EFFECTS OF COMMERCIAL PROBIOTIC PREPARATIONS ON THE GROWTH, EGG LAYING, HAEMATOLOGICAL AND IMMUNOLOGICAL TRAITS OF CHICKENS

BY

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DECLARATION

I, **Juliana Bawah**, hereby declare that the work presented in this thesis is the result of my own effort and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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DEDICATION

This work is dedicated to the glory of God as well as to my husband Mr. Stephen Gibbons Nassam and my parents Mr. and Mrs. Bawah

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ABSTRACT

Two experiments (experiment I and II) were conducted to determine the effects of three commercial probiotic preparations (RE-3, RE-3 plus and P3) on the productive and reproductive performance as well as haematologic characteristics of laying chickens. For experiment I, four hundred 40-week old local-exotic crossbred (96.88% exotic and 3.12% local) layers and forty Lohmann Brown breeder males of the same age were used. They were allotted to four (4) treatments. Layers on the control treatment received a layer diet without probiotics; their counterparts on the three other treatments received the same layer diet which contained 1.5mls of RE3™ solution per kg, RE-3 Plus (fermentation product of RE3™ - 1.5mls of RE-3 Plus solution per kg and T4 (P3 (*Paenebacillus polymyxa*-based probiotic) – 1.0mls of RE-3™ + 0.5mls of *P. polymyxa* solution per kg respectively. Each treatment had four replications with twenty (20) layers and two (2) males. Birds were fed *ad libitum* for twenty-four (24) weeks with a diet containing 18% crude protein and 2754 kcal/kg of energy. Feed intake, feed conversion ratio (FCR), hen-day rate of lay, hen-housed rate of lay and egg weight, total egg hatchability, hatchability of fertile eggs set, dead in shells and saleable chicks were some parameters measured. The following blood parameters total serum protein, serum immunoglobulin A, M, CD 3, CD 4, and packed cell volume were also determined. Differences in feed intake, egg weight, FCR and both hen-day and hen-housed rates of lay between layers on the four treatments diets were not significantly different (P>0.05). Mortality under the four treatments were however, statistically significant (P>0.05). The addition of probiotic to the diets of layer breeders did not significantly (P>0.05) influence the hatchability of the eggs laid. The percentages of saleable chicks from layers under the four treatments were not significantly different from each other (P>0.05) and did not follow any clear trend.

Hematologic parameters (WBC, RBC, HB, PCV, MCV, MCH, MCHC and LYMPH) determined showed no significant differences (P>0.05) among layers on the four treatments.

Layers fed the RE-3 diet (T2) had a significantly higher ($P<0.05$) total protein content than those on basal, RE-3 Plus and P3 diets. However, albumin and globulin did not differ significantly ($P>0.05$) among layers fed the four treatment diets. The immunological parameters determined did not differ significantly ($P>0.05$) among layers fed the four treatments diets. However, layers on the treatment 2 diet (RE-3) had higher numerical values for CD3 and lower numerical values for CD4. Additionally, the numerical values for IgA were lower in layers fed the probiotic included diets compared to those on control diet. The bacteria isolated in the fecal samples were *E-coli* and *Proteus* for all the treatments.

For experiment II, growth performance data and sexing was determined for the growers (7200) which were obtained from hatching eggs from the layers in experiment I. Feed with crude protein of 20.34% and energy of 2769.2 kcal/kg and water were provided *ad libitum.* All the growth parameters were not significantly different ($P>0.05$) for all the growers under the four treatments. However, there was a significant difference ($P<0.05$) in the sex ratio of growers and mortality. The results of these studies showed that the three commercial probiotics (RE-3, RE-3 plus and P3) preparations can be included at a level of 1.5mls in every kilogram of layer diet without any adverse effect on the performance, reproduction and haematologic traits of layers but supplementation resulted in more female chicks being produced and the inclusion of probiotic in the grower diet did not affect the growth and survivability of chicks..

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LIST OF ABBREVIATIONS USED

Abbreviations	Full meaning
AGP	Antibiotics Growth Promoters
ANOVA	Analysis of variance
ARI	Animal Research Institute
ARIBO	Animal Research Institute Broiler
BASO	Basophils
BEST	Basic Environmental System Technology
°C	Degree Celsius
CRD	Completely Randomized Design
DFM	Direct-Fed Microbial
DNA	Deoxyribonucleic acid
E. faecium	*Escherichia faecium*
E.coli	*Escherichia coli*
EO	Eosinophil
EU	European Union
FCR	Feed Conversion Ratio
G	Gram
GF	Germ Free
GIT	Gastrointestinal tract
HB	Haemoglobin
HCL	Hydrochloric acid
HU	Haugh unit
IgA	Immunologlobulin A
IgM	Immunologlobulin M
Kcal/kg	Kilocalorie/kilogram

Kg	Kilogram
KNUST	Kwame Nkrumah University of Science and Technology
LAB	Lactic acid bacteria
LPIU	Livestock Planning and Information Unit
LSD	Least Significant Difference
LYM	Lymphocytes
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MOFA	Ministry of Food and Agriculture
MONO	Monocytes
MPV	Mean Platelet Volume
NEUT	Neutrophils
P	Probability-value
P3	*Paenebacillus polymyxa*
PBP	Penicillin Binding Protein
%	Percentage
PLT	Platelets
PVC	Packed Cell Volume
RBC	Red Blood Cell
RE3 PLUS	Rumen Enhancer 3 Plus
RE3	Rumen Enhancer 3
SED	Standard Error of Difference
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3

T4	Treatment 4
UK	United Kingdom
USA	United States of America
USFDA	United States Food and Drug Administration
WBC	White Blood Cell
WY	Whole Yeast
YE	Yeast Extract

CHAPTER ONE

1.0 INTRODUCTION

Poultry production has been recognized as a short-term solution to meeting the protein needs of the people (Obi and Sonaiya, 1995). However, poultry production is hampered to a large extent by production losses due to diseases and high cost of medication (Appiah, 1993). Prevalent diseases include bacterial (e.g. Chronic respiratory disease), viral (e.g. Newcastle disease and Gumboro) and protozoan (e.g. Coccidiosis) which affect birds at various stages of life (Koney, 1993). Farmers rely heavily on vaccines, antibiotics and coccidiostats to maintain the health and productivity of these birds (Aning, 2006). The addition of sub-therapeutic doses of antibiotics to the diets of farm animals, according to Buchanan *et al*., (2008) was approved in the 1950's due mainly to the increase in demand for animal produce which called for the reduction in floor space of individual animals so as to allow the existing structures to accommodate more animals and the resulting problem of compromised sanitation and environmental conditions like improper ventilation (Doyle, 2001). Cook (2004) explained that the addition of sub-therapeutic doses of antibiotics to the diets of farm animals was accepted worldwide since these small doses of antibiotics did not only reduce the chances of disease outbreaks but also promoted the growth of farm animals leading to the reduction of time required for farm animals to reach market weight. The use of antibiotics however can result in the development of resistant strains of microorganisms with associated increase in cost of control (Dibner and Richards, 2005). Attempts to minimize the use of veterinary drugs include adherence to hygienic standards and use of probiotics as feed additive (Simon, 2005).

Feed additives are substances of non-nutritive nature (e.g. chemicals, microbiological products including probiotics or microorganisms, hormones and drugs) used in minute amounts for the improvement of farm animal performance (Kamra and Pathak, 1996). Feed

additives may be added to the ration to increase weight gain, aid in controlling infections, or help in controlling parasites (Gillespie, 1983).

Probiotics are viable microbial and microbial fermentation products which exert their beneficial effects by decreasing the undesirable micro-flora population in the gastro-intestinal tract (Chiang and Hseih, 1995) and build-up resistance against diseases by stimulating the immune system (Cheeke, 1991). Probiotics have also been an approach that has been reported to have the potential to reduce enteric disease in poultry and subsequent contamination of poultry product (Chapman, 1989; Patterson and Burkholder, 2003). Guilot (2000) observed that there has been a renewed interest in the incorporation of probiotics as a result of reduction in the use of antibiotics as feed additive in animals. Many probiotics are isolated from gastro intestinal tract of healthy animals and hence natural which makes them devoid of unhealthy side effects to the animal and subsequently to the consumer (WU *et al.,* 2008). Bonsu *et al.* (2012) found that the inclusion of a probiotic product, RE-3, in the diets of layers and broilers resulted in considerable reduction of body fat and serum cholesterol content (up to 16%) in broilers, a 15% decrease in cholesterol level of eggs, improved egg weight and reduced mortality in both broilers and layers. Dei *et al.,* (2010) also indicated that the addition of RE-3 to the diets of grower birds significantly reduced mortality compared to birds on a control diet containing no probiotic. Probiotic (RE-3) has also been found to have a positive influence on average daily gain in pigs (Okai *et al*., 2010).

Probiotic-based products are continually being developed and studied. Some of these are RE-3 Plus, which is a fermentation product of RE-3, and P3, which is a *Paenebacillus polymyxa*-based probiotic product (BEST, Canada). The study therefore sought to assess the effects of these probiotic products (RE-3, RE-3 PLUS and P3) on egg production performance, mortality, fertility and hatchability of eggs.

1.1 General Objective

The general objective for the two experiments was to assess the effects of commercial probiotic preparations on the growth, egg laying, haematological and immunological traits of chickens.

1.2 Specific Objectives

1. To investigate the effects of RE-3, RE-3 PLUS and a combination of RE-3 and P3 on growth performance of layer and growers.
2. To assess the reproductive performance (egg-laying) of point-of-lay birds fed the three probiotic-containing diets and the sex ratio of growers.
3. To investigate the blood profile (haematological, biochemical and immunological) responses of chickens to the three probiotic-containing diets.
4. To investigate the composition of microbes in the gut of the birds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 The Poultry Industry in Ghana

The poultry industry consists of the traditional sector which caters for poultry meat and egg needs of the great majority of rural people and a commercial sector, based on imported hybrid layer and broiler strains, which supply the needs of urban dwellers (Koney, 1993). Kwarteng and Towler (1994) have also reported that poultry provide job opportunities and income for several people in Ghana. Certain industries (e.g. the baking industry) use eggs as raw material in many preparations. Poultry droppings are also used as fertilizers in vegetable production and fish farming. According to MOFA/DFID (2002), poultry serves as a safety net, providing ready cash for emergency needs.

According to Flake and Ashitey (2008) the poultry industry in Ghana grew rapidly during the 1980's to 1990's, developing into a vibrant agricultural sector and supplying about 95% of chicken meat and eggs in the country. This was due to the government's initiative in the 1960's to promote commercial poultry production as the greatest potential for addressing the acute shortfall in the supply of animal protein. The growth of the poultry industry was initially slow, due to irregular supply of imported day-old chicks and other inputs and frequent outbreaks of poultry diseases which discouraged potential farmers.

However, in the 1970's the government, as part of its support for the development of the poultry industry, removed custom duties on poultry inputs (feed, additives, drugs and vaccines). In addition poultry producers had access to veterinary services provided by both the government agencies and private practitioners. Since independence, agriculture has been the major employer of the labour force in Ghana (GSS, 2008).

Statistics indicate that since 2000, Ghana's poultry industry has experienced a steep decline due to the very high cost of production (feed, inputs and energy) and lack of credit (Flake and Ashitey, 2008). Ghana imported 26,000 metric tons of chicken in 2002, mostly from the European Union where farmers receive generous subsidies (Kudzodzi, 2008). Two years later, this figure doubled to about 40,000 metric tons (ISODEC, 2004). The annual poultry import bill in 2005 was about 30 million dollars (Kudzodzi, 2008). In contrast, the domestic poultry sector which supplied 95% of Ghana's poultry requirements in 1992 only provided a dismal 11% by 2002 (GOG, 2009). This indicates the contribution of imported chicken to the collapse of broiler production in Ghana (Aning, 2008) and therefore the need for government to intervene in the poultry market through import duties and quotas in order to promote the growth of domestic poultry farming (GOG, 2009).

2.1.1 Demand and Supply of Poultry Products

The official document of Ministry of Food and Agriculture, (2002) estimated the annual poultry production to be 14,000mt of meat and 200 million eggs, respectively. Table 1 and 2 below gives figures of poultry meat and egg production, import and demand as compiled by the Food and Agriculture Organisation.

Table 1: Poultry Meat, demand and supply in Ghana (x 1000 mt)

Year	**Meat (chicken, turkey, guinea fowl, etc)**			
	Production	Export	Import	Demand
2001	20 963	0	12 262	33 225
2002	23 403	823	27 302	51 529
2003	25 545	823	56 090	80 812
2004	28 271	424	51 790	80 485
2005	28 763	64	52 570	81 269
2007	41 730	29	80 551	122 252
2008	44 460	0	76 957	121 417
2009	47 970	18	80 775	128 727
2010	51 675	0	112 145	163 820
2011	56 550	-	-	-

Source of data: Ministry of Trade and Industry. 2011

According to Livestock Planning and Information Unit (LPIU), Ministry of Food and Agriculture, (MOFA, 2002) data, the demand for local and imported poultry meat between 2001 and 2004 shown in Table 1 above represent between 18% and 24% only of the total meat demands of Ghanaians. It is important to note, however that meat, both livestock and poultry contributes only 40% of the national animal protein supply, the rest coming from fish (MOFA, 2002).

Table 2: Poultry Egg, demand and supply in Ghana (x 1000 mt)

Year	Eggs			
	Production	Export	Import	Demand
2001	22 260	0	96	22 356
2002	23 322	0	80	23 402
2003	24 380	196	62	24 246
2004	24181	170	104	24455
2005	25 183	0	107	25 290
2007	31 270	16	51	31 305
2008	33 655	26	45	33 674
2009	36 700	4	20	36 716
2010	36 700	3	36	36 733
2011	36 750	-	-	-

Source of data: Ministry of Trade and Industry. 2011

Commercial egg production is next in importance to village chicken keeping in the Ghanaian poultry industry (Okantah, *et al*, 2003). Egg sales face relatively minor competition on the market compared to poultry meat. The national egg production for 2011 is estimated conservatively, to be 10964 million (Table 3).

Table 3: Egg Production in Ghana (2011)

Region	Number of Farms Estimated	Number of Eggs (million)
Greater Accra	421	542
Ashanti	697	5321
Brong Ahafo	510	3980
Central	312	437
Western	159	247
Eastern	213	358
Volta	98	68
Total	**1075**	**10964**

Source: Field Data Akunzule 2011

2.1.2 Marketing and distribution channels of poultry products in Ghana

Marketing is finding out what customers want and supplying it at a profit. The process is customer oriented. The potential for increased profits offers the main incentive to develop and supply a variety of products to tempt the customer. Activities involved in marketing include the collection, evaluation and dissemination of marketing information; planning and scheduling of production; establishing contracts between buyers and sellers; constant improvement of all post- harvest activities; and co-ordinating inputs, including transport, processing, storage, credit, health care etc. Gaedeke and Tootelian (1983), stated that marketing in the poultry industry is important because of changing demographic patterns. Populations generally move from the villages into the towns, perhaps leaving fewer people in the main agricultural production regions. The urban folks are able to make their contribution to society without the need to consider constant agricultural activity. Growing more food may be easier for those with land, facilities and cash but the farmer with a smallholding may find it difficult to take advantage of a larger market, particularly where there is competition from a

larger producer. An improved marketing strategy, however, may enable him to reduce certain costs, perhaps by joining with others, leaving him with better profits.

The Agricultural Development Bank (ADB) reported in 2003 that poultry products marketing in Ghana include live broilers, spent layers, dressed birds, eggs and sometimes litter (ADB, 2003). The live broilers and spent layers are sold by direct sales method at the gate of poultry farms or are displayed in cages at market places, road side or lorry stations. In the Northern sector of Ghana guinea fowl is most consumed as compared to the consumption of chicken in the Southern sector. Day old chicks are packaged in perforated boxes upon purchase at the farm gate. Dressed birds are sold in kilos by retailers and in big cartons with respect to bulk purchases by retailers. In Ghana dressed birds are purchased on daily basis as compared to live ones. The major distribution points of these dressed birds are hotels, restaurants and chop bars with few going to the fast food sellers and individuals. The cut portion of these dressed birds are categorised into thigh, breast, drumstick, and the wings. Marketing of poultry meat and eggs reaches its peak in Ghana during festive seasons such as Christmas, Easter and Moslem festivals. Eggs are mostly sold in creates or boxes lined with sawdust. Eggs are marketed base on size and colour; the sizes are large, medium, and small and the colours are white and brown eggs. Most Ghanaians attach superstition to white eggs and thus purchases of the browns are higher than white shell eggs. In spite of these, eggs are produced all year round. Droppings from farms are sold to farmers or given out for free. Major buyers of these droppings are onion and shallot farmers in the Northern and Volta Regions of Ghana respectively. However, there are other commercial and backyard crop farmers as well as fish pond farmers who also patronize this product (Osei, 2003).

2.1.3 Some challenges facing the poultry industry in Ghana

Iddrisu (1994) reported that to a large extent, government policies have had both positive and negative effect on the poultry industry by bringing in imported poultry and livestock products to compete against the local products. According to Alhassan (1994), agricultural research by investigating and providing solutions to the nation's agricultural problems is the driving force of increased production, but it is a major problem in Ghana. The report pointed the lack of coordination and cooperation among researchers in the same institutions, different organizations and between researchers, extensionists and farmers. Oddoye (2002), citing a report by Aboe (1998) stated that extension technology/information transfer to the farmers was biased towards crops as compared to livestock. Some other challenges facing the poultry industry in Ghana aside government policies are importation of poultry products, high cost of feed, diseases, housing, high cost of vaccines among others.

2.1.3.1 Effect of poultry products importation on the poultry industry in Ghana

According to Do (1976), Ghana depends mainly on imported feeds (concentrates, mash, and some premixes) for feeding her poultry. Some disadvantages of importing feed, among others are irregular supplies, spoilage of feed by the time importers receive them and all imports involving foreign exchange which constitute a serious drain on Ghana's resources. According to LPIU (1992), only 23% of the estimated total meat demand of about 195,000 metric tons comes from domestic sources. Ministry of trade and industry (2011) reported that substantial quantities of cheap European Economic Community (EEC) subsidized beef and poultry (not directly EEC subsidized) continues to be imported to the detriment of local production. Citing a report by MOFA, Ministry of trade and industry (2011), indicated that poultry meat imports amounted to 6,757,035 mt (26.90% total imports).

Table 4: Imported/exported figures for frozen chicken from 2002 to 2010 (metric tons)

Year	Quantity Imported	Quantity Exported
2002	24782	25
2003	54238	66
2004	44851	54
2005	49916	64
2006	51403	0
2007	75373	0
2008	71731	0
2009	78837	18
2010	109179	0

Source: Ministry of Trade and Industry. 2011

Atarah (2005) reported that in 2002 alone, more than 26,000 mt of chicken was imported into the country, mostly from the European Union where farmers receive generous subsidies for their products. In 2004, however, that figure was estimated to be as high as 40,000 mt costing millions of cedis (Table 5).

Table 5: Imports of poultry in kilograms and costs of importation

YEAR	KGS	US $
2002	175,801,849	17,046,304
2003	84,456,234	25,462,209
2004	66,810,934	46,771,994
2005	52,158,191	72,079,812
2006	61,136,589	46,531,049
2007	4,455,381	3,282,498

Source: Aning, 2006

According to Martin Khor citing Corpwatch (2005), in 1992 domestic poultry farmers supplied 95 percent of the Ghanaian market, but by 2001 their market share had shrunk to 11% and this occurred in just over a decade. The imported chicken is available (wholesale) at a price that is only slightly more than half of the wholesale price of local chicken. Locally grown broilers as at 2006 were being sold at 15 cedis per kilo, whereas poultry imported from the EU was priced at only 5 cedis per kilo, less than the local cost of production (Khor, 2006). In 2005, locally grown broilers were being sold at 2.80 cedis (£1.60) per kilo, whereas poultry imported from the EU was priced at only 1.60cedis (£0.92p) per kilo, which is less than the local cost of production.(Khor, 2006 citing Christian Aid, 2005).

In 2007, Ghana's balance of trade was negative, with domestic production accounting for only about 42 percent of consumption. This share had declined from 72 percent of consumption in 2000. The country produced 1.27 kg of chicken meat per capita in 2008, lower than the per capita average of 1.71 kg for all of West Africa. The per capita chicken meat and egg production has remained fairly steady from 2004-2006 (Khor, 2006).

Table 6: Average market prices of locally-produced and imported poultry meat (100/kg)

Year	**Locally- produced**	**Imported**
2008	6.43	2.64
2009	7.50	2.07
2010	8.21	3.50
2011	9.29	4.21

Source: Amas Farm Ltd, 2011

2.1.3.2 Diseases affecting poultry production in Ghana

Diseases are major constraint to animal production in the tropics and can ruin animal industry when outbreaks are severe or dramatic. Acker and Cuninngham (1991) estimated that, losses

caused by animal disease in the U.S. annually amount to as much as 15% of the potential gross income from animals and animal products. Of these, 2% to 4% of the broilers and turkeys die before they reach market weight and laying hens typically have a mortality rate of about 1% per month. Prevalent diseases include bacterial (e.g. Chronic respiratory disease), viral (e.g. Newcastle disease and Gumboro) and protozoan (e.g. Coccidiosis) which affect birds at various stages of life (Koney, 1993). Newcastle disease occurs worldwide; it is enzootic with severe outbreak (Allan *et al.*, 1978) and is characterized by difficult breathing, rattling, coughing and sneezing with muscular incoordination and partial paralysis (James and David, 1994). There are vaccines and vaccination programmes which when properly used are very effective in controlling the disease (Buamah, 1992). Infectious bursal disease (Gumboro) is another disease affecting young chickens up to the age of six weeks (Jordan and Pattison, 1996). In infectious bursal disease, chickens develop a whitish diarrhoea, become dehydrated and may show darkening of the muscle (James and David, 1994) which results in mortality rates of 10-100% and 10-50% respectively (Jordan and Pattison, 1996) in poultry industry in Ghana. Farmers hence rely heavily on vaccines, antibiotics and coccidiostats to maintain the health and productivity of their birds (Aning, 2006).

2.1.3.3 Feed as a factor affecting the poultry industry

Gillespie (1983) reported that feed accounts for about 70 percent of the cost of production in poultry and this cost a little higher in Ghana than what it is in many countries. This is because of the high cost of maize which forms the largest proportion (about 75%) of the mixed feed. He added that, with this high costs of feed, the margin of profit remains low, and this continues to be a big disincentive to poultry farmers in Ghana. It was reported by Osei (1990) that the reason for high feed cost is quite obvious – primarily an inadequate production of the main fed ingredients, maize and fishmeal, to meet human demand, much less the demand of the poultry industry. Other feed ingredients such as soya bean cake and vitamin-mineral

premix are imported (Table 7), and so is poultry drugs and vaccines. Medium and small-scale commercial farms rely on feed milling companies. Whether to buy ready-made feed from the feed mill or prepare your own feed depends on cost (Tachie-Menson, 1991).

Table 7: Import of feed ingredients (mt)

Year	Soybean meal	Concentrate	Fishmeal	Premix
2010	316	9,058	10,639	672
2011	16,924	5,516	12,624	1,030
2012	51,817	41,954	38,880	6,136

Source: Animal Production Directorate of MoFA, 2013

2.2.0 Feed Additives

According to Kamra and Pathak (1996) feed additives are non-nutritive substances added in minute amounts to animal diets for the promotion of performance. These are not nutrients; however they are added to the feed mix to cause animals to grow faster or to control some diseases. Usually, these substances do not become a part of the body cell but remain in the tissue of the body unlike nutrients which are necessary for cells to live, grow and function properly.

According to Kamra and Pathak (1996), a feed additive should have the following characteristics;

- should be rapid in metabolism and excretion from the body,
- should not be harmful in accumulation in edible carcass
- it should not be harmful on prolonged feeding.
- it must be a normal inhabitant of the gut,
- it must be able to adhere to the intestinal epithelium to overcome potential hurdles, such as the low pH of the stomach, the presence of bile acids in the intestines, and the

competition against other micro-organisms in the gastro-intestinal tract (Blum *et al.*, 2002; Nurmi *et al.*, 1983).

Feed additives may be classified into the following groups:

- chemical compounds like arsenicals and copper sulphate, tranquilizers, antioxidants, antibiotics and other drugs,
- hormones including synthetic hormones,
- and miscellaneous substances like colours, flavours etc as reported by Kamra and Pathak (1996).

2.3.0 Antibiotics

Antibiotics are chemical compounds synthesised wholly or partly by specific strains of microorganisms usually a fungus or a bacterium; capable of inhibiting the growth of or killing other microorganisms (Duane and Marle, 1991; Gracey *et al.*, 1999). According to Sharma and Adlakha (1996) some anti-microbial agents such as those that inhibit cell wall synthesis may be able to kill susceptible bacteria without the intervention of luminal or cellular immune defense. This process is termed bactericidal activity (Sharma and Adlakha, 1996), others such as sulphonamides simply inhibit essential metabolic systems without killing them and are said to be bacteriostatic (Van den Bogaard and Stobberingh, 1999). Antibiotics are primarily used for the treatment and control of infectious diseases (Van den Bogaard, 1997). There are broad-specttrum antibiotics that target more than one microorganism while others are narrow-spectrum which target a specific organism for action. Apart from therapeutic use by veterinarian, antibiotics have over the years gained important recognition and use in sub-therapeutic doses as growth and performance promoters (Brorsen *et al.*, 2002).

2.3.1.0 Antimicrobial resistance

Resistance to antimicrobials and other toxic chemicals, according to Bezoen *et al.,* (1999) is an adaptation or survival mechanism exhibited by bacteria and other microbes in general to all forms of biochemical stress. The acquisition of resistance by bacteria follows several mechanisms including

- alteration or modification of target sites (penicillin binding proteins (PBP)) so that they are no longer bound by the antibiotics,
- inactivation of the antibiotics by enzyme hydrolysis before they reach target sites; modification of cell wall permeability such that they are either impermeable to the antibiotics or so large as to enhance the pumping-out of antibiotics which have already entered the cell and target bypass

Džidic *et al.*, 2008 and Hooper, (2001) classified resistance into two different forms, namely:

Intrinsic/Inherent resistance or Insensitivity and Acquired resistance

2.3.1.1 Intrinsic resistance

Intrinsic resistance is the innate ability of bacteria to resist the activity of a particular antimicrobial agent through its inherent structural or functional characteristics. These allow tolerance of a particular drug or antimicrobial class. Russell and Chopra (1990) indicated that because this form of resistance is inherent or due mainly to some features of the bacteria, it cannot be passed on from one bacterium to another but only from a bacterium to its offspring. The mechanism of action of intrinsic resistance in bacteria, according to Ibezim (2005); Bezoen *et al.*, (1999); Russell and Chopra (1990) are:

- The production of enzymes which inactivate the antibiotics e.g. *Klebsiella spp.* produces the enzymes beta-lactamases that destroy ampicillin before the drug can reach the penicillin binding protein (PBP) target.
- The inaccessibility of the drugs into the cell components due to barriers impermeable to the antibacterial agent on the cell wall e.g. the outer membrane of gram–negative bacteria can prevent the entrance of some β–lactams into the cell.
- The extrusion of the antimicrobial by chromosomally encoded active exporters.
- The lack of affinity of the antimicrobial for the bacterial target.

Intrinsic resistance is the form of resistance that microorganism had even before the advent of antibiotics and other antimicrobial agents. This form of resistance or characteristics of individual species of bacteria serves as bases in the manufacturing of antibiotics (Russell and Chopra, 1990).

2.3.1.2 Acquired resistance

This form of resistance occurs when a microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. According to Bezoen *et al.,* (1999) acquired resistance is the more serious problem since it can also be the cause of several uncontrollable diseases and epidemics. Thus it calls for research into new drugs which may also be ineffective on the pathogens with time. There are two known mechanisms by which bacteria acquire resistance. They are

a. Mutation of chromosomes (Birošová and Mikulašová, 2005) and
b. The horizontal or lateral gene transfer (Maiden, 1998).

2.3.1.2.1 Resistance through chromosomal mutation

Chromosomal mutation which is an inheritable alteration from the normal DNA mutation (Maiden, 1998) occurs in bacteria as an alteration or change in the sequence of nucleotides in the DNA. Hooper (2001) emphasized that, the potential for obtaining resistance by bacteria through mutating their chromosome is essential if the bacteria will survive the harsh environmental condition in their delicate state. Therefore, bacteria and for that matter microorganisms in general, keep on mutating with the objective of achieving a near perfect state which ensures survival from extinction. Chromosomal mutation, according to Bezoen *et al.,* (1999), can occur at anytime with or without the presence of an antibiotic; however, certain chemicals can facilitate the rate at which mutations occur (Birošová and Mikulašová, 2005). As a result of these changes in gene expression, previously bacteriostatic and bacteriocidal agents may no longer be as effective since their targets within the bacteria may no longer be in existence or there may even be over production of target sites such that the normal dosages may be ineffective (Birošová and Mikulašová, 2005). Mutation can change cell wall characteristics such that pores within the cell walls are no longer permeable to certain antibiotics (Birošová and Mikulašová, 2005). Mutation may either deteriorate or improve the condition of the bacteria. Some researchers according to (Denamur and Matic, 2006) emphasized that; some form of mutation can even help to control some bacteria through hitchhiking with the adaptive mutations they generate.

2.3.1.2.2 Resistance through horizontal or lateral gene transfer

Horizontal gene transfer which is favoured by the presence of antibiotics occurs when bacteria picks up functional DNA from either the environment or from other bacteria (Bezoen *et al.*, 1999). This form of resistance is of most importance to scientists since according to Džidic *et al.,* (2008), its occurrence is facilitated by the misuse of antibiotics by man and also by the nutritive/sub-therapeutic and therapeutic use of antibiotics in animal production. There

are basically three forms of horizontal gene transfer; transformation, transduction and conjugation.

2.3.1.2.3 Transformation

The uptake of naked DNA is a common mode of horizontal gene transfer that can mediate the exchange of any part of a chromosome; this process is most common in bacteria that are naturally transformable; typically only short DNA fragments are exchanged and are termed transformation (Davison, 1999). According to Bezoen *et al.*, (1999), when a bacterium dies, its DNA if left behind intact in the surroundings can be picked-up by a competent bacterium and then incorporate parts of it into its own chromosome. In situations where the acquired DNA contains resistance genes, the bacteria then obtain the resistance and then pass it on to subsequent generations.

2.3.1.2.4 Transduction

Bacteriophages are viruses that infect and disintegrate bacteria. Studies however have shown that bacteriophages infect bacteria by only introducing their DNA into the bacteria (Davison, 1999) and may then breakdown the host (bacteria) DNA into segments before packaging some of the bacteria DNA and its own DNA. In some situations, according to Džidic *et al.*, (2008), there may be errors in the packaging and the bacteriophage may then pick up only a bacteria DNA. After lyses of the bacteria, the bacteriophages may then go on to infect other bacteria but because some of the bacteriophages are made up of only bacteria DNA, they may enter into another bacteria and only form part of that bacterium's DNA. If the gene sequence transported by the bacteriophage contains resistance genes, then, according to Bezoen *et al.*, (1999), the bacteria may obtain resistance. It is however worth stating that, this form of acquisition of resistance may be between only closely related bacteria since bacteriophages are known to have a narrow spectrum of host on which they depend (Bezoen *et al.*, 1999).

2.3.1.2.5 Conjugation

Bezoen *et al.,* (1999) defined conjugation as the transfer of DNA by direct cell-to-cell contact. Džidic *et al.,* (2008) define bacterial conjugation as the promiscuous DNA transfer mechanism between bacteria. Conjugation can occur between closely related and unrelated bacteria and therefore is said to be the main cause of the spread of antibiotic resistance among pathogenic bacteria (Dale and Park, 2004).

Conjugation basically requires the direct contact between cells after which a channel (F-pilus) emerges between the 2 cells through which fragments of DNA are sent from a donor cell into a recipient cell (Bezoen *et al.,* (1999). Davison, (1999) stated that, when plasmids containing resistance genes are transferred and they are replicated and transcribed successfully, the donor gains the resistance and this resistance can then be passed on to its offspring.

2.3.2 Antibiotics as feed additive

Antibiotics are among the mostly used feed additives in poultry and livestock and have been used primarily because their use results in more rapid growth, improved feed efficiency and improved general health, primarily in young animals when fed continuously at sub-therapeutic doses (Kellems and Church, 2002). The United States Food and Drug Administration approved the use of antibiotics as animal feed additive without veterinary prescription in 1951. Other European countries also approved antibiotics use around 1950 to 1960 (Coates, 1962; Jones and Ricke, 2003). Witte (1998) and Joerger (2003) however reported that after many years of research, it became evident that the residues of antibiotics in animal products were responsible for the increase in resistance of some pathogenic bacteria found in humans; and also for the toxicity and potential allergic reaction in humans when large quantities are contained in the animal products consumed (Gracey *et al.*, 1999). A regulating body was put in place to regulate the use of antibiotics. This regulation permitted the use of specific antibiotics like Chlortetracyline, Penicillin and Oxytetracyline to be used

as additive at a level of not more than 1 part of antibiotics to 10000 part of the feeding stuff (Coates, 1962). The early establishment of legislature regulating the use of antibiotics gave an indication that although the beneficial effects of antibiotics as feed additive was not doubted, there has always been fear and uncertainty of a possible residual effect from their usage. These developments led to the ban on the sub-therapeutic use of some antibiotics in some countries and the need to find alternatives to the use of antibiotic in animal feeding. Probiotics, prebiotics, synbiotic and some hormones have been used in place of antibiotics as feed additives.

2.3.3 Antibiotics in Poultry Production

Antibiotics have been added to poultry diets to maintain health and production efficiency in the last three decades (Rosen, 1995). However, because of the development of resistance by pathogenic bacteria (Dibner and Richards, 2005) which can have an impact on public health when meat with antibiotic residues are consumed, antibiotics are being taken out of poultry diets around the world (Dibner and Richards, 2005). Bedford (2003) also pointed out that the growth-promoting effects of antibiotics in animal diets are clearly related to the gut microflora because they exert no benefits in the performance of germ-free (GF) animal.

2.3.4 The ban on the use of antibiotics growth promoter in animal production

Džidic *et al.*, (2008) reported the evidence in support of the fact that antibiotic resistant bacteria from animals like pigs and poultry enter into the human food supply chain and end up colonizing the digestive tracts and transpose resistance genes into the microflora within the intestines. The United Kingdom was the first to ban the use of antibiotic in animal production in 1970 by banning the use of penicillin and tetracycline as growth promoters (Buchanan *et al.*, 2008) followed by Sweden, Denmark and European Union (Dibner and Richards 2005) respectively. Dibner and Richards (2005) and Vondruskova *et al.*, (2010)

have explained that with the current health awareness of consumers and pressure on food safety, there is the high likelihood of the US and other parts of the world banning the use of all AGP's and therefore the need to investigate and find the best alternative to AGP's which will also be safe within the food chain.

2.4.0 Synbiotics

Synbiotics is the use of a combination of probiotics and prebiotics (Gibson and Roberfroid, 1995). The live bacteria must be used with specific substrates for growth. Therefore, the colonization by an exogenous probiotic could be enhanced and extended by simultaneous administration of a prebiotic being specifically used by the probiotic strain as a substrate in the intestinal tract (Rolfe, 2000). Few studies have shown that feeding a diet with synbiotics to young pigs increased *Lactobacillus* and *Bifidobacterium* levels when compared to prebiotics and probiotics alone (Nemcová *et al.*, 1999). It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. However, there is little information on synbiotics and their possible mechanisms in young poultry.

2.5.0 Prebiotics

Gibson and Roberfroid (1995) defined prebiotics as non-digestible food additives which beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance. The growth of endogenous microbial population groups such as bifidobacteria and lactobacilli is specifically stimulated and these bacteria species are perceived as beneficial to animal health. Prebiotics have the advantage, compared with probiotics, that bacteria are stimulated which are normally present in the GIT of that individual animal and therefore already adapted to that environment (Snel *et al.*, 2002). The

dominant prebiotics are fructo-oligosaccharide products (oligofructose and insulin) (Patterson and Bukholder, 2003); gluco-oligisaccharides, stachyose, malto-oligosaccharides and oligochitosan have also been investigated in broiler chickens (Zhang *et al.,* 2005; Gao and Shan, 2004 and Jiang *et al.,* 2006). Reports on the effects of prebiotics on the activity of microflora of broilers are limited and the effects are variable depending on the type of prebiotic. Fructo-oligosaccharides were shown to support the growth of beneficial bacteria such as lactobacilli (Xu *et al.,* 2003; Yusrizal and Chen, 2003; Zhang *et al.,* 2005 cited in Vidanarachchi *et al.,* (2006)), but failed to stimulate the growth of bifidobacteria (Vidanarachchi *et al.,* 2006). However, there are many considerations in supplementing prebiotics in animal feed. These include the type of diet (*i.e.* the content of non-digestible oligosaccharides); the type and inclusion level of the supplements, the animal characteristics (specie, age, stage of production); and the hygiene status of the farm (Verdonk *et al.,* 2005). The primary ones are the inclusion level of the supplements as high dosage of prebiotics can have negative effects on the gut system and retard the growth rate of birds as observed by Biggs *et al.,* (2007).

2.6.0 Probiotics

Probiotics include viable microbial and microbial fermentation products which are beneficial to decrease the undesirable micro flora population in the gastro-intestinal tract of chicks (Chiang and Hseih, 1995), build up resistance against diseases by stimulating the immune system (Cheeke, 1991; Patterson and Bukholder, 2003) and contain microorganisms working in consonance with the host animals (Fuller, 1989). Microorganisms are naturally present in the digestive system of the animals. Some of the microbes aid digestion, others can potentially cause pathogenesis. Therefore one of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for poultry production (Patterson and Bukholder, 2003) which helps in the buildup of the beneficial

bacteria in the intestine and competitiveness excluding the pathogenic bacteria. In addition, probiotics may contribute to the improvement of health status of birds by reducing ammonia production in the intestines (Chiang and Hseih 1995). These beneficial bacteria also release enzymes, which help the digestion of feed. Sarkar (2011) also stated that probiotics may be involved in allergy prevention, enhancement in the bioavailability of nutrients, induction of hypocholesterolemic effects and improvement in digestion.

The use of probiotics or direct fed microbial in animals feed particularly poultry is slowly becoming popular. The common organisms in probiotic products are *Aspergillus oryzae, Lactobacillus acidophilus, bulgaricus, L. plantarium, Bifidobacterium bifidum, Streptococcus lactis and Saccharomyces cerevisiae* (Wenk, 2000). These can be administered through water or incorporated in the feed. Probiotics have been particularly useful in the early stages of chick growth since the gut of the newly hatched chick is sterile and administering probiotics through water at this stage helps to build up beneficial bacteria much faster than the normal course (Wenk, 2000).

Probiotics are becoming accepted in animal nutrition as potential alternatives to antibiotics for use as growth-promoters, and in select cases, for control of specific enteric pathogens ((Anadón *et al.,* 2006; Boyle *et al.,* 2007; Cartman *et al.,* 2008; Vila *et al.,* 2009 and Williams *et al.,* 2009) cited in Tellez *et al.,* 2011). Currently, there is no universal class of probiotic bacterium although the most common types that have been indisputably effective involve lactic acid bacteria. These bacteria are found normally in the gastrointestinal tract (GIT) of vertebrates and invertebrates, and the use of some lactic acid bacteria cultures is able to restore the natural microflora within the gut (Shahani and Ayebo, 1980). Lactic acid bacteria (LAB) include the genera Lactobacillus, Pediococcus, and others that have long been associated with health benefits and which have been used for fermentation of certain foods. While speciation of members of these genera is difficult and inconsistent, these organisms are uniformly safe and not associated with disease in healthy animals or humans (Tellez *et al.,*

2006). Second class of probiotic cultures are those microorganisms that are not normally found in the GIT (allochthonous flora). For example, Saccharomyces boulardii has been shown to be effective in preventing the recurrence of Clostridium difficile infections (Czerucka *et al.*, 2007) and some colibacillosis in humans (Czerucka and Rampal, 2002). Other allochthonous probiotic microbes are the spore-forming bacteria, normally members of the genus Bacillus.

2.6.1 Types of probiotics

Different kinds of probiotics have been used so far in the diets or otherwise of animals for stimulating production and/or feed utilization efficiency. Examples include: RE-3 (*Lactobacillus* sp., *Bacillus* sp., *and Saccharomyces* sp.), RE-3 Plus, P3, Prima Lac, Maz, PoultryStar, Lacto-Sacc etc. The difference in these probiotics comes with the strain of bacteria that was used, dosage, mode of application, time of application etc. Probiotic products may contain different genera, different species, or even different strains of the same species, and not all products should be expected to work the same. Therefore, claims of efficacy should be target specific and should be made only for products that have been found efficacious in carefully designed studies.

2.6.2 Probiotics as feed additive

Probiotics, Yeast culture and other natural feed additives for poultry and pigs' feeds have gained much attention over antibiotics in the poultry industry. Much of this interest has been generated because of increased public awareness and objection to the use of antibiotics as growth promoting feed additives in those industries (Chapman, 1989 and Hong *et al*., 2005). Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality and the associated contamination of poultry products for human consumption. With increasing concerns about antibiotics resistance, the ban on sub

therapeutic antibiotic usage in Europe and in the United States, there is increasing interest in finding alternative to antibiotics for poultry production (Patterson and Burkholder, 2003).

Patterson and Burkholder (2003) have shown in numerous in vivo and in vitro studies that the commensal intestinal microbiota inhibits pathogens. A variety of microbial species have been used as probiotics in animal feed and are mainly bacterial strains of Gram positive bacterial including *Lactobacillus acidilactici, farciminis, rhamnosus, Enterococcus faecium, mundtii, Pedicoccus acidilactici, Bacillus cereus, licheniformis, subtilis, Bifidobacterium, Streptococcus* and a variety of microscopic fungi such as strains of yeast belonging to the *Saccharomyces cereevisiae* species (Guilot, 2000 and Simon *et al*., 2001). Among these species of probiotics, *Lactobacillus, Bacillus, Bifidobacterium,* yeast and *Enterococcus* have extensively been used (Patterson and Burkholder, 2003).

In assessing the value of a probiotics or direct-fed microbial, Hutcheson (1987) and Guilot (2000) enumerated characteristics necessary for a probiotics to be effective. These criteria include the following;

- Must be a normal inhabitant of the intestine,
- Must have a short regeneration time,
- Must produce antimicrobial substance (eg lactic acid, bacteriocins, etc),
- Must be durable enough to withstand the duress of commercial manufacturing, processing and distribution so the product can be delivered alive to the intestine and
- Must be free of diffusible antibiotic resistance gene, non pathogenic and non toxigenic for target species and for man under expected conditions for use.

  The most efficient probiotic bacteria are likely to be strains that are robust enough to survive the harsh physio-chemical conditions present in the gastro intestinal tract (Fooks and Gibson, 2002). The probiotic bacteria that survive usually do not colonise the intestinal mucosa for long periods of time and are generally eliminated within few

days of the cessation of their ingestion (Martear *et al*., 2004) necessitating continuous supplementation.

Although there are enormous benefits to derive from addition of probiotics in the animal feed, the greatest benefits are observed when the animals are stressed by various factors such as transportation, overcrowding, vaccination, chilling and/or overheating (temperature). These conditions create an imbalance in the intestinal microflora and a lowering of body's defense mechanism (Suzuki *et al*., 1989).

2.6.3 Mode of action of probiotics

Two basic mechanisms by which probiotics act to maintain a beneficial microbial population are competitive exclusion and immune modulation. Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogens, and competition for attachment sites (Jin *et al*., 1997; Simon *et al*., 2001; Ghadban, 2002 and Edens, 2003). Immune modulation is the alteration of the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen that initiated their production (Ghadban, 2002). Based on these mechanisms, probiotics have been tested for their efficacy at controlling *Salmonella* colonization in broilers and the results are positive and consistent. For example, Higgins *et al.,* (2007) performed a research on the prevention of *Salmonella* colonization using probiotics on 840 chickens and this resulted in 60% reduction in the colonization of *Salmonella*. Stern *et al*. (2001) also carried out the same research on 210 chickens and there was 44% reduction in the colonization of *Salmonella*. Probiotic supplementation, especially with *Lactobacillus* species, has also shown beneficial effects on resistance to the other infectious agents such as *Clostridium* (Decroos *et al.,* 2004) and *Campylobacter* (Stern *et al.,* 2001). Regarding the gut microbiota of normal birds, the results of probiotic supplementation

are variable because of the difference in origin, strain as well as species of probiotics. Reduced caecal coliform populations were noticed in chickens given a diet supplemented with lactobacilli strains, isolated from chicken intestine, but the populations of other kinds of bacteria were not affected (Watkins and Kratzer, 1984; Jin *et al.*, 1998a, 1998b). In contrast, Murry *et al.*, (2006) reported that birds supplemented with botanical probiotic containing lactobacilli had higher lactobacilli but lower *C.perfringens* compared to the control birds. By directly interacting with mucosal immune system, probiotics can modulate either innate or acquired immunity, or both (Dugas *et al.*, 1999). Further, specific immune modulatory effects of probiotics are dependent on the strain or species of bacteria included in the probiotics (Edens, 2003 and Huang *et al.*, 2004). Lactobacilli, the most studied strain of probiotic in both animal and human, have been implicated to increase the activity of certain innate immune functions, specifically the activity of macrophages and natural killer cells (McCracken and Gaskins, 1999). Koenen *et al.*, (2004) reported that feeding *L. paracasei* to broilers enhanced the phagocytic activity of the gut cells (caecum, ileum). However, *L. plantarium*, rather than *L. paracasei*, exerted stronger stimulating effect on antigen specific titre (Koenen *et al.*, 2004). In general, feeding probiotics could improve antibody titres against Newcastle disease; infectious bursal disease virus and/or sheep red blood cell (SRBC, Panda *et al.*, 2000; Zulkifli *et al.*, 2000 and Huang *et al.* 2004) but no responses were also observed by Panda *et al.* (1999). The results from Zulkifli *et al*., (2000) further indicated that these effects of probiotics could be affected by the age and strain of broilers. In addition *Lactobacillus* based probiotic may strengthen gut defense function via activation and enhancement of local cell-mediated immunity to against certain enteric pathogen (Dalloul *et al.*, 2003). However the exact mechanisms for probiotics to enhance immune function remain largely unknown. Probiotics did not consistently improve growth performance and/or mortality rate of birds. According to According to Liu *et al.* (2007) birds that were on probiotics supplemented diets had a higher body weight gain (g/bird) of 1920 whilst the

control birds had a lower weight gain of 1892 g. Also birds on probiotics had 1.74 g of FCR whilst the control had a feed conversion ratio of 1.75 g when an experiment conducted.

Conversely, Murry *et al.*, (2006) also had 2720 g in body weight gain of birds on probiotics and 2784 g of body weight gain of birds on control diet, 1.63 g of feed conversion ratio on birds fed with probiotic and 1.62 g on control birds. Mortality was 4.76% for birds fed with probiotic and 7.02 on control birds. Zulkifli *et al.* (2000) had body weight gain of 1545 g on birds fed with probiotic and 1379 g on birds fed with control diet. Feed conversion ratio on control birds was 2.08 g and that on probiotics was 2.17 g. Mortality of control birds was 1.7 and that of birds on probiotic was 2.2. Jin *et al.* (1998a) recorded mortality of 5.3% on birds on probiotic and 6.7 on birds on control. The inconsistency may become more complex because of rearing environment. For example, under heat stress condition, lactobacilli probiotic supplementation improved body weight gain of female birds by 12% but increased FCR (feed conversion ratio) and mortality rate by 4% and 29% respectively (Zulkifli *et al.*, 2000). However, Edens (2003) reported that, the growth-promoting effects of probiotics are dependent on the specific probiotics, the application level of probiotics, the age of birds as well as the delivery method (*i.e.* via water and/or feed). Reports on the use of probiotics and its attendant benefits in poultry production have been generally inconclusive. In review of published results on the use of probiotics in poultry diet or water, Stavric and Kornegay (1995) concluded that, results were generally inconsistent, while a few results indicated a beneficial effect in terms of weight gain, egg production and feed efficiency in broilers and layers.

2.6.4 Mode of administration and timing on the efficacy of probiotics

Probiotics may be administered to the host animal in a variety of ways. It may be given as a powder, tablets, liquid suspension, capsule, paste or spray. Moreover, the amount and interval between doses may vary (Chesson, 1994). Probiotics may be given only once or periodically

at daily or weekly intervals (Timmerman *et al.,* 2006). Little is known about the minimum dose required for the probiotic effect but trials in rats, humans and pigs indicate that the effect falls off after administration of the probiotic ceases (Cole and Fuller, 1984; Goldin and Gorbach, 1984). It therefore seems very likely that the effect obtained will be affected by the amount and frequency of dosing.

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.

2.6.5 Probiotics in health and medicine

The manipulation of gut microbiota following the administration of probiotics influences the development of the immune response (McCraken and Gaskins, 1999). The exact mechanisms for this influence are not clearly understood. Christense *et al.,* (2002); Lammers *et al.,* (2003) and Maassen *et al.,* (2000) have however, shown that probiotics stimulate several subsets of immune system cells to produce cytokines, necessary for the induction and regulation of immune responses.

Stimulation of human peripheral blood mononuclear cells with *Lactobacillus rhamnosus* strain GG *in vitro* resulted in the production of interleukin 4 (IL-4), IL-6, IL-10, tumor necrosis factor alpha, and gamma interferon (Shultz *et al.,* 2003). Other studies have provided confirmatory evidence that Th2 cytokines, such as IL-4 and IL-10, are induced by lactobacilli (Christense *et al.,* 2002; Lammers *et al.,* 2003 and Rokoff-Nahoum *et al.,* 2004). The outcome of the production of Th2-type (a subset of the most prolific cytokine producers) cytokines is the development of B cells and the immunoglobulin isotype switching required

for the production of antibodies. The production of the mucosal IgA response is dependent on other cytokines, such as transforming growth factor β (Lebman and Edmiston 1999). Importantly, various species and strains of lactobacilli are able to induce the production of transforming growth factor β, albeit to various degrees (Blum *et al.,* 2002). Probiotics, especially lactobacilli, could modulate the systemic antibody response to antigens in human (Kabir *et al.,* 2004; Apata, 2008; Haghighi *et al.,* 2006; Mathivanan and Kalaiarasi, 2007; Huang *et al.,* 2004 and Koenen *et al.,* 2004 as cited in Kabir, 2009). Probiotics have been beneficial in other areas including delaying Pseudomonas colonization and infection in critically ill patients (Forestier *et al.,* 2008) clearing of vancomycin-resistant enterococci colonization (Manley *et al.,* 2007) treatment of mastitis during lactation (Jimenez *et al.,* 2008) prevention of recurrent urinary tract infection in women (Falagas *et al.,* 2006) and vulvovaginal candidiasis (Falagas *et al.,* 2006) A Cochrane review suggested that there is a lack of sufficient evidence for/against recommending probiotics in the treatment of bacterial vaginosis and emphasized the need for well-designed randomized controlled trials. However, the authors noted a favourable outcome in the treatment of bacterial vaginosis with the use of metronidazole/probiotic and probiotic/estriol regimens (Senok *et al.,* 2009). The potential role of genetically engineered probiotics as vaginal microbicides to prevent HIV is provocative; however, it is in the early stages of development and further research in this field is needed (Hemmerling and Cohen, 2011).

2.6.6 Some side effects of probiotic in medicine

It has been well established that the intestinal microflora plays an important role in the metabolic activity and immune system of the host, and probiotics help to promote microflora. However, it can also be argued that manipulation of the normal microflora by probiotic use may theoretically increase the risk of adverse metabolic and immunomodulatory effects. Some minor adverse effects, including thirst and constipation with S. boulardii use

(McFarland *et al.*, 1994), bloating and flatulence with L. rhamnosus GG use (Lawrence *et al.*, 2005) nausea, vomiting, abdominal pain, rash, diarrhoea and constipation, have been reported (McFarland, 2009). Although serious complications from probiotic use are exceedingly rare, given that probiotics are live microorganisms, it is conceivable that they may rarely result in invasive infections. Mackay and others reported a case of Lactobacillus endocarditis with probiotic use in a patient with underlying mitral valve disease (Mackay *et al.*, 1999). A case of recurrent Bacillus subtilis septicemia has also been reported in an immunocompromised patient (Oggioni *et al.*, 1998) after the use of probiotics containing B. subtilis. There have also been several reported cases of S. boulardii fungemia associated with probiotic use. Most cases of invasive infections associated with the use of probiotic have occurred in patients with intravenous catheters (Hennequin *et al.*, 2000) the elderly (Cherifi *et al.*, 2004) and immunocompromised population (Cesaro *et al.*, 2000).

2.6.7 Probiotic in layer chicken production

Avian coccidiosis is the major parasitis diseases causing mortality, malabsorption, inefficient feed ultilisation, impaired growth rate and reduced egg production in layers (Haddadin *et al.*, 1996). Davis and Anderson (2002) reported that a hen receiving a Direct-Fed Microbial treatment diet exhibited greater egg weight (61.72g) and percentages of extra large egg (XLE 52.0.6 %) compared to the control (61.12g and 48.98) respectively. The use of DFM therefore resulted in a shift from smaller to larger eggs regardless of the stocking density. Given the current interest in reducing the dietary intake of cholesterol by humans (Anonymous, 1986), producing eggs with reduced cholesterol concentration might prove attractive as an aid to marketing table eggs (Haddadin *et al.*, 1996). Mohan *et al.*, (1995) also observed a significant ($P<0.05$) reduction in serum cholesterol concentration of probiotics containing L. *acidophilus, L. casei, B. bifidum, A. oryzae* and *Torulopsis.* Mean egg yolk

cholesterol value was decreased and attributed to reduce absorption, synthesis or both of cholesterol in the GIT.

2.6.8 Effects of Probiotics on egg production

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 and Panda *et al.,* 2008).

2.6.9 Mechanisms through which probiotics improve feed conversion ratio

Probiotics improve feed conversion efficiency through several mechanisms including alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo,1997). Therefore, the major outcomes from using probiotics include improvement in growth (Yeo, 1997), reduction in mortality (Kumprecht and Zobac, 1998), and improvement in feed conversion efficiency (Yeo, 1997). Gil de los Santos *et al.,* (2005) demonstrated that improved feed conversion rate by 12 and 11 per cent was obtained when broiler control diet was supplemented with a probiotic based on *Bacillus cereus* and *Saccharomyces boulardii* respectively. Moreover, after 47 days, average live weight was significantly higher (16 and 7 per cent, respectively) in birds fed the two types of probiotics in comparison to the control group.

2.6.10 Influence of Probiotics on feed intake of poultry birds

Santoso *et al.,* (2001) reported reduction in feed intake of birds given probiotics in the diet. It has been observed that microorganisms in probiotics perform well in warm season, since they are able to multiply and work very well in the body of the bird thereby increasing feed intake of the birds. Wenk (2000) also reported the usefulness of probiotic supplementation in the early stages of chick growth; since the gut of the newly hatched chick is sterile. Zulkifli *et al.,* (2000) however, indicated that under heat stress condition, *Lactobacilli* probiotic supplementation increased feed conversion efficiency. In other experiments involving broiler chickens, inconsistent results of feed intake were reported. Liu *et al.,* (2007) and Murry *et al.,* (2007) found no significant differences in feed intake between the control and birds fed probiotics; whereas Jin *et al.,* (1998a) and Zulkifli *et al.,* (2000) observed significant differences between the control and their counterparts when probiotic was incorporated into their diets. This inconsistency could be as a result of the complex rearing environment according to Zulkifli *et al.,* (2000). The growth-promoting effects of probiotics are dependent on the specific probiotics, the application level of probiotics, the age of birds as well as the administration method (*i.e.* via water and/or feed) as reported by Edens (2003).

2.6.11 Effects of Probiotics on weight gain of poultry birds

Studies on the beneficial impact on poultry performance have indicated that probiotic supplementation can have positive effects. According to Kabir *et al.,* (2004) live weight gains were significantly (P<0.01) higher in experimental birds as compared to control ones at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated and non vaccinated birds when probiotic was supplemented in feed. On the other hand, Lan *et al.,* (2003) found higher (P<0.01) weight gains in broilers subjected to two probiotic species. Huang *et al.* (2004) demonstrated that inactivated probiotics, disrupted by a high-pressure

homogenizer, have positive effects on the weight gains of broiler chickens when used at certain concentrations. According to Santos *et al.,* (2001) the administration of the multi-strain probiotic (PoultryStar) in the drinking water significantly improved live weight (4 per cent) and daily weight gain (4 per cent) in comparison with a negative control in layer birds. Torres-Rodriguez *et al*., (2007) also reported a 1.5 and 2.0 per cent improvement in average daily weight gain and feed conversion rate, respectively in turkey, when diets were supplemented with a blend of different probiotic strains originating from *Bacillus* or *Enterococcus* representing an economic alternative to improve turkey production. In a study by Mountzouris *et al.,* (2006) with broilers, the above mentioned multi-strain probiotic additive also increased average daily weight gain and feed efficiency (by three and two per cent, respectively, in two applications).

2.6.12 Effects of probiotic on meat quality

Kabir, (2008) and Kabir *et al.,* (2005) evaluated the effects of probiotics on the sensory characteristics and microbiological quality of dressed broiler meat and reported that supplementation of probiotics in broiler ration improved the meat quality both at pre-freezing and post-freezing storage. Mahajan *et al.,* (2000) stated that the scores for the sensory attributes of the meat balls appearance, texture, juiciness and overall acceptability were significantly ($p<0.001$) higher and those for flavour were lower in the probiotic (Lacto-Sacc) fed group. Simultaneously, Mahajan *et al.,* (2000) reported that meat from probiotic (Lacto-Sacc) fed birds showed lower total viable count as compared to the meat obtained from control birds. On the other hand, Loddi *et al.,* (2000) reported that probiotic did not affect sensory characteristics (intensity of aroma, strange aroma, flavour, strange flavour, tenderness, juiciness, acceptability, characteristic colour and overall aspects) of breast and leg meats. Zhang *et al.,* (2005) conducted an experiment with 240, day-old, male broilers to investigate the effects of *Saccharomyces cerevisiae* (SC) cell components on the meat quality

and they reported that meat tenderness could be improved by the whole yeast (WY) or *Saccharomyces cerevisiae* extract (YE).

2.6.13 The use of probiotics in the food and beverage industry

A probiotic may also be a functional food (Scheinbach, 1998). Functional foods are defined as foods that contain some health-promoting component(s) beyond traditional nutrients'. Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods and medifoods. Probiotics can be added to foods to modify it to become functional (FAO/WHO, 2002). New food products have been formulated with the addition of probiotic cultures. Different types of food matrices have been used such as various types of cheese, ice creams, milk-based desserts, powdered milk for newborn infants, butter, mayonnaise, powder products or capsules and fermented food of vegetable origin (Tamime *et al*., 2005). Dairy products are especially considered as ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract. Yoghurts with high fat content showed inhibitory effects against probiotic cultures, particularly *B. bifidum* BBI (Vinderola *et al.,* 2000). The supplementation with vitamins (*e.g.* ascorbic acid) has been reported to improve the viability of *L. acidophilus* in yoghurts (Dave and Shah, 1997). The addition of substances such as whey protein may also enhance the viability of some probiotics, probably due to their buffering property. The survival of probiotics has been assayed in soymilk and this substrate has shown to be efficient for the growth of species such as *L. casei* (Garro *et al.*, 1999), *L. acidophilus* (Wang *et al.,* 2002), *B. infantis*, and *B. longum* (Chou and Hou, 2000). In addition, the antioxidative activities of soymilk can be increased after fermentation by lactic acid bacteria and bifidobacteria (Vinderola *et al.*, 2000). This has led to the designing of the probiotic soybean yoghurt (Wang *et al.*, 2006).

When *B. lactis* were microencapsulated, incorporated into African fermented beverages (amasi and mahewu) and assayed for physiological conditions of the stomach, they showed a

high survival rate, *i.e.* the microencapsulation enhanced the viability in comparison with free cells (McMaster *et al.,* 2005). It was found that these same bacteria grew in beet juice (Kyung *et al.,* 2005). Arora *et al*., (*2010*) found an enhancement of 14 and 11 % in thiamine and niacin contents, respectively, when food mixture based on germinated barley flour with whey powder and tomato pulp were autoclaved and fermented by *L. acidophilus*.

2.7.0 Works done with probiotics (RE3) in the poultry industry in Ghana

Investigations conducted on the probiotic, RE 3 in Ghana using different animal models have generated great responses in the form of growth rate improvement, efficiency of feed utilization in pigs and poultry, superior egg production and characteristics as well as lowered mortality in laying birds, weight gain and delayed weight loss under feed-stress conditions in sheep (Osei *et al.,* 2008; Okai, 2010). The use of RE3 did not affect total feed intake of broilers. This was because there was no significant difference between the control and treatment diets although birds on the RE3 diet tended to consume slightly more feed than their counterparts on the basal diet (Osei *et al.,* 2008). The results in Table 8 showed that, there was a significant difference in weight gain and final body weight between the basic diet and the RE3 diet. That was, birds on the RE3-based diet recorded higher weight gains when compared with the birds on the basic diet. When RE-3 was fed to the birds, there was a significant difference ($P<0.05$) in feed conversion efficiency between the basic diet and the RE3 diet. This was as a result of the treatment birds being able to convert and utilize, better than the control birds (Osei *et al.,* 2008; Okai, 2010). Birds on the basal diet recorded higher mortality rate whilst birds on the RE3-based diet recorded no mortality. However, Dei *et al.*, (2010) and Bonsu *et al.,* (2012) fed broilers diets supplemented with RE-3, birds on RE-3 consumed less feed ($P<0.05$) compared to their counterparts on diets with no probiotic.

Table 8: Performance of broilers fed probiotics

Parameter	Basal diet	Basal diet + RE3
Total feed intake	5669 [a]	5809 [a]
Daily feed intake, g	101 [b]	104 [a]
Final body weight, g	2302 [b]	2571 [a]
Body weight gain, g	2342 [b]	2493 [a]
Feed conversion efficiency (feed/gain ratio)	2.53 [b]	2.35 [a]
Mortality, g (4-8 wks)	6.25	0.00

Source: Osei *et al.,* (2008)

2.8.0 Inferences from literature

Probiotics or Direct Fed Microbials (DFM's) are viable, bacterial or fungal cultures which are able to improve the balance of intestinal flora and exert beneficial effects on the individual in which it has been administered (Benno and Mitsuoka, 1992; Rolfe, 2000). In Ghana several researches have been done on the effects of probiotics on different production indices within farm animals; some have shown significant improvement in animal growth (Okai *et al.*, 2008) whilst most of the researches (Brown, 2009 and Owusu-Amoah, 2010) have failed to indicate any clear cut headways. Bonsu *et al.*, (2012) found that the inclusion of a DFM product, RE-3, in the diets of layers and broilers resulted in considerable reduction of body fat and serum cholesterol content (up to 16%) in broilers, a 15% decrease in cholesterol level of eggs, improved egg weight and reduced mortality in both broilers and layers. Dei *et al.*, (2010) also indicated that the addition of RE-3 to the diets of grower birds significantly reduced mortality compared to birds on a control diet containing no DFM. DFM (RE-3) has also been found to have a positive influence on average daily gain in pigs (Okai *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Duration of Experiment

Two experiments (experiment I and II) were conducted at the Poultry Section of the Animal Science Department in the College of Agriculture and Natural Resources of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. It lasted for six (6) months (June-December, 2012).

Kwame Nkrumah University of Science and Technology Poultry Section is within the semi-deciduous forest zone of Ghana. Kumasi is located within latitude $0^0$43'N and longitude $01^0$36'W (Jollans, 1960) as cited by Bonsu *et al.*, (2012). Yearly average rainfall is 1570±344.9mm and maximum and minimum temperatures of 33 and 22°C, respectively throughout the year. The maximum temperature of 33^0C is recorded in February or March and the minimum temperature of about 22^0C is recorded in May. Relative humidity varies from 75% in the afternoon to 90% in the mornings (Bonsu *et al.*, 2012). Winds are consistently from South-West, except in part of December and January when they are from the West-East. The climate in Kumasi is generally described as hot and humid.

3.2 Experiments carried out for layers and growers

There were two different experiments, carried out as follows:

Experiment I - Starter-Grower (Growth performance and sexing) experiment

Experiment II - Layer experiment

3.3.0 Experiment 1

3.3.1 Experimental birds, treatments, diet composition and experimental design

Three hundred and twenty 40-week old layers and thirty-two (32) cocks of the same age were obtained from Akate Farms and Trading Company Limited for the experiment. The layers

were female parents of a local-exotic crossbred (96.88% exotic and 3.12% local) and the males were male parents of Lohmann brown cocks. Each pen contained twenty two (22) birds (20 layers and 2 males) for the first eight (8) weeks of the experiment and thereafter twenty (20) layers in a replicate till end of the experiment. There was a double-compartment laying nest (each compartment was 15 cm × 9 cm in measurement) in each pen. The birds were allowed a one-week adaptation period before the start of the experiment. Each treatment was replicated four times in a Completely Randomized Design (CRD).

There were four (4) treatments which were named: T1, T2, T3 and T4. Each treatment had four replications with twenty (20) layers and two (2) males in each replicate. The descriptions of the four treatments are as follows:

T1: Birds on this treatment received the layer diet with no added probiotics (Table 9)

T2: Birds were fed the same layer diet to which was added 1.5mls of RE3™ solution in every kg diet

T3: 1.5mls of RE3 Plus solution was added in every kg of the same layer diet for birds on this treatment

T4: Birds on this treatment received 1.0mls of RE3™ + 0.5mls of *P. polymyxa* (*Paenebacillus polymyxa*-based probiotic) solution in every kg of layer diet

Table 9: Feed composition and calculated analysis of layer diet on percent dry matter basis

Ingredient	Quantity (kg)
Maize	54.0
Fishmeal	6.0
Soybean Meal	15.0
Wheat Bran	16.0
Oyster Shell	8.0
Vitamin/Mineral Premix*	0.5
Sodium Chloride	0.5
Total	100
Nutrient composition (calculated)	
Crude Protein (%)	18.00
Crude Fibre (%)	2.79
Ash (%)	2.65
Metabolizable Energy ($MJkg^{-1}$)	2754
Lysine (%)	0.98
Methionine (%)	0.34
Calcium (%)	3.42
Phosphorous (%)	0.74
Cystine (%)	0.52

*Composition of vitamin/ trace mineral premix per kg diet:
Vitamin premix per kg diet: Vitamin A (8x 10^3IU), Vitamin D3 (2.0 IU); Vitamin E (10.0 IU); Vitamin K3 (1.5 mg); Vitamin B_2 (2x10mg); Vitamin B_{12} (0.5mg); Folic acid (0.6mg); Nicotinic acid (5 mg); Calcium panthotenate (4mg); Choline (0.078mg).Trace elements: Mg (5x10mg); Zn (5x10mg); Cu (2.5mg); Co (0.5mg); I (2mg); Se (0.2mg). Antioxidants: Butylated hydroxytoluene (0.625mg).Carrier: Calcium carbonate q.s.p (0.25kg).

3.3.2 Management of layers

Birds were housed in an open sided deep litter pen of dimension 3.00m X 1.8m given a total area of 5.4m^2/pen and affording a space per bird of 0.216m^2 with wood shavings as the litter material. The pen had a part elevated at a height of 0.85m and covered with welded mesh such that droppings from birds will passed through the mesh and drop outside the pen. All pens were cleaned and disinfected before the start of the experiment. Subsequently, floors of the pens were swept and water troughs were washed daily and fresh water provided every morning. Feeding troughs were also cleaned daily before feed was provided. Drinkers were labelled and placed at the elevated part of the pen to ensure drinking water is devoid of litter contamination. Feed and water were given *ad libitum*. Birds were given dewormer, Piperazine Citrate (KELA, Belgium) and Newcastle vaccine, Newcavac (INTERVET, Holland) during the first and ninth weeks of the experiment respectively. Foot bath was provided at the entrance to the pen to prevent contamination.

Egg laying nests were placed in each pen and eggs collected twice daily, 9.00hr and 3.00hr. Ten days after introducing the males to the females, egg collection for weekly incubation started.

3.3.3 Source of Probiotics

Direct–Fed Microbial was donated by Basic Environmental System and Technology Inc. (BEST), Canada. Probiotics were kept in a fridge upon receipt and stored there throughout the period of the experiment.

3.3.4. Incubation

This is the process by which fertile eggs are subjected to conditions suitable for the initiation and sustaining of embryonic development and the hatching of strong, healthy chickens.

Weekly incubation of fertile eggs began ten (10) days after cocks were introduced to hens and was done consecutively for seven (7) weeks. Eggs collected from layers for the week were sent to a commercial hatchery for hatching after dirty, irregular shape, cracked, very small/large eggs were removed. Hatchery practices such as fumigation, candling, setting and other activities were carried out at the commercial hatchery. The first hatch was pulled three (3) weeks after the first batch of eggs were set and subsequently every week for seven consecutive weeks. The following parameters were measured: hatchability of fertile eggs, total egg hatchability, dead in shells and saleable chicks.

3.3.5 Data Collection

The following parameters were measured weekly: initial and final weights, feed intake, feed conversion ratio (FCR), hen-day rate of lay, hen-housed rate of lay and egg weight.

3.3.5.1 Mean feed intake

Feed consumption of birds was measured weekly. Feed consumed per birds per pen was measured by subtracting feed left over in trough from total feed supplied for a week using a digital electronic scale (Jadever, JPS-1050). It was then divided by the number of birds in a replicate and number of days to obtain mean feed intake per bird per day.

3.3.5.2 Live weight

A week before the treatment diets were introduced and at the end of the experiment, birds in each pen were weighed together using a metric scale (Vintage Avery 3205 ABA, England) by putting them in container with known weight and weighing on scale. The weight was divided by the number of birds in the pen to obtain the mean live weight per bird. Live weight was then calculated by getting the difference in final and initial weight of birds.

3.3.5.3 Mean egg weight

Eggs collected weekly were weighed treatment by treatment using an electronic scale (Scout Pro SP7129141298, USA) with 0.01g accuracy. The mean egg weight was calculated as the total weight of eggs collected in a week divided by the number of eggs collected in a week. This was also expressed in grams (g).

3.3.5.4 Feed conversion ratio (FCR)

The efficiency of utilization of feed was obtained as the amount of feed utilized to produce 1kg of egg.

3.3.5.5 Hen day production

The number of eggs laid by the hens per replicate was recorded daily; from this, hen-day was calculated by dividing the number of eggs laid in a day by total number of hens in the pen and multiplied by 100. This was expressed as a percentage (%).

3.3.5.6 Hen House Production

Hen house production was calculated on the basis of the number of birds placed in the laying house at point of lay. This was expressed as a percentage (%).

3.3.5.7 Mortality

Mortality was determined as a percentage of the number of dead birds divided by the number of birds at the start of the experiment.

3.3.5.8 Hatchability of fertile eggs

Hatchability of fertile eggs was calculated by dividing the number of total eggs set minus infertile eggs by the number of total eggs set and multiplying by 100. This was expressed as a percentage (%).

3.3.5.9 Total egg hatchability

Total egg hatchability was also calculated as the ratio of the number of chicks hatched by the number of total eggs set and multiplies by 100. This was expressed as a percentage (%).

Total egg hatchability = (Chicks hatched/Total eggs set) × 100

3.3.5.10 Dead in shell

Dead in shell is when the embryo in an egg develops mid-way but dies without hatching**.** This was expressed as a percentage (%).

3.3.5.11 Saleable chicks

Saleable chicks were calculated by subtracting abnormal and deformed chicks from the total hatch by visual observation. It was expressed as a percentage (%).

3.3.5.12 Blood and faecal sampling and analysis

Blood and faecal samples were collected randomly from two birds from each replicate on the starting date before probiotics were introduced and were measured subsequently once every three months. Faecal samples were collected directly from the cloaca. Blood samples were collected (before feed and water was given) from wing vein into anticoagulant (heparin) bottles and analyzed for total red blood cells (RBC), haemoglobin (HB), packed cell volume (PCV), white blood cells (WBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin

(MCH), Mean Cell Haemoglobin Concentration (MCHC), Platelets, Mean Platelet Volume (MPV) and serum cholesterol using a Haematological Auto Analyzer. The haematology procedure used was the Complete Blood Count (Tiezt, 1995). Blood samples were filled into micro-capillary tubes, sealed and fixed in a slot of a Thermo-spectronic machine (Mindray Automatic Hematology Analyzer BC 5300, China). They were centrifuged at 3000 rpm for five (5) minutes.

Biochemical parameters that were analyzed included: total protein, albumin, and cholesterol. Blood samples were taken at 1, 90, and 180d of age from two birds randomly selected from each replicate pen assigned to the 4 treatments, for total serum protein, albumen, serum immunologlobulin A, and M, cluster of differentiation 3 and 4 and packed cell volume.

The chemical analysis of the blood samples taken was carried out at the Biochemistry Laboratory Komfo Anokye Teaching Hospital using the colometric method (Tietz, 1995). Blood samples in the vacutainer tubes were allowed to clot by leaving them at room temperature for about 2-3 hours. They were centrifuged and spun at 3000 rpm for 5 minutes. The serum was pipetted into clean dried bottles, labelled accordingly and stored in a freezer at -20°C until the test was ready to be done. Prior to the test, the samples were allowed to thaw. This method makes use of samples, reagents, standard solutions and colorimeter capable of measuring absorbance at a specified wavelength. These parameters were used to assess the health status of the animals under the various treatments.

3.3.6 Statistical Analysis

Data from experiment were subjected to analysis of variance (ANOVA) using Genstat (2009). The performance of birds under the four treatments was compared at 5% level of significance.

3.4.0 Experiment II

Starter-Grower experiment (Growth performance and sexing)

3.4.1Treatments description

There were four (4) treatments which were named: T1, T2, T3 and T4. The descriptions of the four treatments are as follows:

T1: Birds on this treatment received the grower diet with no added probiotics (Table 9)

T2: Birds were fed the same grower diet to which was added 1.5mls of RE3™ solution in every kg diet

T3: 1.5mls of RE3 Plus solution was added in every kg of the same grower diet for birds on this treatment

T4: Birds on this treatment received 1.0mls of RE3™ + 0.5mls of *P. polymyxa* (*Paenebacillus polymyxa*-based probiotic) solution in every kg of grower diet

3.4.2 Source of chicks, management, diet composition and experimental design

A total of 800 day-old layer chicks were used for experiment II. They were allocated to four treatments with two hundred (200) chicks in a treatment. Each treatment was replicated four times in a Completely Randomized Design (CRD) with fifty (50) chicks in a replicate. They were fed with chicks' starter diet (Table 10).

All four groups were housed in different brooder houses with wood shaving as litter and incandescent bulbs which provided light and heat for 24 h for the first three weeks after which they were moved to their permanent pens and intensity of heat reduced. Medication and vaccines were also given. Sexing was done at the 8th week to determine the ratio of males to females for each treatment using the difference between the combs and the feathers. Food and water was given *ad libitum* in a chick-type feeder and drinker.

Table 10: Feed composition and calculated analysis of starter-grower diet on percent dry matter (0-9 wks)

Ingredient Quantity	(kg)
Maize	60.0
Fishmeal	10.0
Soybean Meal	15.0
Wheat Bran	12.0
Oyster Shell	2.0
Vitamin/Mineral Premix*	0.5
Sodium chloride	0.5
Total	100
Nutrient composition (calculated)	
Crude Protein (%)	20.34
Crude Fibre (%)	3.4
Ash (%)	2.61
Metabolizable Energy ($MJkg^{-1}$)	2769.2
Lysine (%)	1.17
Methionine (%)	0.44
Calcium (%)	1.29
Phosphorus (%)	0.76
Cystine (%)	0.54

*Composition of vitamin/ trace mineral premix per kg diet:
Vitamin premix per kg diet: Vitamin A (8x 10^3IU), Vitamin D3 (2.0 IU); Vitamin E (10.0 IU); Vitamin K3 (1.5 mg); Vitamin B_2 (2x10mg); Vitamin B_{12} (0.5mg); Folic acid (0.6mg); Nicotinic acid (5 mg); Calcium panthotenate (4mg); Choline (0.078mg).Trace elements: Mg (5x10mg); Zn (5x10mg); Cu (2.5mg); Co (0.5mg); I (2mg); Se (0.2mg). Antioxidants: Butylated hydroxytoluene(0.625mg).Carrier: Calcium carbonate q.s.p (0.25kg).

3.4.3 Data Collection

Parameters such as feed intake, initial weight, daily weight gain, final life body weight, feed conversion ratio (FCR), sexing (sex ratio) and mortality were determined.

3.4.3.1 Feed intake

Feed consumption of birds was measured weekly. Feed consumed per birds per pen was measured by subtracting feed left-over in trough at the end of the week from total feed supplied for a week using a kitchen scale (Jadever, JPS-1050). It was then divided by the number of birds in a replicate and number of days to obtain mean feed intake per bird per day. It was expressed in grams (g).

3.4.3.2 Initial weight

Before the treatment diets were given, chicks in each treatment pen were weighed using a metric scale (Vintage Avery 3205 ABA, England) by putting them in container with known weight and weighing on scale. The weight was divided by the number of chicks in the pen to obtain the initial weight per chick. Initial weight was expressed in grams (g).

3.4.3.3 Daily weight gain

The chicks were weighed using a metric scale (Vintage Avery 3205 ABA, England) by putting them in container with known weight and weighing on scale. The weight was divided by the number of chicks in the pen and then by 7 to get the daily weight. It was expressed in gram (g).

3.4.3.4 Final Live Body Weight

Birds in each pen were weighed at the end of the experiment. The weight was divided by the number of birds in the pen to obtain the mean live weight per bird. This was expressed in grams (g).

3.4.3.5 Feed Conversion Ratio (FCR)

The efficiency of utilization of feed was obtained as the amount of feed utilized to produce 1g of body weight.

3.4.3.6 Sexing

Separation of the male chicks (cockerels) from the female chicks (pullets) was done using their combs, body, wattles, and feathers after the 8th week of brooding. After the 8th week of brooding the comb, body and wattles of the cockerels were visibly bigger in appearance than that of the pullets and the males had relatively shorter wing feathers than the females. The female's covert feathers were shorter than the primary feathers and in the males, the covert feathers were as long as/or longer than the primary feathers making separation easy. Sexing was not done on day of hatching because it was difficult to distinguish the above features on the males from the females. This was expressed in percentages (%).

3.4.3.7 Mortality

Mortality was determined as a percentage of the number of dead birds divided by the number of birds at the start of the experiment.

3.4.4 Statistical Analysis

Data from experiment were subjected to analysis of variance (ANOVA) using Genstat (2009). The performance of birds under the four treatments was compared at 5% level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Layer experiment (Experiment I)

4.1.0 Production studies

Table 11 is the summarized layer performance data for the twenty-four weeks of the experiment.

4.1.1 Initial and Final body weights

Both initial and final weights did not differ significantly (P>0.05) among the layers fed the different probiotics (Table 11). This is in contrast to the findings of Fritts *et al.,* (2000), Gil de los Santos *et al*., (2005) and Samad *et al.,* (2011) who concluded that the feeding of probiotic-supplemented diets to chicken increased the final body weight of chickens by 16, 5 and 7 per cent, respectively. The positive effects of probiotics supplementation could be due to decrease in the multiplication of harmful bacteria resulting from improvement in gut environment and enhanced nutrient utilization (Miles, 1993). These however were not achieved in this experiment.

4.1.2 Feed intake

Differences in feed intake between layers on the various treatment were not significantly different (P>0.05, Table 11). However, birds on the basal diet tended to consume the least (116.86g) amount of feed with layers on RE3 Plus diet consuming the highest (118.18g). Results are contrary to that observed by Dei *et al.,* (2010) and Bonsu *et al.,* (2012) where broilers fed diets supplemented with RE-3 consumed less feed (P<0.05) compared to their counterparts on diets with no probiotic. This probably is because they used broilers for their studies whilst birds used in this experiment were layer. Bonsu *et al.,* (2012) however attributed the reduction in intake to the improved nutrient retention and utilization arising

from a better gastrointestinal tract (GIT) environment enabled by the beneficial microorganisms. The results also are in contrast with Anukam *et al.,* (2005) who recorded increased intake when rats were given feed supplemented with a DFM product containing *Lactobacillus* strains. It has been reported that probiotic-supplemented diets enhances digestion through the production of enzymes (Anukam *et al.,* 2005).

Table 11: Effect of diets on egg production and growth performance of layers

PARAMETERS	TREATMENTS[#]					
	Basal	RE-3	RE-3 Plus	P3	Lsd	P
Initial weight (kg)	1.62	1.58	1.62	1.62	0.11	0.841
Final weight (kg)	1.64	1.60	1.60	1.60		0.865
Feed intake/bird/day (g)	116.86	117.05	118.18	116.96	5.75	0.823
Rate of lay, hen-day (%)	64.80	70.31	71.21	68.14	10.95	0.159
Rate of lay, hen-house (%)	61.60	65.20	69.50	64.70	9.70	0.150
Average egg weight (g)	59.05	59.01	59.08	59.03	1.34	0.999
FCR (Feed intake/kg egg weight)	3.07	2.83	2.83	2.97	0.39	0.189
Mortality (%)	7.5[a]	10.0[a]	2.5[b]	11.25[a]	7.57	0.009

[#]Treatment means were compared at 5% level of significance (P<0.05); L.S.D: least significant difference; P: P-value

[a–b]Means with different superscripts in a row differed significantly (P < 0.05).

4.1.3 Hen-day and Hen-house production

Hen-day and hen-housed rates of lay did not differ significantly (P>0.05) among the layers on the four treatments (Table 11). Nonetheless, layers fed the probiotic-supplemented diets did well numerically. This outcome is similar to those reported by Lalev *et al.,* (2011) and Yörük *et al.,* (2004), who recorded non-significant increases (P>0.05) in the rate of lay of layers fed

diets supplemented with probiotics. North (1984) attributed the non-significance to the dose level, type of strain, diet composition, feeding strategy and form of the probiotic supplemented.

4.1.4 Egg Weight

The mean weight of eggs produced by layers were not statistically significant (P>0.05), (Table 11). Probiotic-supplementation (59.01 (RE-3 diet), 59.08 (RE-3 Plus diet) and 59.03g (P3 diet) respectively) did not affect egg weight (59.05g, basal diet) and this could be attributed to the dose (1.5ml probiotic per kg feed) of probiotic used or the feeding strategy. This agrees with the results recorded by Lalev *et al*., (2011), Yoruk *et al*., (2004) and Hosseini *et al*., (2006) where differences recorded in egg weight were not significantly different (P>0.05) with the supplementation of probiotics. On the contrary, Davis and Anderson (2002) and Bonsu *et al*., (2012) recorded significantly (P<0.05) heavier egg weights with the inclusion of probiotics. These inconsistencies could be attributed to the differences in strains of bacteria/birds, dose level, diet composition, feeding strategy, management practices, feed form and interaction with other dietary feed additives (Chesson, 1994).

4.1.5 Feed Conversion Ratio (FCR)

FCR values among the layers kept under the four treatments were not significantly different (P>0.05), however, the numerical differences showed that layers on the basal (3.07kg/egg weight) diet compared to those on the probiotic-supplemented (2.83-2.97kg/egg weight) diets had the highest value . Arpasova *et al*., (2012), Liu *et al.*, (2007) and Murry *et al.*, (2007) had similar results when diets for laying hens were supplemented with probiotic and their results confirm what Chumpawadee *et al.*, (2008), quoting various sources, had intimated that,

probiotic use is ineffective in animals housed in clean environments as was the situation in this experiment because researchers kept a well hygienic environment.

Penkov and Hristova, (2004) and Bonsu *et al.,* (2012) however, reported better FCR for probiotic fed birds compared to the non-supplemented group and Bonsu *et al.,* (2012) attributed it to the increased feed retention and nutrient utilization arising from a better GIT environment devoid of entero-pathogenic micro-organisms. According to (Sissons, (1989) and (Jin *et al.,* 2000) improved feed conversion might be explained by the increased intestinal amylase activity when lactic acid bacteria are fed to fowl.

4.1.6 Mortality

Mortality was significantly lower (P>0.05) in birds fed with the diet containing the fermentation product of RE-3 (2.5%) compared to those fed the other treatments (7.5, 10.0 and 11.25% respectively). According to Arpasova *et al.,* (2012) probiotics increases resistance to infectious diseases and reduces risk of mortality caused by the presence of infectious diseases; they further explained that this effect may be strain specific and that some probiotic strains may not exhibit such effects. Bonsu (2010) also found that the inclusion of probiotic (RE-3) in the diets of broilers and layers reduced mortality by 4%. Lalev *et al*., (2011), Kritas *et al.,* (2008) and Dei *et al*., (2010) similarly observed significant reduction in mortality with the addition of DFM to the diets of broiler breeders compared to those fed a basal diet without probiotic. From the statistically insignificant variations in mortality rates observed among all the treatment groups, it would be out of place to attest to the comparative effectiveness of RE-3 in maintaining the natural defense mechanism of layers introduced the product as part of the diet regimen by the elimination of entero-pathogenic micro-organisms through 'competitive exclusion' aided by the production of antimicrobial substance (lactic acid) by the RE-3 as confirmed by (Nurmi and Rantala, 1973; Stern *et al*., 2001 in Bonsu *et al.,* 2012). A similar observation was made in relation to pigs given 1.5 ml RE-3/kg feed

when Owusu-Amoah, (2010) supplemented their feed with RE-3 and recorded no significant difference. Cause of death in this experiment was not attributed to diseases. In the case of the probiotic treatment deaths, it was associated with reproductive disorder especially impacted oviduct. This condition was goaded by increased egg size of these treatments group, birds then pecked affected birds to death especially in the absence of caretakers. Bonsu *et al.,* (2012) reported a similar incident with layers when probiotic was supplemented in their diet.

4.1.7 Hatchability of fertile eggs

The addition of probiotic to the diets of birds had no significant (P>0.05) effect on the hatchability of the fertile eggs set (Table 12). This is in accordance with Lalev *et al.,* (2011) who also recorded no significant differences in the hatchability of fertile eggs although, numerical differences favoured birds on the supplemented diets. This was explained by Chesson, (1994) that the effect of probiotics may be due to several aspects of the probiotic such as strains of bacteria, dose level, diet composition, feeding strategy, feed form and interaction with other dietary feed additives.

Table 12: Reproductive records for layers on the four treatments

PARAMETERS	**TREATMENTS**[#]						
	Basal	RE-3	RE-3 Plus	P3	Sed	Lsd	P
Hatchability of fertile eggs set (%)	85.87	86.69	84.08	84.59	4.67	10.55	0.940
Dead in shell of fertile eggs (%)	34.35	33.09	34.97	29.53	5.57	12.60	0.769
Total egg hatchability (%)	65.65	66.91	65.03	70.47	5.57	12.60	0.769
Saleable Chicks of fertile eggs set (%)	64.99	66.84	64.29	69.57	5.42	12.27	0.771

[#]Treatment means were compared at 5% level of significance (P<0.05); S.E.D: standard errors of differences; L.S.D.: least significant difference; P: P-value; [a–b]Means with different superscripts in a row differed significantly (P < 0.05).

4.1.8 Dead-in-shell

The percentage values for dead-in-shell were not significantly different (P>0.05) among the various treatments (Table 12). This disagrees with Altan *et al*., (1995) who obtained increased dead in shell in the eggs from the treatment birds. They attributed the increased dead in shell to egg size (>80g) and explained that larger eggs have greater difficulty achieving adequate embryonic temperature at the initial stages of incubation as a result losing embryonic metabolic heat during later stage of incubation with increased difficulty of heat dissipation and a resultant higher embryo mortality (Altan *et al*., (1995). Their explanation does not apply to this experiment because medium (55.0-79.9g) and clean eggs were carefully selected for the incubation. All eggs that were above 79.9g of weight were rejected. A study by King'ori (2011) also attributed dead in shell to a number of factors including lethal genes, insufficient nutrients in the egg and exposure to conditions that do not meet the needs of the developing embryo.

4.1.9 Total egg hatchability

The probiotic had little effect (P>0.05) on the hatchability of the eggs set (Table 12). Roque and Soares (1994) explained that the addition of probiotics to the ration of birds improves the shell thickness of eggs by facilitation of the absorption of minerals, leading to higher hatchability values. These properties of probiotic cannot be said to have been realized in this experiment or not because egg shell thickness was not measured. Hatchability was generally low, which could be attributed to the high dead- in-shell values encountered.

4.1.10 Saleable chicks as a percentage of total hatchability

The percentage of saleable chicks (Table 12) from layers under the four treatments was not significantly different (P>0.05) and did not follow any clear trend. There is dearth of information on the effect of probiotics on percentage saleable chicks.

4.2.0 Blood analysis

Blood analysis was made up of the haematological, biochemical and immunological parameters of the layers in experiment I.

4.2.1 Haematological Studies

A summary of haematological data is presented in Table 13. Overall, probiotic-supplementation had no significant effect on any of the haematologic traits measured (P>0.05). However, treatment 2 (RE-3) had significantly higher value (P<0.05) for blood platelets midway the experiment than the basal and RE-3 Plus supplemented diet but no significant difference at the end of the experiment. This is similar to what Chen *et al.,* (2005) reported that hematology and serum chemistry parameters, RBC, WBC and lymphocyte were not affected by the dietary treatments (p>0.05). According to La Ragione *et al.,* 2001, Dimcho *et al.,* (2005) and Knowles *et al.,* (2000) the addition of DFM did not affect RBC, WBC, haemoglobin, haematocrit and platelet, total protein and total cholesterol concentrations significantly. However, values obtained from the haematological analysis were within the normal physiological ranges and this is in conformity to Blood and Studdert, (1999) data for gilts and layers.

Table 13: Effects of diet on hematological parameters of layers

Parameters	Time	Treatment[#]					
		Basal	RE-3	RE3 Plus	P3	Lsd	P
WBC {10^3/ul}	1	510	515	522	469	100.30	0.661
	2	598	589	589	610	71.20	0.904
	3	568	551	554	590	75.10	0.675
RBC {10^6/ul}	1	2.19	2.23	2.21	1.95	0.37	0.374
	2	2.49	2.55	2.55	2.55	0.29	0.964
	3	2.27	2.26	2.11	2.33	0.36	0.607
HB {g/ul}	1	7.46	7.15	7.10	7.06	2.25	0.979
	2	8.16	8.35	8.18	8.29	0.85	0.954
	3	7.90	7.74	7.90	7.94	0.76	0.939
PCV {%}	1	28.06	27.62	27.61	25.16	4.11	0.435
	2	29.93	30.84	30.07	30.52	2.95	0.901
	3	29.76	29.15	27.86	29.79	4.18	0.727
MCV {fL}	1	128.59	124.92	125.75	127.03	5.19	0.469
	2	120.42	121.20	118.39	121.65	4.72	0.475
	3	131.90	128.90	132.20	128.00	7.53	0.548
MCH {Pg}	1	34.20	32.25	32.92	33.51	2.15	0.284
	2	32.79	32.49	31.84	32.96	1.99	0.635
	3	34.90	34.20	39.80	34.10	7.69	0.357
MCHC {g/ul}	1	26.62	25.79	26.19	25.93	1.06	0.374
	2	27.23	27.05	26.88	27.02	0.69	0.749
	3	26.48	26.52	30.12	26.64	5.83	0.471
PLT {10^3/ul}	1	3.06^b	3.69^b	3.51^b	5.62^a	1.81	0.043
	2	2.62^b	4.00^a	2.50^b	3.38^{ab}	1.12	0.043

	3	7.38	5.25	5.75	4.12	5.39	0.630
LYMPH {%}	1	54.20	51.90	49.40	44.50	9.13	0.171
	2	53.09	52.56	52.69	52.74	3.79	0.991
	3	50.44	51.66	49.51	47.36	4.88	0.315

[#]Treatment means were compared at 5% level of significance (P<0.05); L.S.D.: least significant difference; P: Probability value;

[a–b]Means with different superscripts in a row differed significantly (P < 0.05). 1: Start of Experiment; 2: Midway into Experiment; 3: End of Experiment

4.2.2 Biochemical parameters

Layers fed the RE-3 added diet (Treatment 2) had a higher mean value in total protein and albumin compared to those on basal diet, RE-3 Plus diet (T3) and P3 diet (T4). However, total protein, albumin and globulin did not differ significantly (P>0.05), (Table 14).

Table 14: Effect of dietary probiotic on biochemical parameters of layers

Parameters	Time	Treatment[#]					
		Basal	RE-3	RE-3 Plus	P3	Lsd	P
Total- Protein {g/L}	1	46.8	46.2	52.1	54.8	10.9	0.294
	2	49.7[b]	61.7[a]	50.4[b]	54.6[b]	8.9	0.045
	3	52.6	54.0	50.4	53.7	4.6	0.340
Albumin {g/L}	1	15.1	14.4	15.7	17.1	5.9	0.790
	2	32.5	39.1	33.4	36.2	5.9	0.114
	3	34.5	36.9	34.9	36.6	4.5	0.568
Globulin {g/L}	1	32.0	31.1	36.4	37.8	10.6	0.469
	2	17.2	22.5	15.8	18.3	7.2	0.252
	3	18.1	17.0	15.5	16.3	6.1	0.824

[#]Treatment means were compared at 5% level of significance (P<0.05); L.S.D.: least significant difference; P: Probability value;

[a–b]Means with different superscripts in a row differed significantly (P < 0.05).

1: Start of Experiment; 2: Midway into Experiment; 3: End of Experiment

4.2.2.1 Total -Protein

Significantly (P>0.05) the probiotics did not affect total protein values and this is in agreement with reports by Owusu-Amoah, (2010) and Al-Saiady (2010) when RE3 was fed to pigs and calves respectively. Dimcho *et al.,* (2005) also found no significant difference (P>0.05) in blood haemoglobin, total protein and total cholesterol concentrations when they fed probiotic as a supplement to Muskovy ducks.

4.2.2.2 Albumin

Albumin values in all the treatments although layers fed probiotics generally recorded higher values (36.99, 34.86 and 36.61g/l for RE-3, RE-3 Plus and P3 respectively) than layers on the basal diet (34.54g/l) were not significant. In accordance with this experiment Chen *et al.,* (2005) reported that probiotic supplementation did not have any effect on albumin, globulin and haematological parameters. Owusu-Amoah, (2010) on the other hand recorded a significant (P<0.05) differences in albumin values when he fed probiotic as a supplement to pigs. These inconsistencies of the effect of probiotics may be attributed to several aspects of probiotics such as strains of bacteria, dose level, diet composition, feeding strategy, feed form and interaction with other dietary feed additives (Chesson, 1994).

4.2.2.3 Globulin

Globulin values recorded for all the treatments were not statistically significant (P>0.05) from each other; however, layers on the basal diet recorded superior values to birds that were on other probiotic supplemented diets. This result conforms to Al-Saiay, (2010) who reported that probiotic supplementation did not have any effect on globulin and haematological parameters of calves.

4.2.3 Immunological parameters

The immunological parameters measured did not differ significantly among layers on the four treatments (Table 15).

Table 15: Effect of probiotic supplementation on immunological parameters of layers

Parameters	Time	Treatment[#]					
		Basal	RE-3	RE-3 Plus	P3	Lsd	P
Cd3 cells/l	1	1652	1598	1539	1564	268.60	0.814
	2	1948	2039	1850	1844	382.30	0.654
	3	1787	1896	1798	1870	347.60	0.878
Cd4 cells/l	1	2109	2694	4067	6000	2974.90	0.062
	2	2273	1359	3290	3441	2898.70	0.400
	3	1895	1260	1909	1317	1444.60	0.643
IgA{0.031-2.0ug/ml}	1	0.46	0.54	0.52	0.55	0.15	0.490
	2	1.45	1.29	1.29	1.28	0.44	0.800
	3	1.55	1.56	1.82	1.63	0.51	0.584
IgM{0.031-2.0ug/ml}	1	0.61	0.67	0.66	0.64	0.16	0.841
	2	1.34	1.39	1.43	1.41	0.11	0.377
	3	1.64	1.61	1.88	1.72	0.42	0.539

[#]Treatment means were compared at 5% level of significance (P<0.05); L.S.D.: least significant difference; P: Probability value; 1: Start of Experiment; 2: Midway into Experiment; 3: End of Experiment

4.2.3.1 Cluster of differentiation 3 and 4 Cells (CD3 and CD4)

Significantly, there were no differences (P>0.05) in the values obtained for all the four treatment although numerically basal diet (1787/L) and RE-3 (1260/L) treatments recorded the least values for CD3 and CD4 respectively (Table 15). On the contrary Edens (2003)

reported significant (P<0.05) differences among treatments when probiotic was fed to turkey. Bai *et al.,* (2012) also recorded higher proportions of CD3, CD4 and $CD8^+$ T-lymphocyes in the probiotic supplemented diet group when broilers were fed probiotics. Chesson, (1994) explained the inconsistency as due to several aspects of probiotics such as strains of bacteria, dose level, diet composition, feeding strategy, feed form and interaction with other dietary feed additives. The probiotic did not affect the CD3 and CD4. Birds were healthy throughout the experiment.

4.2.3.2 Immunologlobulin A (IgA)

Significantly (P>0.05) no difference in IgA for the four treatments were obtained although RE-3 diet recorded better values (1.29 and 1.52µg/ml) midway into the experiment and at the end of the experiment respectively with RE-3 Plus treatment recording the highest value of 1.821µg/ml at the end of the experiment. The numerical values IgA for midway the experiment were lower in layers fed on the probiotic included diets compared those on the basal diet (Table 15). These results agree with Scharek *et al.,* (2005) who recorded no statistical difference in their IgA level over time. However, values recorded for layers on basal, RE-3 and RE-3 Plus treatments do not conform with Fukushima *et al.,* (1998) report that when infants were fed probiotics for 20 days the IgA production peaked at d 8 and declined thereafter and Scharek *et al.,* (2005) reported that in piglets, fecal IgA levels after 14 d of age declined when probiotic was fed as a supplement.

4.2.3.3 Immunologlobulin M (IgM)

Numerically the basal diet (T1) recorded the lowest values (0.610 and 1.343) in the beginning and midway the experiment with RE-3 Plus (T3) recording the highest (1.430 and 1.879) values in midway and end of the work but none were significant (P>0.05). The administration of probiotic bacteria in chicken was shown to enhance specific, systemic antibody response

and to stimulate the production of natural antibodies such as serum IgG and IgM (Haghighi *et al.,* 2006). Previous studies have indicated that the modulation of innate and adaptive immunity by probiotic is a dose and strain-dependent phenomenon (Perdigon *et al.,* 1999; Galdeano and Perdigon 2004; Alberda *et al.,* 2007). Hays (1969) also suggested that the responsive degree of additives such as antibiotics was associated with the general health of experimental animals. This principle might apply to the use of probiotics said Chen *et al.,* (2005). In this work although there was no significant difference layers on basal diet did better compared to layers on the other treatments in terms of immunity.

4.3.0 Microbes isolated from faecal sample

The bacteria isolated in the faecal samples were *E-coli* and *Proteus* (Table 16) for all the birds under the four treatments and there were no significant (P>0.05) difference either in the *E.coli* or the *Proteus* values for all the treatments. However, numerically RE-3 Plus (T3) recorded the highest (37.1) for both the *E.coli* and the *Proteus* with RE-3(T2) and P3 (T4) recording (31.4 and 33.4 respectively) as the lowest values for *E.coli* and *Proteus*.

Table 16: Microbial count of faecal matter

Microbes Identified	Treatments[#]				P value	SED
	Basal	RE-3	RE-3 Plus	P3		
E. coli (10^8)org/ml	36.2	31.4	37.5	36.1	26.36	0.960
Proteus(10^8)org/ml	35.9	36.7	37.0	33.4	43.80	0.998

[#]Treatment means were compared at 5% level of significance (P<0.05); S.E.D: standard errors of differences; P: Probability value

Fuller (1989) has indicated that, one of the mechanisms of action of probiotics is depriving pathogens of sites in the intestinal wall and also the needed nourishment. Table 16 which is a

summary of the total viable counts (TVC) of *E.coli* and *Proteus* found in the faecal matter of the birds throughout the experiment indicates that, although insignificant (P>0.05), the amount of *E. coli* and *Proteus* found in the faecal sample of the birds did not follow any clear trend. The results of this experiment is in accordance with Rao (2007) who did not record any significant differences (P>0.05) in the quantities of *E.coli* in the gut of pigs supplemented with probiotic although the quantities of E. coli recorded were smaller compared to animals on a control. The ability of the natural intestinal complex and dynamic microbial ecosystem to fight intestinal infections according to Corcionivoschi *et al.,* (2010) is not always effective and supplementation with probiotic bacteria has proven to support as well as aid treating infections. Probiotics reduces faecal shedding of *Escherichia coli* in lambs (Lema *et al.,* 2001 and La Ragione *et al.,* 2001) and *Salmonella* colonization in poultry, and prevents antibiotic-associated diarrhoea in humans as reported by Fuller, (1999) but this is contrary to the results of this experiment. However, Chen *et al*., (2005) suggested that the responsive degree of additive such as probiotic was associated with the general health of experimental animals. This can be attributed to the outcome of this experiment because experimental birds were healthy before and during the experiment a reason why the probiotic might not have been effective.

4.4.0 Experiment II

4.4.1 Starter-grower experiment

Growth performance during the 8 weeks of starter-Grower phase is shown in Table 17.

Table 17: Probiotic supplementation on the growth performance of growers

Parameters	Treatments[#]					
	Basal	RE-3	RE-3 Plus	P3	Lsd	P
Initial weight (g)	39.2	40.0	39.5	41.0	2.84	0.12
Daily feed intake (g/day)	42.00	41.00	40.50	41.50	3.42	0.26
Daily weight gain (g)	16.0	14.64	14.38	15.84	4.50	0.85
Final Live Body Weight (g)	935.20	859.84	844.78	816.04	164.55	0.75
FCR (kg feed /kg live body weight gain)	2.61	2.77	2.82	2.62	0.58	0.92
Sex ratio of normal chicks (%): Female	63.4[a]	71.5[a]	61.2[a]	47.7[b]	13.73	0.016
Male	36.6[a]	28.5[b]	38.8[a]	52.3[a]	13.73	0.016
Mortality (%)	7.4[a]	10.1[a]	2.7[b]	11.23[a]	7.57	0.009

[#]Treatment means were compared at 5% level of significance (P<0.05); L.s.d: least significant difference; P: P-value

[a–b]Means with different superscripts in a row differed significantly (P < 0.05).

Significantly no differences (P>0.05) in initial weight, daily weight gain, daily feed intake and feed conversion ratio among the treatments were obtained. Murry *et al.*, (2007) had similar results when diets for laying hens were supplemented with probiotic. Chumpawadee *et al.*, (2008), quoting various sources, had intimated that, probiotic use is ineffective in animals housed in clean environments as was the situation in this experiment because

watering troughs were washed and house swept every day. The inclusion of probiotic did not affect significantly the growth and survivability of chicks. There were however, significant ($P<0.05$) differences in the sex ratios amongst the layers and the cocks (Table 17). RE-3 (T2) recorded the highest (71.5%) number of females but least (28.5%) number of males. On the other hand P3 (T4) supplemented diet recorded the highest (52.3%) number of males but least (47.7%) number of females. In general there were more females than there were males. On the contrary, Samad *et al.*, (2011) obtained a non significant difference ($P>0.05$) in sex ratio, feed intake and FCR between birds on basal and treatment diet when probiotic was supplemented in the diet of birds. Mortality among birds raised under the four treatments were significantly lower ($P>0.05$) in birds fed with the diet containing the fermentation product of RE-3 (2.7%) compared to those fed the other treatments (7.4, 10.1 and 11.23% respectively). According to Arpasova *et al.*, (2012) probiotics increases resistance to infectious diseases and reduces risk of mortality caused by the presence of infectious diseases; they further explained that this effect may be strain specific and that some probiotic strains may not exhibit such effects. Additionally, Bonsu *et al.*, (2012) found that the inclusion of RE-3 in the diets of broilers and layers reduced mortality in both broiler and layer (by 4% in both broilers and layers) birds. A similar observation was made in relation to pigs given 1.5 ml RE 3/kg feed (Owusu- Amoah, 2010).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of these studies showed that the three commercial probiotics (RE-3, RE-3 plus and P3) preparations can be included at a level of 1.5mls in every kilogram of layer diet without any adverse effect on the performance, reproduction and haematologic traits of layers however, feeding probiotic resulted in more female chicks being produced. The inclusion of probiotic in grower diet did not affect the growth and survivability of growers.

5.2 Recommendation

- Further studies should be conducted to confirm the results that probiotic (RE-3 and RE-3 plus) can be incorporated in the diet of layers at 1.5mls in every kilogram feed to obtain more female chicks and larger eggs.

- It is recommended that feeding trials be conducted to evaluate the effects of probiotic supplementation of the diet of poultry under conditions where environmental factors such as sanitation, stress, feeding and other management practices are difficult to control.

- Further research work supplementing probiotic at varying levels should be considered to assess its effect on production and reproduction performance.

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APPENDICES

APPENDIX 1

Production data for Layer experiment

Week One of Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake						
Hen Day	62.7	71.9	75.3	69.8	10.44	0.112
Hen House	62.7	71.9	75.3	69.8	10.44	0.112
Av. Egg weight						
FCR						
Mortality	0	0	0	0	0	0

Week two of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	119.32	122.39	122.32	121.48	6.14	0.678
Hen Day	62.7	71.9	75.3	69.8	10.44	0.112
Hen House	62.7	71.9	75.3	69.8	10.44	0.112
Av. Egg weight	58.46	57.88	57.41	57.62	0.69	0.035
FCR	3.27	2.96	2.85	3.04	0.44	0.261
Mortality	0	0	0	0	0	0

Week three of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	122.90	124.80	126.40	129.20	6.60	0.254
Hen Day	65.50	74.30	77.90	72.50	9.60	0.155
Hen House	58.50	58.26	57.56	57.87	1.28	0.088
Av. Egg weight	58.50	58.26	57.56	57.87	1.28	0.425
FCR	3.18	2.89	2.83	3.09	0.14	0.082
Mortality	1.25	0	0	0	1.93	0.426

Week four of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	115.80	123.10	123.60	120.7	8.84	0.252
Hen Day	60.50	73.40	76.60	72.50	9.57	0.016
Hen House	59.10	73.40	76.6	72.50	9.58	0.009
Av. Egg weight	58.44	58.36	58.05	58.26	1.42	0.937
FCR	3.69	2.90	2.79	2.86	0.67	0.038
Mortality	2.50	0	0	0	2.22	0.073

Week five of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	121.50	122.90	126.60	126.50	9.43	0.566
Hen Day	65.00	75.2	73.20	73.00	11.93	0.301
Hen House	66.70	76.2	73.20	73.90	12.33	0.411
Av. Egg weight	59.78	58.69	57.00	58.81	1.87	0.045
FCR	3.150	2.79	3.07	2.96	0.402	0.278
Mortality	2.50	1.25	0	0	2.94	0.248

Week six of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	106.22	112.15	105.91	106.25	4.99	0.050
Hen Day	58.10	74.20	63.00	66.7	8.72	0.012
Hen House	57.50	69.2	63.00	66.7	9.10	0.075
Av. Egg weight	58.44	58.43	58.51	58.61	0.99	0.978
FCR	3.15	2.59	2.92	2.72	0.47	0.107
Mortality	3.75	3.75	0	0	2.72	0.01

Week seven of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.18	117.70	115.12	133.33	4.72	0.216
Hen Day	70.70	75.00	65.60	71.30	15.84	0.644
Hen House	69.80	73.20	64.60	70.70	15.39	0.672
Av. Egg weight	59.74	60.06	61.40	60.39	4.54	0.869
FCR	2.79	2.64	3.08	2.65	0.89	0.690
Mortality	3.75	3.75	1.25	1.25	3.85	0.310

Week eight of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	115.50	118.00	115.00	114.20	7.33	0.697
Hen Day	61.80	80.30	74.20	65.30	11.29	0.015
Hen House	58.60	76.30	72.30	62.90	10.97	0.015
Av. Egg weight	59.74	60.06	61.40	60.39	4.54	0.869
FCR	3.14	2.46	2.57	2.91	0.38	0.007
Mortality	3.75	3.75	1.25	2.50	4.01	0.495

Week nine of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	111.50	112.80	107.30	108.30	7.75	0.400
Hen Day	53.20	55.80	60.60	53.20	14.62	0.662
Hen House	50.50	53.00	58.90	51.40	14.37	0.595
Av. Egg weight	61.53	58.43	56.70	59.22	2.81	0.019
FCR	3.42	3.68	3.17	3.17	1.19	0.706
Mortality	3.75	3.75	1.25	2.50	4.01	0.495

Week ten of Layer Experiment

PARAMETERS	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	122.70	113.30	116.00	119.80	7.55	0.083
Hen Day	56.60	63.10	61.40	58.80	9.08	0.453
Hen House	54.50	60.70	60.50	57.30	8.64	0.378
Av. Egg weight	59.18	59.25	58.12	59.41	2.45	0.662
FCR	3.76	3.05	3.27	3.44	0.71	0.216
Mortality	3.75	3.75	1.25	2.50	4.01	0.495

Week eleven of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	122.40	119.70	118.50	118.60	9.95	0.815
Hen Day	49.50	64.70	64.30	62.40	13.27	0.086
Hen House	47.00	61.40	62.70	60.00	12.36	0.058
Av. Egg weight	58.13	58.02	57.95	61.02	4.27	0.364
FCR	4.28	3.30	2.25	3.13	0.84	0.041
Mortality	3.75	3.75	1.25	2.50	4.01	0.495

Week twelve of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.80	116.70	115.40	120.30	8.38	0.640
Hen Day	46.00	59.40	52.50	57.50	11.32	0.096
Hen House	43.70	56.40	51.10	54.80	11.68	0.139
Av. Egg weight	59.58	58.85	57.78	59.06	2.67	0.535
FCR	4.33	3.38	3.88	3.60	0.81	0.114
Mortality	3.75	3.75	1.25	3.75	4.97	0.627

Week thirteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.59	118.14	118.13	118.13	1.98	0.619
Hen Day	57.50	62.87	55.75	55.75	5.26	< .001
Hen House	55.40	58.90	53.00	53.00	7.17	0.003
Av. Egg weight	59.78	58.69	58.81	58.81	1.47	0.361
FCR	3.43	3.18	3.61	3.61	0.23	< .001
Mortality	3.80	6.20	1.20	5.0	7.12	0.491

Week fourteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	119.05	116.38	114.53	118.15	3.04	0.031
Hen Day	60.64	62.72	67.25	63.50	3.10	0.003
Hen House	58.40	56.45	66.41	60.33	5.55	0.011
Av. Egg weight	58.74	58.24	57.66	59.92	2.33	0.240
FCR	3.35	3.19	2.85	3.09	0.209	0.002
Mortality	3.80	10.00	1.20	5.0	6.85	0.090

Week fifteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	118.53	116.77	119.09	118.09	3.03	0.419
Hen Day	58.61	64.32	67.82	61.42	5.97	0.031
Hen House	55.70	58.80	66.90	58.20	6.95	0.022
Av. Egg weight	59.00	59.07	59.46	59.27	1.92	0.954
FCR	3.44	3.08	2.97	3.25	0.28	0.017
Mortality	3.80	8.80	1.20	5.00	7.12	0.196

Week sixteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.70	117.67	118.15	117.97	2.22	0.958
Hen Day	53.03	60.39	63.64	56.93	3.39	< .001
Hen House	50.38	55.19	62.85	54.09	5.88	0.004
Av. Egg weight	61.20	59.70	59.62	59.25	1.85	0.157
FCR	3.64	3.17	3.09	3.49	0.26	0.002
Mortality	3.80	8.80	1.20	5.00	7.12	0.196

Week seventeen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.79	115.81	118.79	117.42	2.87	0.205
Hen Day	61.64	62.61	68.47	60.82	4.71	0.016
Hen House	58.60	57.20	66.80	57.80	6.92	0.034
Av. Egg weight	59.53	59.02	60.12	59.91	3.80	0.921
FCR	3.23	3.13	2.89	3.22	0.29	0.087
Mortality	5.00	8.80	2.50	5.00	6.94	0.317

Week eighteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	118.71	117.96	118.13	118.32	1.77	0.816
Hen Day	60.15	61.68	63.75	58.61	3.29	0.029
Hen House	57.15	56.25	62.14	55.70	4.98	0..057
Av. Egg weight	58.47	57.96	59.67	58.36	1.49	0.128
FCR	3.38	3.21	3.09	3.42	0.14	< .001
Mortality	5.00	8.80	2.50	5.00	6.94	0.317

Week nineteen of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	119.50	116.28	117.82	116.24	3.32	0.160
Hen Day	60.70	64.60	68.00	64.00	6.76	0.189
Hen House						
Av. Egg weight	61.20	59.90	59.25	58.45	2.25	0.106
FCR	3.22	3.01	2.93	3.11	0.28	0.170
Mortality	5.00	8.80	2.50	6.20	6.48	0.257

Week twenty of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	116.89	114.55	115.79	113.73	5.29	0.595
Hen Day	63.91	62.73	67.61	59.18	5.18	0.029
Hen House	60.70	56.40	65.90	55.50	6.37	0.015
Av. Egg weight	58.33	58.49	59.15	58.52	2.19	0.857
FCR	3.14	3.09	2.89	3.29	0.35	0.163
Mortality	5.00	8.80	2.50	6.20	6.48	0.257

Week twenty-one of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	118.44	117.66	117.18	118.03	1.67	0.437
Hen Day	59.83	66.32	65.64	63.57	4.76	0.047
Hen House	55.36	59.66	62.67	56.43	3.94	0.007
Av. Egg weight	58.50	58.53	58.31	57.87	1.43	0.733
FCR	3.39	3.04	3.21	3.21	0.23	0.016
Mortality	7.50	10.00	2.50	11.25	4.85	0.009

Week twenty-two of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.79	116.81	115.04	116.92	2.63	0.197
Hen Day	60.36	66.44	65.03	61.96	4.61	0.051
Hen House	55.74	59.82	63.39	55.01	4.69	0.007
Av. Egg weight	58.46	57.88	57.41	57.65	0.69	0.036
FCR	3.36	3.05	3.08	3.28	0.24	0.041
Mortality	7.50	10.00	2.50	11.25	4.85	0.009

Week twenty-three of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	118.25	116.53	117.87	116.24	2.73	0.340
Hen Day	60.29	65.86	68.00	64.82	4.80	0.027
Hen House	55.70	60.03	65.37	57.59	3.46	< .001
Av. Egg weight	59.09	58.59	60.02	59.18	0.86	0.024
FCR	3.30	3.03	2.89	3.04	0.26	0.028
Mortality	7.50	10.00	2.50	11.25	4.85	0.009

Week twenty-four of Layer Experiment

PARAMETER	TREAMENT					
	TI	T2	T3	T4	LSD	P
Initial Weight	1.62	1.58	1.62	1.62	0.11	0.841
Final Weight	1.64	1.59	1.59	1.59	0.12	0.865
Feed Intake	117.00	114.61	114.61	115.18	3.47	0.497
Hen Day	61.78	66.46	66.46	65.68	5.43	0.207
Hen House	57.11	59.83	49.83	64.06	6.06	0.043
Av. Egg weight	59.08	59.09	59.09	59.27	1.57	0.882
FCR	3.21	2.93	2.93	2.97	0.25	0.079
Mortality	7.50	10.00	2.50	11.25	4.85	0.009

APPENDIX 2

Anova tables for performance parameters (Layers)

Table1: Mortality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	179.688	59.896	6.05	0.009
Residual	12	118.750	9.896		
Total	15	298.438			

Table 2: Egg Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.676	0.225	0.22	0.999
Residual	12	12.413	1.034		
Total	15	13.088			

Table 3: Feed Conversion Ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.22857	0.07619	2.89	0.189
Residual	12	0.31618	0.02635		
Total	15	0.54474			

Table 4: Feed Intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	12.793	4.264	0.84	0.823
Residual	12	60.757	5.063		
Total	15	73.550			

Table 5: Final Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.004425	0.001475	0.24	0.865
Residual	12	0.073150	0.006096		
Total	15	0.077575			

Table 6: Hen-Day Production

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	65.93	21.98	1.77	0.159
Residual	12	149.13	12.43		
Total	15	215.05			

Table 7: Hen-House Production

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	171.49	57.16	3.69	0.150
Residual	12	185.91	15.49		
Total	15	357.40			

Table 8: Initial Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	0.004525	0.001508	0.28	0.841
Residual	12	0.065250	0.005437		
Total	15	0.069775			

APPENDIX 3

Anova tables for sex ratio of growers

Female Chicks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	1459.0	486.3	4.64	0.016
Residual	16	1677.1	104.8		
Total	19	3136.1			

Males Chicks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	1459.0	486.3	4.64	0.016
Residual	16	1677.1	104.8		
Total	19	3136.1			

APPENDIX 4

Anova tables for microbes identified in both blood and feacal sample

E. coli

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	85.6	28.5	0.10	0.960
Residual	12	3513.4	292.8		
Total	15	3599.0			

Proteous

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	3	31.2	10.4	0.01	0.998
Residual	12	9696.7	808.1		
Total	15	9727.9			

APPENDIX 5

Haematologic reference values for poultry

Haematologic parameters	**Range**
Haemoglobin (g/l)	100-160
Haemogram (μmol/L)	6.2-9.9
Haematocrit/PCV (L/L)	0.32-0.50
Red Blood Cell ($x10^6/\mu l$)	5-8
Mean Cell Volume (fl)	50-68
Mean Cell Haemoglobin (pg)	17-21
Mean Cell Haemoglobin Concentration (g/L)	300-340
Reticulocytes ($x10^9/L$) $^{\Psi}$	0-80
White Blood Cell ($x10^3/\mu l$)	11-22
Neutrophils (mature) ($x10^9/L$)	3.1-10.5
Neutrophils (band) ($x10^9/L$)	0-0.9
Lymphocytes ($x10^9/L$)	4.3-13.6
Monocytes ($x10^9/L$)	0.2-2.2
Eosinophils ($x10^9/L$)	0.1-2.4
Basophils ($x10^9/L$)	0-0.4
Platelets ($x10^9/L$)	320-720
Plasma proteins (g/L)	60-80
Fibrinogen (g/L)	1-5

Source: Blood DC, Studdert VP: Saunders, comprehensive veterinary dictionary, ed. 2, Philadelphia, 1999, WB Saunders, p 1252. Reference values may be influenced by the method of measurement and by the animal's breed, sex, age and environment; hence, these values are guidelines only.
ΨAggregate reticulocytes derived from Fan LC., Dorner JL., Hoffman WE: J Am Anim. Hosp. Assoc, 14: 219, 1978.