was incorporated in a litre of water. Feed and water were provided *ad- libitum*. At 28 days of age, two hundred and forty birds (240) each were randomly selected and divided into four groups, each group constituting a treatment with four replicates per treatment in a completely randomised design (CRD). Each replicate group of fifteen birds (5 males and 10 females) each was maintained in a coop. The two trials lasted for 56 days each and each of the four groups of birds received one of the dietary treatments for 8 weeks. Feed and water were provided *ad-libitum.* The compositions of the first and second experimental broiler diets and their chemical compositions are presented in Tables 7 and 8.

Table 7: Chemical Composition of Experimental Broiler Diets (DFM)

	TREATMENTS:	Direct-Fed Microbials (DFM)		
Ingredients (g kg^{-1})	**Control (T1)**	**RE3 (T2)**	**RE3+ (T3)**	**P3 (T4)**
DFM(ml)	0	150.00	150.00	150.00
Maize	60.00	60.00	60.00	60.00
Soyabean meal	18.00	18.00	18.00	18.00
Fish meal	10.00	10.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	10.00
Oyster shell	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.25	0.25	0.25	0.25
Vit/mineral premix*	0.50	0.50	0.50	0.50
Salt (NaCl)	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100
Chemical Composition (g kg^{-1}DM)				
Crude protein	22.20	22.20	22.20	22.20
Crude fibre	3.43	3.43	3.43	3.43
Ether extract	4.00	4.00	4.00	4.00
Ash	8.26	8.26	8.26	8.26
Moisture	11	11	11	11
Nitrogen Free Extract	51.11	51.11	51.11	51.11
Lysine	13.06	13.06	13.06	13.06
Methionine	5.05	5.05	5.05	5.05
Cystine	3.44	3.44	3.44	3.44
ME (kcal/kg) calculated	2857.40	2857.40	2857.40	2857.40

*Premix supplied (kg^{-1}diet); 10,000 IU Vit A; 2000 IU Vit D3; 10 IU Vit E; 3 mg Vit K; 2.5 mg Riboflavin; 0.05 mg Cobalamin; 5 mg Panthothenic acid; 12.5 mg Niacin; 175 mg Choline; 0.5 mg Folic acid; 2.8 mg Manganese; 0.5 mg Iron; 2.5 mg Zinc; 625 mg Cobalt.

Table 8: Chemical Composition of the Experimental Broiler Diets (DFM)

	TREATMENTS: Direct-Fed Microbials (DFM)			
Ingredients (g kg^{-1})	**Control (T1)**	**RE3 (T2)**	**RE3+ (T3)**	**P3 (T4)**
Maize	60.00	60.00	60.00	60.00
Soyabean meal	18.00	18.00	18.00	18.00
Fish meal	10.00	10.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	10.00
Oyster shell	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.25	0.25	0.25	0.25
Vit/mineral premix*	0.50	0.50	0.50	0.50
Salt (NaCl)	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100
Chemical Composition (g kg^{-1}DM)				
Crude protein	22.80	22.80	22.80	22.80
Crude fibre	3.31	3.31	3.31	3.31
Ether extract	5.00	5.00	5.00	5.00
Ash	8.00	8.00	8.00	8.00
Moisture	11	11	11	11
Nitrogen Free Extract	50.11	50.11	50.11	50.11
Lysine	13.06	13.06	13.06	13.06
Methionine	5.05	5.05	5.05	5.05
Cystine	3.44	3.44	3.44	3.44
ME (kcal/kg) calculated	2833.20	2833.20	2833.20	2833.20

*Premix supplied (kg^{-1}diet); 10,000 IU Vit A; 2000 IU Vit D3; 10 IU Vit E; 3 mg Vit K; 2.5 mg Riboflavin; 0.05 mg Cobalamin; 5 mg Panthothenic acid; 12.5 mg Niacin; 175 mg Choline; 0.5 mg Folic acid; 2.8 mg Manganese; 0.5 mg Iron; 2.5 mg Zinc; 625 mg Cobalt.

Routine and periodic management practices such as vaccination, drug administration and maintenance of cleanliness within and outside the poultry pens were carried out. Birds were vaccinated against Gumboro, Newcastle disease and the control were medicated against Coccidiosis at 3 days of age and again at third week using Sulfadimidine Sodium 33% (Bremer Pharma GMBH, Germany) via the drinking water.

3.2.1. Chemical Analysis:

Proximate analysis of each experimental diet was carried out at the Department of Animal Science as described by the (AOAC, 1990). Metabolizable energy was computed using the equation of NRC (1985):

ME (kcal/kg) = (35x % CP) + (85x % CF) + (35x % NFE).

3.3. Parameters Measured

3.3.1. Feed Intake, Weight Gain, Feed Conversion Ratio and Live Weight Changes

Data pertaining to performance traits such as growth, feed conversion ratio, percent mortality, and body weights were recorded by weighing individual chicks at weekly interval up to 8 weeks of age for comparative evaluation and interaction effects of all treatments. Chicks were fed *ad-libitum*. Difference in initial and final body weight represented the weight gained by chicks over the corresponding period. Weighed amounts of diet were provided to chicks. Feed consumed and weight gains were recorded weekly. The percent mortality was also regularly recorded for each group. The biweekly records of the feed offered and residual amounts of weigh backs were maintained for each replicate to calculate the feed consumption per bird. All birds in each replicate were weighed at biweekly intervals using a scale and a weighing cage and then the weight divided by the total number of birds in each coop to get a representative mean weight for each bird to calculate for body weight gains. Feed Conversion Ratio (FCR) was calculated by the standard formula using feed eaten (g) / bird divided by weight gain (g). To know the status of mortality daily observations were made to record the occurrence of deaths in each experimental treatments.

3.4. Blood Collection and Assays

At 56 days of age, blood samples from 2 birds (male and female) per replicate in each experiment were collected for haematological assay using a sterile syringe. These included red blood cells (RBC) count, white blood cells (WBC) differential, haemoglobin (Hb) and haematocrit (PVC). Five millilitres (5ml) of blood was collected from each broiler into vacuutaner tubes containing ethylene diamine tetracetic acid (EDTA) as an anticoagulant. Haemoglobin was determined using the cyanmethemoglobin method described by Cheesbrough (2001). Haematocrit was determined using the microcapillary method (Mukherjee, 2005), RBC by Dacie and Lewis (2000) method and WBC by the method described by Holfbrand and Petit (2000). Each determination was made in duplicate and the mean calculated. Various haematological indices like mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were calculated from results obtained. Total Protein (T.protein), Total Cholesterol (T.Chol), Triglycerides (TGS), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) using auto analyzer called Sysmex KX-21N (Japan) and Flexor Junior (Netherlands), respectively in the estimation.

3.5.1. Microbiological Faecal Analysis

Faecal samples for microbiological analysis were taken at the end of each experiment using disposable hand gloves to prevent self-and-sample contamination. The Most Probable Number (MPN) method was used to determine total and faecal coliforms in the samples. Serial dilutions of 10^{-1} and 10^{-4} were prepared by weighing 1g of the sample into 10 ml sterile distilled water. One milliliter aliquots from each of the dilutions were incubated into 5 ml of MacConey Broth for 35^{o}C for total coliforms

and 44ºC faecal coliforms for 18-24 hours. Tubes showing colour change from purple to yellow after 24 hours were identified as positive for faecal coliforms. Counts per 100 ml were calculated from MPN tables. Additionally, the Gram stain technique was used to facilitate microscopic examination of morphological characteristics of the various bacteria.

3.5.1.1 E. coli (Thermotolerant Coliforms)

From each of the positive tubes identified, a drop was transferred into a 5 ml test tube of tryptone water and incubated at 44ºC for 24 hours. A drop of Kovacs' reagent was then added to the tube of tryptone water. All tubes showing a red ring colour development after gentle agitation denoted the presence of indole and recorded as presumptive for thermotolerant coliforms *(E. coli).* Counts per 100 ml were calculated from MPN tables.

3.5.1.2 Faecal Enterococci

Serial dilutions of 10^{-1} and 10^{-4} were prepared by measuring 1ml of the sample into 9ml sterile distilled water. One milliliter aliquots from each of the dilutions were inoculated on a Slanetz and Barltey Agar prepared on sterile petri dishes. The petri dishes were preincubated at a 37ºC for 4hours to aid bacterial resuscitation. The plates were then incubated at a 44ºC for further 44 hours. After incubation, all red, maroon and pink colonies that were smooth and convex were counted and recorded as faecal enteroccoci.

3.5.1.3 Salmonella

Serial diluted sample was added to 10 ml Buffered Peptone Water (BPW) and incubated at 37ºC for 24 hours. Then 0.1ml of the sample from the BPW was

transferred into 10ml of selenite broth in universal bottle and incubated at 44ºC for 48 hours. Swaps from the bottle onto SS agar and incubated at 37ºC for 48 hours. Blank colonies on the SS agar indicate the presence of salmonella.

3.6. Carcass Analysis

At the end of each experiment, 2 chickens (1 male and 1 female) were taken from each replicate, which represented the average weight of the group for carcass evaluation. Preslaughter live weight for each chicken was taken. Dressing percentage and weight of organs were measured. The organs were expressed as a percent of live weight.

3.7. Economics of Production

Economics of production was based on the feed cost per kilogram diet and feed cost to produce a kilogram (kg) body weight. Feed cost per kilogram for each of the experimental diets was estimated based on the prevailing prices of the feed ingredients at the time of each trial. Feed cost to produce a kg body weight was calculated as the product of the feed cost per kg and feed conversion ratio for individual dietary treatments.

3.8. Statistical Analysis:

The data collected was subjected to one-way analysis of variance (ANOVA) using GenStat (2012) Version (12) and the least significant difference (Lsd) was used to separate the treatment means.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1. EXPERIMENT ONE: DFM (RE3, RE3+ and P3) in Feed for Broiler Chickens.

4.1.2. Effect of Probiotic on Growth Performance and Carcass Parameters of Broiler Chickens.

Data on the general performance of the broiler chickens fed diets containing RE3, RE3+ and P3 are summarized in Table 9.

4.1.3. Feed Intake

From the experiment, it was realized that there was no significant ($p>0.05$) difference in feed intake, but the birds on the control diet tended to eat more than their counterparts on the probiotic treated diets.

In a previous rat study, using the same DFM product, there were no significant differences ($P>0.05$) in the mean feed intake among the dietary treatments (Okai, 2008). Furthermore, other researchers had reported similar results for mean daily feed intake of broilers (Bonsu, 2009; Dei, 2010). Broilers on the Control diet recorded the highest total feed intake followed by P3, RE3+ and RE3 though there were again no significant ($P>0.05$) differences among them.

4.1.4. Body Weight, Weight Gain and Feed Conversion Ratio

There were no significant differences ($p>0.05$) among the mean values for the final body weight, total weight gain and feed conversion ratio of broiler birds fed the DFM diets and those devoid of the DFM. However, numerical differences exist among the DFM-fed diets and the control. These results were clearly evident from the findings of

many investigators who demonstrated no beneficial effects (Goodling *et al.*, 1987; Maiolino *et al.*, 1992; Owings, 1992; Karaoglu and Durdag, 2005) of DFM on boby weight, weight gain and feed conversion ratio.

4.1.5. Percentage Mortality

Mortality of the broiler chickens was not significantly (P>0.05) affected by the dietary treatments. However, there were numerical differences in mortality among the DFM-fed experimental animals and the control groups. A total of eleven (11) birds were recorded dead, Six (6), one (1), two (2) and two (2) for control, RE3, RE3+ and P3 respectively representing 1.50, 0.25, 0.50 and 0.50% respectively. This result is in agreement to the findings of Bonsu (2010), Dei *et al*., (2010), Lalev *et al*., (2011), and Arpasova *et al*., (2012); who observed that, probiotics increases resistance to infectious diseases and reduces risk of mortality caused by the presence of infectious diseases. Research has shown that when animals are fed certain strains of bacteria, the activity of their immune systems increases (Choudhari *et al*., 2008) and this must have accounted for non occurrence of any pathogenic disease.

Table 9. Effect of DFM on Growth Performance and Carcass Parameters of Broiler Chickens.

PARAMETERS	TREATMENT					
	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	Lsd	FPr
Initial Weight (g)	43.00	43.00	43.00	43.00	-	-
Total Feed Intake (g)	4715	4685	4672	4708	44.5	0.264
Final Body Weight (g)	2350	2555	2448	2278	275.9	0.199
Total Weight Gain (g)	2307	2512	2405	2235	275.6	0.194
FCR	2.04	1.87	1.94	2.11	0.24	0.163
Mortality (%)	1.500	0.250	0.500	0.500	1.238	0.183
Carcass characteristics						
Carcass yield (% of LBW)	0.75	0.73	0.86	0.85	1.481	0.06
Organ weights (g)						
Gizzard Weight.	76.349	74.923	74.918	69.693	16.48	0.825
Intestine Weight.	147.685	150.226	153.148	156.050	29.38	0.932
Liver Weight.	42.288	45.471	46.815	43.965	8.14	0.661
Heart Weight.	10.815	8.898	10.423	10.568	1.634	0.094
Economy of gain						
Cost/kg(GH₵)	1.26	1.30	1.30	1.30	0.045	-
Cost/kg weight gain	2.57	2.43	2.52	2.74	0.310	0.102

4.1.6. Carcass Characteristics and Organ Weights of Broiler Chickens

The relative organ weights of the probiotic-fed broilers did not differ significantly ($P < 0.05$) from their control counterparts, as no differences were observed in the other carcass parameters too. Carcass yield percentages were higher for the probiotic-fed broilers than for the control. The results are in agreement with the work done by Willis *et al.*,(2007), that DFM supplementation did not significantly (P>0.05) affect carcass weight of broiler birds.

4.1.7. Effect of Probiotic on Haemato-Biochemical Parameters of Broiler Chickens.

The results of the study indicated that haematological parameters were not significantly different between treatments (P>0.05) except for total protein, albumin and globulin as presented in Table 10. However, the results obtained were within the normal range for healthy birds as stated by Aiello and Mays, (1998), Awaad and Zouelfeker, (2001), Campbell *et al*., (2003) and Pampori, (2003). In addition, Haghighi *et al.* (2005) demonstrated that administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. Blood cellular and biochemical indices of chickens provide valuable information on the immune status of animals (Kral and Suchy, 2000) as well as serve as indicators of physiological state of birds.

Table 10. Effect of DFM on Haemato-Biochemical parameters of broiler chickens.

PARAMETERS	TREATMENTS					
Haematology	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	LSD	FPr
WBC (mm^3x10^3)	246.2	240.2	246.2	241.5	14.31	0.716
RBC ($3x10^6/l$)	2.450	2.300	2.500	2.300	0.3176	0.426
HB (g/dl)	10.15	9.25	9.80	9.15	1.245	0.301
PCV (%)	33.35	30.32	32.73	30.45	3.430	0.176
MCV (fl)	135.00	129.25	131.75	131.00	4.943	0.135
MCH (Pg)	41.25	39.48	39.45	39.40	1.884	0.142
MCHC (g/dl)	30.43	30.40	31.75	30.05	1.811	0.236
Blood chemistry						
Albumin ($g\ l^{-1}$)	11.25^b	14.00^a	13.75^a	14.75^a	2.279	0.030
Globulin ($g\ l^{-1}$)	14.50^b	17.25^a	17.25^a	17.50^a	2.156	0.032
HDL ($mmoll^{-1}$)	1.250	1.200	1.000	1.225	0.3261	0.363
LDL ($mmoll^{-1}$)	0.875	0.825	1.100	1.000	0.3708	0.400
TGS ($mmoll^{-1}$)	1.000	1.100	0.850	0.875	0.3199	0.335
T-CHOL ($mmoll^{-1}$)	2.62	2.80	3.35	3.10	0.677	0.149
T-PROT ($g\ l^{-1}$)	26.00^b	31.25^a	31.00^a	32.25^a	4.346	0.036

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$).

HB =Haemoglobin, PCV = Packed Cell Volume or HCT= haematocrit, RBC = Red Blood Cell, WBC = white blood cell, MCV = mean cell volume, MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, T.protein = Total Protein, T.Chol = Total Cholesterol, TGS = Triglycerides, HDL =High Density Lipoprotein, LDL = Low Density Lipoprotein, LSD=Least Significant Difference, P-Value=Probability Value.

4.1.8. Effect of Probiotic on the Intestinal Microbiota of Broiler Chickens

From the experiment, it was realized that significant ($p<0.05$) differences were observed among the faecal enterococci. The results are in agreement to the findings of (Rada *et al*., 1995; Jin *et al*., 1998; Line *et al*., 1998; Pascual *et al*., 1999; Kabir *et al*., 2005; Yaman *et al*., 2006; Higgins *et al*., 2007; Mountzouris *et al*., 2007); who observed that, in broiler nutrition, probiotic species belonging to *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus*, *Candida*, and *Saccharomyces* have a beneficial effect on modulation of intestinal microflora and pathogen inhibition. Their results revealed competitive antagonism. However there were some numerical differences between the other intestinal microbiota of the control and the DFM-treated experimental animals as shown in table 11.

Table 11. Effect of DFM on Intestinal Microbiota of Broiler Chicken

PARAMETERS	TREATMENT					
	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	LSD	FPr
E.Coli {cfu}	$2.4*10^7$	$6.0*10^6$	$1.3*10^6$	$6.2*10^6$	$3.8*10^7$	0.587
Enterococci {cfu}	199^a	46^b	68^b	86^b	153.5	0.005
Salmonella {cfu}	$7.5*10^4$	$4.8*10^4$	$1.8*10^4$	$2.3*10^4$	$3.2*10^4$	0.091

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$).

4.1.9. Feed Cost and Economy of Gain

The feed cost per kg of the Control, RE3, RE3+ and P3 diets were GH¢ 1.26, GH¢ 1.30, GH¢ 1.30 and GH¢ 1.30 respectively. The differences in the cost values were attributed to high increment of DFM at the commencement of the experiment. Broilers on the RE3 diets were more efficient with respect to feed to gain ratio (Table 9), however, it could be deduced that, it was more economical to raise broilers on the probiotic containing diets. Feed costs per kg gain of the various diets were GH¢ 2.57 (Control), GH¢ 2.43 (RE3), GH¢ 2.53 (RE3+) and GH¢ 2.47 (P3). There was no significant difference (P>0.05) between the value recorded for RE3 and the other dietary treatments (Table 9).). Feed accounts for up to 80% of the costs in production of poultry in Ghana and other developing countries (Adesehinwa, 2007). It is therefore essential that farmers regulate the level of administration in feed formulation so as to optimize profit.

4.2. EXPERIMENT TWO: DFM (RE3, RE3+ and P3) in water for Broiler chickens.

4.2.1. Effect of Probiotic on Growth Performance and Carcass Parameters of Broiler Chickens.

A summary of the growth performance and carcass characteristics of the bird population for experiment two is shown in Table 12

4.2.2. Feed Intake

The results from the experiment indicated no significant ($P > 0.05$) effects of DFM on feed intake (Table 12). Average feed consumption varied between diets, but was not statistically different.

Many factors affect feed consumption in animals including physical texture, presence of anti-nutritive factors, dietary energy and protein contents (Donkoh *et al.,* 2012). The mean values for total feed intake were 4647g, 4608g, 4628g and 4637g for dietary treatments Control, RE3 and RE3+ and P3 respectively (Table 12). There were no significant differences ($P>0.05$) among the treatment means. In a previous rat study, using the same DFM product, there were no significant differences ($P>0.05$) in the mean feed intake among the dietary treatments (Okai, 2008). Furthermore, other researchers had reported similar results for mean daily feed intake of broilers (Bonsu, 2009; Dei, 2010). Broilers on the Control diet recorded the highest total feed intake followed by P3, RE3+ and RE3 though there were again no significant ($P>0.05$) differences among them.

4.2.3. Body Weight, Weight Gain and Feed Conversion Ratio

Contrary to the results of the DFM feeding trial (Experiment 1), significant ($p < 0.05$) differences in total weight gain, final body weight and feed conversion ratio of birds were observed during the study (Table 12). These results concur with the findings of

the following researchers (Jernigan *et al.*, 1985; Tortuero and Fernandez, 1995; Jin *et al.*, 1997; Yeo and Kim, 1997; Jin *et al*., 1998; Collinder *et al*., 2000; Zulkifli *et al*., 2000; Kalavathy *et al*., 2003; Lan *et al.*, 2003., Alexopoulos *et al.*, 2004; Islam *et al*., 2004; Kabir *et al.*, 2004; Kralik *et al*., 2004; Gil De Los Santos *et al*., 2005; Kamruzzaman *et al.*, 2005; Sun *et al*., 2005; Mountzouris *et al*., 2007; Nayebpor *et al.*, 2007; Vicente *et al*., 2007; Apata, 2008; Ashayerizadeh *et al.*, 2009) who found that live weight gains were significantly ($P<0.01$) higher for the DFM experimental birds as compared to their control counterparts as shown in Table 12. Huang *et al.* (2004) demonstrated that inactivated probiotics, disrupted by a high-pressure homogenizer, have positive effects on the production performance of broiler chickens when used at certain concentrations. In addition, Torres-Rodriguez *et al*. (2007) reported that administration of the selected probiotic (FM-B11) to turkeys increased the average daily gain and market BW, representing an economic alternative to improve turkey production.

4.2.4. Percentage Mortality

No health - related problems were observed during the experiment that could be attributed to the effectiveness of the various probiotics. A total of two (2) birds were recorded dead only in the control treatments with no mortality in the DFM treated groups. This result is in agreement to the findings of Bonsu (2010), Dei *et al*., (2010), Lalev *et al*., (2011), and Arpasova *et al*., (2012); who observed that, probiotics increases resistance to infectious diseases and reduces risk of mortality caused by the presence of infectious diseases. Research has shown that when animals are fed certain strains of bacteria, the activity of their immune systems increases (Choudhari *et al*., 2008) and this must have accounted for non occurrence of any pathogenic disease.

4.2.5. Carcass Characteristics and Organ Weight of Broiler Chickens

Similar to the body weight gain and feed conversion ratio, the carcass yields of broiler chickens supplemented with or without DFM were similar ($p > 0.05$). At the termination of the trial, examination of some organs (gizzard, liver, heart and intestine) obtained from all sacrificed birds revealed no macroscopic deviation from the normal in terms of gross tissue changes and that there were no significant differences among them. The results from the carcass evaluation relate well with those obtained in performance characteristics and it was observed that superior values were obtained for all the parameters evaluated. This is in agreement to the work done by Willis *et al.*,(2007), that DFM supplementation did not significantly ($P>0.05$) affect carcass weight of broiler birds.

Table12: Effect of DFM on Growth Performance and Carcass Parameters of Broiler Chickens.

PARAMETERS	TREATMENT					
	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	Lsd	FPr
Initial weight (g)	40.00	40.00	40.00	40.00	-	
Total Feed Intake (g)	4647	4608	4628	4637	74.4	0.718
Final Body Weight (g)	2842[c]	3175[a]	3017[b]	3042 [ab]	158.0	0.005
Total Weight Gain (g)	2802[b]	3135[a]	2977[ab]	3002 [a]	167.5	0.027
FCR	1.66[b]	1.47[a]	1.55[ab]	1.54 [a]	0.190	0.019
Mortality (%)	0.50	0.00	0.00	0.00	0.770	0.426
Carcass characteristics						
Carcass yield (% of LBW)	0.81	0.81	0.79	0.81	0.029	0.570
Organ weights (g)						
Gizzard Weight	72.75	81.25	82.75	75.00	15.087	0.439
Intestine Weight	128.38	127.25	129.00	117.00	26.542	0.735
Liver Weight	52.63	53.50	52.00	47.25	7.253	0.287
Heart Weight	14.50	14.75	13.38	13.88	2.044	0.481
Economy of gain						
Cost/Kg (GH₵)	1.26	1.30	1.30	1.30	-	-
Cost/kg weight gain	2.09	1.91	2.02	2.00	0.180	0.102

[a,b,c] Means within columns with no common superscript differ significantly ($P < 0.05$).

4.2.6. Probiotic Effect on Haemato-Biochemical Parameters of Broiler Chickens

From the study, haematological parameters were not significantly different between treatments (P>0.05) except for LDL as presented in table 13. However, the results obtained were in harmony with the normal range for healthy birds as stated by Jain (1993), Aiello and Mays, (1998), Awaad and Zouelfeker, (2001), Pampori (2003) and Campbell *et al*., (2003).

Table 13. Effect of DFM on Haemato-Biochemical Parameters of Broiler Chickens.

PARAMETERS	TREATMENT					
Haematology	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	Lsd	Pr
WBC (mm^3x10^3)	303.2	296.8	294.8	284.0	20.63	0.284
RBC ($3x10^6$/l)	2.825	2.800	2.675	2.500	0.3708	0.258
HB (g/dl)	11.45	10.95	10.55	10.40	1.407	0.403
HCT (%)	36.62	35.38	33.35	33.33	4.732	0.382
MCV (fl)	128.25	126.00	125.00	132.25	5.790	0.077
MCH (Pg)	40.17	39.10	39.62	41.35	1.705	0.071
MCHC (g/dl)	33.00	30.95	31.65	31.23	2.903	0.455
Blood chemistry						
T-PROT ($g\ l^{-1}$)	48.0	42.0	47.2	42.8	8.78	0.367
Albumin ($g\ l^1$)	17.50	17.00	16.50	16.75	3.041	0.903
Globulin (gl^{-1})	30.5	25.0	30.8	26.0	6.74	0.189
T-CHO{$mmoll^{-1}$}	3.98	3.70	3.85	3.62	0.672	0.682
TGS ($mmoll^{-1}$)	1.62	1.57	1.45	1.50	0.663	0.940
HDL ($mmoll^{-1}$)	2.950	2.950	2.700	2.675	0.5777	0.594
LDL ($mmoll^{-1}$)	0.525^a	0.100^b	0.150^b	0.275^b	0.2201	0.005

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$).

HB =Haemoglobin, PCV = Packed Cell Volume or HCT= haematocrit, RBC = Red Blood Cell, WBC = white blood cell, MCV = mean cell volume, MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, T.protein = Total Protein, T.Chol = Total Cholesterol, TGS = Triglycerides, HDL =High Density Lipoprotein, LDL = Low Density Lipoprotein

Serum cholesterol levels were numerically lower in broilers supplemented with DFM in water (Table 13) than those of the control birds. A similar reduction of serum cholesterol levels has been found in broilers (Mohan *et al*., 1996), layers (Tortuero *et al*., 1975; Abdulrahim *et al*., 1996), germ-free pigs (Mott *et al*., 1973), rats (Grunewald, 1982), and humans (Harrison and Peat, 1975) fed diets supplemented with *Lactobacillus*. The decrease in cholesterol level could be due to cholesterol assimilation (or uptake) by the *Lactobacillus* cells (Gilliland *et al*., 1985; Buck and Gilliland, 1994), or to the coprecipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993).

4.2.7. Effect of Probiotic on the Intestinal Microbiota of Broiler Chickens

It was realized from the study that significant ($p<0.05$) differences were observed in salmonella and faecal enterococci among the treatment and the control groups. This results concurs to the findings of (Rada *et al*., 1995; Jin *et al*., 1998; Line *et al*., 1998; Pascual *et al*., 1999; Dalloul *et al*., 2005; Kabir *et al*., 2005; Yaman *et al*., 2006; Higgins *et al*., 2007; Mountzouris *et al*., 2007) who observed that, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a beneficial effect on modulation of intestinal microflora and pathogen inhibition. The DFM's method of application used in the present study had a strong ability to attach to the intestinal epithelium of chicken (Jin *et al*., 1996d), are resistant to the bile and acidic conditions and are able to antagonize and competitively exclude some pathogenic bacteria *in vitro* (Jin *et al*., 1996b,c). However, there were numerical differences in *E. coli* between the control and the DFM-treated experimental animals as shown in table 14.

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Table 14. Effect of DFM on Intestinal Microbiota of Broiler Chickens

PARAMETERS	TREATMENTS				
	Control(T1)	RE3(T2)	RE3+(T3)	P3(T4)	Lsd
FPr					
E. coli (cfu)	571250	230000	493750	665000	395822.9
0.151					
Enterococci (cfu)	885[a]	110[c]	598[b]	132[c]	77.8
<0.001					
Salmonella (cfu)	65000[a]	0[b]	10000[b]	10000[b]	31132.4
0.003					

[a,b,c] Means within columns with no common superscript differ significantly ($P < 0.05$).

On the other hand, Chichlowski *et al.* (2007) compared the effects of providing a direct-fed microbials (DFM) with the feeding of salinomycin on intestinal histomorphometrics, and microarchitecture and they found less mucous thickness in DFM-treated chickens and the density of bacteria embedded in the mucous blanket appeared to be lower in DFM-treated chickens than in the control in all intestinal segments. Watkins and Kratzer (1983) reported that chicks dosed with *Lactobacillus* strains had lower numbers of coliforms in cecal macerates than the control. Francis *et al.* (1978) also reported that the addition of *Lactobacillus* product at 75 mg/kg of feed significantly decreased the coliform counts in the ceca and small intestine of turkeys. Using gnotobiotic chicks, Fuller (1977) found that host-specific *Lactobacillus* strains were able to decrease *Escherichia coli* in the crop and small intestine.

4.2.8. Feed Cost and Economy of Gain

Feed cost per kg was lower as the control birds were not given the DFM. The diets which contained the DFM were a little more expensive that is, GH¢ 1.30, GH¢ 1.30, GH¢1.30 and GH¢ 1.26 per kg for dietary treatments RE3, RE3+, P3 and Control respectively. This was solely due to the price disparities between the DFM and the Control diets at the commencement of the experiment. Broilers on the RE3 diets were more efficient with respect to feed to gain ratio (Table 12), consequently, it was more economical to raise broilers on the probiotic containing diets. The cost of feed to produce a kilogram (kg) live weight gain, was, however, lowest for birds on the dietary treatments which contained the DFM.

CHAPTER FIVE

5.0. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSION

The present study revealed that supplementation of probiotics in feed and in water at the level of 1.5mls for broilers has achieved good results with regard to animal health and growth performance. The probiotic added at the normal recommended rate in the various combinations had superior overall feed utilization efficiency and reduced mortality which certainly cannot be obtained with the use of synthetic substances. Besides these effects there were evidences of lower microbial load in the intestines of probiotic supplemented broilers.

Additionally, every probiotic product is different and efficacy against specific organisms is not always the same. Thus, the producer must be able to very specifically identify the production problem for which specific probiotics must be applied.

5.2. RECOMMENDATIONS

Further studies should be conducted at the same level of administration and method of application to confirm the observations made in these preliminary studies.

Also, further research should be carried-out to evaluate the effectiveness of frequency of application of DFM on broiler performance.

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APPENDICES

APPENDIX 1: ANALYSIS OF VARIANCE (ANOVA) TABLES

EXPERIMENT ONE

TABLE 1: ANALYSIS OF VARIANCE FOR FEED INTAKE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.098275	0.032758	5.05	0.017
Residual	12	0.077900	0.006492		
Total	15	0.176175			

TABLE 2: ANALYSIS OF VARIANCE FOR FINAL BODY WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.045169	0.015056	6.29	0.008
Residual	12	0.028725	0.002394		
Total	15	0.073894			

TABLE 3: ANALYSIS OF VARIANCE FOR TOTAL WEIGHT GAIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.0151687	0.0050562	7.16	0.005
Residual	12	0.0084750	0.0007063		
Total	15	0.0236437			

TABLE 4: ANALYSIS OF VARIANCE FOR FEED CONVERSION EFFICIENCY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.024019	0.008006	3.09	0.068
Residual	12	0.031075	0.002590		
Total	15	0.055094			

TABLE 5: ANALYSIS OF VARIANCE FOR WBC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.005769	0.001923	0.61	0.618
Residual	12	0.037525	0.003127		
Total	15	0.043294			

TABLE 6: ANALYSIS OF VARIANCE FOR RBC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.002919	0.000973	0.44	0.730

Residual	12	0.026625	0.002219	
Total	15	0.029544		

TABLE 7: ANALYSIS OF VARIANCE FOR LDL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.046525	0.015508	2.13	0.150
Residual	12	0.087450	0.007287		
Total	15	0.133975			

TABLE 8: ANALYSIS OF VARIANCE FOR HDL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.0017000	0.0005667	0.70	0.569
Residual	12	0.0097000	0.0008083		
Total	15	0.0114000			

TABLE 9: ANALYSIS OF VARIANCE FOR *E. Coli*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.06570	0.02190	0.99	0.430
Residual	12	0.26510	0.02209		
Total	15	0.33080			

TABLE 10: ANALYSIS OF VARIANCE FOR ENTEROCOCCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.04265	0.01422	1.30	0.320
Residual	12	0.13125	0.01094		
Total	15	0.17390			

TABLE 11: ANALYSIS OF VARIANCE FOR SALMONELLA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.47602	0.15867	3.42	0.053
Residual	12	0.55672	0.04639		
Total	15	1.03274			

EXPERIMENT TWO

TABLE 1: ANALYSIS OF VARIANCE FOR FEED INTAKE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.058725	0.019575	2.66	0.096
Residual	12	0.088450	0.007371		
Total	15	0.147175			

TABLE 2: ANALYSIS OF VARIANCE FOR FINAL BODY WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.03523	0.01174	1.15	0.368
Residual	12	0.12235	0.01020		
Total	15	0.15757			

TABLE 3: ANALYSIS OF VARIANCE FOR TOTAL WEIGHT GAIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.015525	0.005175	1.19	0.354
Residual	12	0.052050	0.004338		
Total	15	0.067575			

TABLE 4: ANALYSIS OF VARIANCE FOR FEED CONVERSION EFFICIENCY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.0624687	0.0208229	21.97	<.001
Residual	12	0.0113750	0.0009479		
Total	15	0.0738437			

TABLE 5: ANALYSIS OF VARIANCE FOR WBC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.026500	0.008833	6.42	0.008
Residual	12	0.016500	0.001375		
Total	15	0.043000			

TABLE 6: ANALYSIS OF VARIANCE FOR RBC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.011269	0.003756	0.40	0.756
Residual	12	0.112875	0.009406		
Total	15	0.124144			

TABLE 7: ANALYSIS OF VARIANCE FOR LDL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.029619	0.009873	1.24	0.338
Residual	12	0.095375	0.007948		
Total	15	0.124994			

TABLE 8: ANALYSIS OF VARIANCE FOR HDL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.007650	0.002550	1.05	0.406
Residual	12	0.029150	0.002429		
Total	15	0.036800			

TABLE 9: ANALYSIS OF VARIANCE FOR *E. Coli*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.795319	0.265106	35.36	<.001
Residual	12	0.089975	0.007498		
Total	15	0.885294			