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EFFECT OF DIRECT-FED MICROBIAL (DFM) AS ADMINISTERED IN WATER ON THE HEALTH AND PERFORMANCE OF POULTRY IN HOT HUMID ENVIRONMENT

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MSc.

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BY

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CERTIFICATION

I certify that this research was carried out by me at the Animal Science Department, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and that this thesis is entirely my own account of the research and has not been submitted to any other University for a degree. However, works of other researchers and authors which served as sources of information were duly acknowledged.

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ABSTRACT

The study aimed at determining the effectiveness of probiotics (RE3) administration in drinking water on growth of broilers and laying performance as well as health status of broiler and layer chicks in a hot humid environment. In Experiment 1, 192 four-week old broiler chickens were randomly allotted to four treatment groups, with 3 replicates of 16 birds per replicate in a Completely Randomized Design (CRD). The RE3 was administered at 1.5 ml/kg in feed for T_2, 1.5 and 2.5 ml/L of drinking water, representing T_3 and T_4. The control group (T_1) did not receive RE3 either in feed or water. 2.5 ml DFM was used in treatment 2 instead of 2.0 ml because of the short duration (4 weeks) for the experiment. The first experiment lasted for five (5) weeks. Parameters measured include, feed consumption, body weight, body weight gain, feed conversion ratio, carcass characteristics (gizzard, liver, intestines and abdominal fat) and blood haematological parameters. The economics of production was also calculated.

The provision of RE3 via feed or drinking water did not have any significant ($P>0.05$) effect on broiler performance and carcass characteristics. However, performance of broiler birds which has 1.5 ml DFM in feed (T_2) and water (T_4) showed slight improvement in performance. The blood parameters followed the same trend, except the platelet values which was significantly ($P<0.05$) higher (7.833) for birds on the higher level of administration of DFM in water (T_4) and significantly ($P<0.05$) lower (6.833) for the control group that received no probiotic. T_2 and T_3 recorded the same platelet value of 7.333.

Three hundred (300) 30-week old layer birds were used in Experiment 2. The birds were randomly allotted to 5 treatments with 3 replicates of 20 birds per replicate in a Completely Randomized Design (CRD). The RE3 (DFM) was administered at the levels of zero in both feed and water for the control (T_1), 1.5 ml kg^{-1}feed, 1.0, 1.5 and 2.0 ml/L of drinking water, representing treatment T_2, T_3, T_4 and T_5 respectively. Parameters measured were, feed consumption, body weight and body weight gain, feed conversion ratio, egg production, egg weight and internal egg quality (albumen height, width and shell thickness) and the economics. Mortalities were recorded as it occurred. The layer experiment lasted for ten (10) weeks.

The administration of RE3 at different concentration either in feed or water did not have any significant impact on the laying performance or egg quality of the layer birds.

Similar to the broiler studies, the performance of layer birds on treatment which contained1.5 ml DFM in feed (T_2) and 2.0 ml DFM in water (T_5) were numerically higher in terms of egg weight, hen-housed, and hen-day egg production. It can therefore be concluded that the administration of DFM (RE3) at a concentration of 1.5 ml/L in drinking water for broiler chickens and 2.0 ml/L in drinking water for layer chickens contributes positively to their performance under hot humid conditions.

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ABBREVIATIONS

ABBREVIATION	FULL MEANING
ANT	Antibiotics
BWG	Body Weight Gain
Ca	Calcium
CE	Competitive Exclusion
CP	CrudeProtein
CS	Corn-Soybean
DFM	Direct-Fed Microbial
DNA	Deoxyribonucleic Acid
FCR	Feed Conversion Ratio
FI	Feed Intake
FAO	Food and Agriculture Organization
g	gram
GDP	Gross Domestic Product
GH¢	Ghana Cedi
GIT	Gastrointestinal Tract
Hb	Haemoglobin
HDP	Hen-Day Production
HHP	Hen-Housed Production
LC	Lactobacillus Culture
mg	Milligram
ml	Millilitre
MSPB	Multistrain Probiotics

MS	Mean of Square
N	Nitrogen
P	Phosphorus
PCV	Packed Cell Volume
RBC	Red Blood Cells
SEM	Standard Error of the Mean
SS	Sum of Squares
US$	American Dollar
VFA	Volatile Fatty Acids
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

Poultry meat and eggs provide an acceptable form of animal protein for most people in the world mostly due to their low cholesterol level (Tweneboah, 2000). Efforts have therefore been made to increase poultry production and consequently its contribution to Ghana's GDP (1.1%)through genetic improvement and the use of additives to reduce the cost of feed which accounts for 70% of the total production cost (Diao, 2009).Despite these interventions, the poultry industry is faced with increased disease challenges due mainly to stress responses. Chickens suffer depressed immune- responsiveness when exposed to extreme environmental conditions and managerial stressors. Increased stressors may weaken immune function and thus predispose broilers to colonization of the gastrointestinal tract (GIT) by bacterial pathogens or other unfavorable micro-organisms, posing a threat to food safety and bird health (Barnes, 1979; Hume *et al*., 2003). Stress causes birds to become more susceptible to various pathogens especially enteropathic microbes such as *E. coli*, *Salmonella spp*., *Clostridium perfringens* and *Campylobacter spp*. The increased susceptibility of chickens to diseases resulted in the use of antimicrobial growth promoters (antibiotics) to enhance gut health and control sub-clinical challenges (Dunkley, 2008).

Antibiotic resistance observed among bacteria such as *E. coli* and *Salmonella spp* and the transfer of antibiotic resistance gene from animal to human microbiota generated the strongest objection to the use of antibiotics (Gustafson and Bowen, 1997; Castanon, 2007).The increasing public concern about bacterial resistance to antibiotics in poultry feed, has limited or eliminated the use of antibiotics in many

countries. As a result, antibiotics as feed additives have become less popular (Dunkley, 2008).

To keep poultry farmers in business, there is the need to get the best alternative to promote the health and growth of poultry birds other than giving more feed. The use of probiotics in poultry production has therefore been suggested as a viable alternative to the use of conventional antibiotic and other feed additives.

In recent years, there has been interest in the use of living micro-organisms (probiotics or Direct Fed Microbials) as therapeutic agents. Probiotic is a live organism that serves as a natural feed supplement which does not develop resistivity like antibiotics (Hargis, 2008). Direct fed microbials (DFM) are beneficial bacteria, which are adapted to the intestinal mucosa and create a medium for complete digestion, absorption, and assimilation of all nutrients being acted upon (Kociova *et al.*, 1990). The bacteria that are used as probiotic organisms have an ecological advantage in the gastrointestinal tract because they can multiply more effectively. The use of DFM in poultry production is economical since the use of DFM in both layers and broilers greatly reduce infection of pathogenic bacteria and subsequently reduce the cost of purchasing coccidiostat, medications, antibiotics, mortality and increases growth and egg production .As a result, better and relatively higher economic returns of using DFM is achieved (Mandal *et al*., 2000; Bonsu *et al*., 2012).

Despite this breakthrough, farmers who compound their feeds on large scale using large equipment and machinery are faced with the problem of losing the livability and efficacy of the DFM during processing and storage of the feed. It therefore became

necessary for an experiment to be carried out to determine the best alternative for administration of DFM to animals without passing it through equipment and machinery. Administration of DFM via drinking water became the next alternative for consideration.

Many studies from Europe, Africa, and in Ghana most especially by Bonsu *et al*. (2012) on the use of DFM administered via feed have shown the beneficial effects of DFM on health and performance of farm animals. In view of the reported potential beneficial effect of the addition of probiotics in poultry diet, a study was undertaken to ascertain whether same or similar improved health and productive performance effects could be achieved using DFM administered via drinking water in a hot humid environment as prevails in Ghana where ambient temperatures during the year generally exceeds 32^{o}C and coincide with declining performance of both broiler and layer chickens (Osei, 1984). The value of administering DFM via drinking water on the economics of production in poultry enterprise as an alternative to DFM administered through feed was also assessed.

1.1 Objectives of Study

The objectives of this study were to evaluate the effect of RE3 (DFM) via water and Feed on the:

i. Growth performance and health status of broiler chicks in a hot humid environment

ii. Performance (egg) and health status of layer chickens in a hot humid environment

iii. Value of DFM in water and feed treatments on the economics of production

CHAPTER TWO

LITERATURE REVIEW

2.1. Feed Additives

Feed additives are products used in animal nutrition for purposes of improving the performance, health of animals and quality of feed by enhancing the digestibility of the feed materials.

Feed additives stimulate growth, improve the efficiency of feed utilization and are beneficial in some manner to the health or metabolism of the animal (Kellems and Church, 2002). Classes of feed additives include the following: Chemical components such as arsenicals and copper sulphates, tranquilizers, surfactants, antibiotics, antioxidants, hormones, probiotics, colors and flavors (Kamra and Pathak, 1996). Feed additives tend to fall into certain categories which describe their action in the feed or in the animals as follows by Didier *et al*. (2011):

1. **Technological additives**. This classification refers to a group of additives that influences the technological aspects of the feed. The nutritional value of the feed is not influenced directly but may do so indirectly by improving its handling or hygienic characteristics or shelf life, for example an organic acid for preservation of feed.
2. **Sensory additives**. This refers to a group of additives which improve the palatability (i.e. voluntary intake) of a diet by stimulating appetite, usually through the effect these products have on the flavor or color of the diet. For example, vanilla extract may well encourage piglets to eat a ration.
3. **Nutritional additives**. Such additives supply specific nutrient(s) required by the animal for optimal growth. Examples are vitamins, amino acids or trace

minerals. In most cases, such additives are simply concentrated forms of nutrients supplied in natural ingredients in the diet.

4. **Zootechnical additives**. These additives do not provide specific nutrients, but enable more efficient use of the nutrients present in the diet. An example of such an additive is an enzyme or a DFM product, both of which enhance the conditions of the intestinal tract, thus enabling more effective nutrient extraction from the diet. In this respect they are often referred to as pro-nutrients, i.e. products which improve the nutritional value of a diet without necessarily providing nutrients directly.
5. **Coccidiostats and Histomonostats**. These products are used to control intestinal health of poultry through direct effects on the parasitic organism concerned. They are not classified as antibiotics.

According to Duane and Merle (1991), feed additives have been extensively used in rations for poultry since the 1950's. Their wide acceptance is attributed to their well established benefits of improving growth rate, feed conversion efficiency and reducing morbidity and mortality caused by sub-clinical and clinical infections.

2.2. Antibiotics as Feed Additives

Over the years, antibiotics have been used as feed additives in poultry and livestock diets and are among the most widely used feed additives. The administration of many of these antibiotics has resulted in a more rapid growth, improved feed efficiency and improved general health, primarily in young animals when fed continuously at sub-therapeutic levels (Kellems and Church, 2002).

It is worth noting that not all antibiotics are used as feed additives. The major ones that have been used over the years in the diet of farm animals, and permitted by the European Union are; bacitracin, bambermycins, chlortetracycline, lasalocid, lincomycin, flavomycin, pencillin, neomycin, monensin, oxytetracycline, virginiamycin and tylosin. These are used alone or in combination with other feed additives (Kamra and Pathak, 1996; Castanon, 2007). Although many antibiotics prevent sub-clinical infections and promote growth rate and feed efficiency, the mode of action for growth promotion has not been fully elucidated (Leser *et al.,* 2000).

Generally, similar antibiotics used for animals are administered therapeutically to humans. Care is therefore advised for their usage in animals. This is because an indiscriminate or uncontrolled use would have adverse effect on the potency and its subsequent use in humans (Donoghue, 2003). As a result, many EU countries have legislations regulating antibiotics usage in their respective countries.

2.3. Constraints to Antibiotics as Feed Additives

The United States Food and Drug Administration approved the use of antibiotics as animal feed additives without veterinary prescription in 1951. Other European countries also approved antibiotics use around 1950 to 1960 (Coates, 1962; Jones and Ricket, 2003). Realizing the possible dangers that antibiotics as feed additives could cause, a legislation regulating the use of antibiotics was soon put in place. In the United Kingdom, it was regulated under the Therapeutic Substance Regulation of 1953 and 1954. This permitted specific antibiotics such as Chlortetracycline, Penicillin and Oxytetracycline to be used as feed additives and with a level of incorporation of not more than 1 part of antibiotics to 10,000 parts of the feed

(Coates, 1962). The early establishment of legislation regulating the use of antibiotics gave an indication that although the beneficial effects of antibiotics as feed additives were not doubted, there has always been the fear and uncertainty of a possible residual effect from their usage.

In Ghana, the use of antibiotics in the animal industry has become so common that, a lot of commercial poultry enterprises are of the opinion that production is not possible without it. It is administered via feed or drinking water with some farmers under-or over-dosing it. There is indeed no regulation enforcing strict adherence to practices. These are pre-disposing factors that can lead to the development of antibiotic resistance. If indeed antibiotic usage as feed additive is causing serious concern as regards human health, then Ghana is by no means exempted from the menace (Bonsu *et al*., 2012). For this reason, if such a ban can be very effective, there must be a replacement, which should be as good and can provide the same benefits derived from antibiotics as feed additives. Some products that have been tested to effectively replace antibiotics as feed additives include probiotics, prebiotics, organic acids, plant extracts and hen egg antibodies (Patterson and Burkholder, 2003; Ricke, 2003).

2.4 Probiotics as Feed Additives

Guarner and Schaafsma (1998) defined probiotics as living microorganisms that upon ingestion in certain numbers exert health effects beyond inherent basic nutrition. It is also the combination of beneficial bacteria adaptable to the intestinal mucosa of all warm blooded animals (Siggers *et al*., 2008). According to FAO/WHO (2001) probiotics are live microorganisms, which when administered in adequate amounts confer health benefits on the host. It was also defined by Havenarr and Huisin'tVeld

(1992) as a mono or defined mixed culture of live microorganisms, which when applied to animal or man beneficially affects the host by improving the properties of the indigenous gastrointestinal microflora.

Many bacteria from several different genera have been used as probiotics since the inception of this form of growth promotion and disease prevention. These include, but are not limited to, *Bacillus, Bifidobacterium, Enterococcus, Escherichia coli, Lactobacillus, Lactococcus, and Streptococcus* (Patterson and Burkholder, 2003). Probiotics can contain one or several strains of bacteria, as well as bacillus spores and yeast species (Ranlyn, 2010). Probiotic cultures consisting primarily of different species *of Lactobacillus* have been shown to improve feed efficiency, enhance growth and aid in preventing some bacterial infections when added to poultry feed or water (Huang*et al*., 2004; Jin *et al*., 2000). Members of the genus *Lactobacillus* are particularly suited for being developed as probiotics and they constitute a diverse group of organisms (Gasson *et al*., 2004). Some of them are also permanent members of the colonic mommensal microflora (Kullen and Klaenhammer, 1999).

Commercially produced probiotic products are usually species-specific, with products intended for use in chickens comprised of bacterial species that would have been isolated from the GIT of chickens. The use of probiotics may provide an alternative to the administration of sub-therapeutic levels of antibiotics (O'Dea *et al*., 2007). Past research has shown that administering probiotics can provide the same protection as a naturally developed commensal Gastrointestinal Tract (GIT) microflora (Pascual *et al*., 1999; Kubena *et al*., 2001).

Unlike antibiotics which have received a ban by the European Commission for use in sub-therapeutic levels in animal feeds, probiotics presently have been endorsed by the European Commission, the United States and backed by legislation. The European Commission Regulation (EC) No.1411/1999 of 29 June 1999 concerning the authorization of new additives use in feedstuffs has authorized the use of feed additives on the market. On the 18th of October 2004, the EU Regulation (EC) No 1831/2003 also endorsed the use of probiotics in poultry production (FAO/WHO, 2001).

The growth promotion effect of intragastric administered probiotics is well documented. There have been several reports of improvement in mean live body weight, body weight gain and feed conversion ratio. However, the magnitude of improvement varies depending on the type of probiotics used (Chapman, 1989; Mohan *et al.*, 1996; Mandal *et al.*, 2000; Sieo *et al.*, 2005; Khan *et al.*, 2007).

2.5. Mode of Action of Direct Fed Microbials (DFM)

According to Edens *et al.* (1997), the colonization of lactic acid bacteria in the intestinal tract of chickens apparently controls the populations of pathogenic microorganisms such as *Salmonella spp.*, *Enterococci spp.* and *E.coli.* Lactic acid bacteria produce significant amounts of bacteria growth inhibitory substances such as reuterin, which has a broad spectrum antimicrobial activity that has proven to inhibit the growth of bad bacteria, fungi and protozoa.

Kociova *et al.* (1990) explained that DFM which contains lactic acid bacteria, when given to animals attach themselves to the villi of the intestinal wall in astronomical

numbers thereby creating an acidic environment. The organisms take over the gut and produce a good state of intestinal health. The healthy appetite created in the animals makes available a medium for complete digestion, absorption and assimilation.

During times of stress, hormonal changes can occur causing the pH of the small intestine to rise. This allows existing bad bacterial to take a foothold in the lining of the intestine resulting in the deterioration of the protective mucus lining. Because of this, 'villi' (little fingers), which normally exist in the small intestine, can be lost. Villi slow the movement of food as it passes through the small intestines so that nutrients can be absorbed through the intestinal wall and also increase the surface area of the small intestine. Increasing good gut bacteria through the use of probiotics will cause the good bacteria to compete against the bad ones, create a good gut environment, allow nutrient absorption, and prevent infection (Theodore, 1999).

Exposure of chickens to extreme conditions in the environment can also induce non specific stress responses leading to depressed immune-responsiveness that will influence gut microbial populations. Unfortunately, the depression in the production of immunoglobulins tends to influence pathogen growth more than beneficial microbials. Many managerial stressors such as beak and claw trimming and other hatchery processes contribute to immune-suppression in poultry (Adler and Rehkopf, 2008**).** Breeding periods are very stressful times in the lives of birds and the vulnerability to diseases increases during those times. Probiotics supplementation helps to repair the deficiencies in the gut flora and maintain a balanced intestinal micro flora enhancing resistance to infection (Soderholm and Perdue, 2001) and

consequently reducing the vulnerability to diseases by increasing the growth of beneficial microbes (Theodore, 1999).

Probiotics treatment has also shown the ability to stimulate appetite and maintain the weight of an ailing bird by stimulating the immunity of the chicken through;

a. The migration and multiplication of flora from probiotics throughout the gut wall.
b. The absorption of antigens released by the dead organism which result in the stimulation of the immune system.

Beneficial microorganisms possess certain favorable characteristics that allow for the expression of several mechanisms that prevent pathogens from colonizing the intestinal tract. These mechanisms are: creation of a micro ecology that is hostile to harmful bacteria species, elimination of available receptor sites for such bacteria, production and secretion of antimicrobial metabolites, and competition for essential nutrients (Havenaar and Spanhaak, 1994).

It is speculated that the benefit derived from probiotics is as a result of the organisms growing and contributing some beneficial function in the intestinal tract. Therefore, one of the most important considerations in achieving the desired effect from using *lactobacilli* as growth promotants is to ensure that the organisms survive passage through the stomach and proliferate in the intestinal tract. To establish successfully in the intestinal tract, bacterial strains must be able to adhere physically and multiply on the intestinal surfaces (Jin *et al.*, 1998a).

Microbial populations within the GIT colonize very quickly after hatching (Guen *et al*., 2004). Contact with microorganisms on the eggshell (Coates and Fuller, 1977) or in feed (Jones and Richardson, 2004) contribute to microbial colonization of the GIT. It is during this early period, when a stable gut microflora has not yet been established, that the chick is most vulnerable to colonization by pathogens, and establishment of a healthy GIT (O'Dea *et al*., 2007).

In assessing the value of a probiotic or DFM, Guillot (2000) enumerated characteristics necessary for a probiotic to be effective.

1. Must be a normal inhabitant of the intestine.
2. Must have a short regeneration time.
3. Must produce antimicrobial substance (eglactic acid, bacteriocins, etc).
4. Must be durable enough to withstand the duress of commercial manufacturing, processing and distribution so that the product can be delivered live to the intestine.
5. Must be free of antibiotic resistance gene, non-pathogenic and non-toxigenic for target species and for man under expected conditions of use.
6. The most efficient DFM bacteria are likely to be strains that are robust enough to survive the harsh physico-chemical conditions present in the GIT tract such as gastric acid and bile secretions (Fooks and Gibson, 2002). The DFM bacteria that survive usually do not colonize the intestinal mucosa for long periods of time and are generally eliminated within a few days of the cessation of their ingestion necessitating continuous supplementation (Marteau *et al.*, 2004).

7. The efficacy of probiotics application depends on many factors such as specie, composition and viability, administration level, application method (eg. spraying, feed or water), frequency of application (eg. once, intermittent or continuous), overall diet, age of birds, overall farm hygiene and environmental stress factors such as temperature and stocking density (Mountzouris *et al*., 2007).

Patterson and Burkholder (2003) summarized the benefits of the use of probiotics as follows;

1. Modify intestinal micro flora.
2. Stimulate immune system.
3. Reduce inflammatory reactions.
4. Prevent pathogen colonization.
5. Enhance animal performance.
6. Decrease carcass contamination.
7. Decrease ammonia and urea excretion.

High inclusion of yeast level has an adverse effect on nutrient digestibility (Romashko, 1999). Ahmad (2004) confirms that the growth pattern of his experimented birds increased relative to the control, up to 1.0 g per 10 kg feed but beyond that the pattern was reversed. This was due to the fact that high inclusion of yeast has an effect on nutrient digestibility (Romashko, 1999).

2.6. Effect of DFM in Water on Layer and Broiler Chickens

Consumption of DFM as an additive in drinking water by birds is expected to result in health improvement, apparently because of competition with pathogenic microflora in

the digestive tract. It has also been reported severally of the improvement in mean live body weight, body weight gain and feed conversion efficiency of broiler and layer chicken given DFM through their drinking water (Yongzhen and Weijiong, 1994).

2.6.1 Effect of DFM in Water on Health

The gastro-intestinal tract of birds may house several pathogenic micro-organisms (Larbier and Leclercq, 1994). Anjum *et al.* (1996) reported a greater bursa and thymus index in commercial broiler chickens given DFM through drinking water and feed. According to this study, DFM supported these two important lymphoid organs that make up the vital component of humoral and cellular immunity. Antibody geometric mean titre (GMT) against Newcastle disease vaccine virus was 6.5 times in broilers given DFM in drinking water, 3.85 times in broilers given DFM in feed and 3.73 times in broilers given both DFM in water and in feed.

An isolate of *L. acidophilus* has been reported to produce 2 bacteriocins, which inhibited growth of 2 non-pathogens: *Lactococcus*and *Pediococcus.* These bacteriocins also inhibited growth of several pathogenic organisms in vitro, from genuses including *Staphylococcus, Enterococcus, Streptococcus, Listeria, Clostridium* and *Bacillus* (Bogovicˇ-Matijasˇic´ *et al*., 1998).

Floramax, a DFM from the Bacillus genus that could work as a drinking water treatment, is observed to enhance performance and reduce food borne disease causing bacteria like *Salmonella enteritidis*in chickens and turkeys (Hargis, 2008). It was observed that the lactic acid secreted by the DFM exerted many positive effects to maintain a healthy intestinal environment. The acid acts as a stimulus for the

development of the acid producing bacteria. Also pathogenic microbes are eliminated as a result of unfavorable intestinal environment through lowering of pH and finally, the acid environment is conducive to increased enzymatic activity within the digestive system (Chapman, 1989).

2.6.2. Effect of DFM in Water on Broilers

Administration of DFM via drinking water improves nitrogen absorption in broilers. An approximately increased live body weight of 2,004 g for broilers given DFM in drinking water was observed after 45 days of DFM treatment in day-old commercial broilers as against 1,978 g for broilers given DFM in feed; 2,022 g for broilers given DFM in both water and feed, and 1,690 g for the control broiler (Yongzhen and Weijiong, 1994).

O'Dea *et al*. (2007) observed a significant increase in body weight of 35-day-old broiler birds given probiotics in water (499 g) as compared to birds given probiotics in feed, (487 g) and in control (489 g). This was obtained in a research to investigate the effects of commercial probiotics on broiler chick quality and production efficiency.

Eckert *et al.* (2010) conducted an experiment to determine the influence of probiotics administration via feed or water on growth parameters of broilers reared on medicated and non-medicated diets. The trial was conducted to determine the effects of post pelleting feed or drinking water application of a Lactobacillus-based probiotic, alone or in combination with a phytogenic product, on growth parameters of broilers. At the end of the experiment, it was observed that broilers fed the phytogenic product or probiotic showed improved performance parameters at multiple time points during

grow-out. Intermittent application of the probiotic via water increased broiler body weight ($P<0.05$) of 520 g on day 15 compared with all the other treatment groups. On the day 40, intermittent probiotic administration via drinking water resulted in increased body weight (2.89 kg) compared with the control (2.84 kg), in feed (2.84 kg) and phytogenic product group (2.83 kg). Based on the observed increases in body weight through the 40-day trial, it was concluded that intermittent application may be the best route of administration of the probiotics (biomin poultry star).

Performance of broilers in terms of body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) improved when probiotics was provided via drinking water, compared to the control group that received no probiotics or antimicrobials and the feed application group that received an inclusion of probiotic at a rate of 1g/kg (Karimi Torshizi *et al*., 2010). These improvements in birds provided with probiotics via drinking water have been suggested to be due to increased feed intake and subsequent nutrient availability (Svihas *et al*., 1997). Karimi Torshizi *et al*. (2010) also observed reduced plasma cholesterol and triglyceride concentrations in broilers given probiotic administration in water at a rate of 0·5 g/l.

An experiment conducted by Chantsavang and Watcharangkul (1998) on the effectiveness of the addition of DFM in drinking water and feed on broiler performance and carcass characteristics are recorded in Table 2.1 and Table 2.2.

Table 2.1**: Performance of Broilers Given DFM in Drinking Water and Feed (0-7wks)**

	DFM Supplementation			
	Control	Water	Feed	Feed/Water
Initial weight (kg)	0.04	0.04	0.04	0.04
Weight gain (kg)	1.83	1.73	1.86	1.74
Feed intake (kg)	3.80	3.71	3.92	3.88
Feed conversion	2.07^{b}	2.21^{a}	2.10^{b}	2.24^{a}
Mortality (%)	5.55^{b}	8.33^{ab}	13.89^{a}	2.78^{b}

Means in the same row with different superscript were significantly different at $P<0.05$
Source: Chantsavang and Watcharangkul (1998)

Chantsavang and Watcharangkul (1998) observed no significant effect in feed intake, weight gain of broilers given DFM in drinking water and/or feed. On the other hand, mortality was high (13.89) in birds who had DFM in their feed and low (2.78) in birds given DFM in feed and water. At the end of the 7 weeks, the experiment revealed that there were no significant effects on the carcass characteristics and qualities of broiler meat and fat.

Tortuero (1973) reported an increase in growth rate in chicks given a *Lactobacillus acidophilus* probiotic culture in drinking water for 11 days. Similarly, Jin *et al*. (1998a) and Huang *et al*. (2004) also reported improved performance characteristics in broilers receiving a *Lactobacillus* probiotic culture.

Table 2.2**: Carcass Characteristics of Broilers Given DFM in Drinking Water and Feed**

	DFM Supplementation			
	Control	Water	Feed	Feed&Water
Number of chickens	12	12	12	12
Live weight (kg)	1.909	1.953	2.022	1.955
Carcass weight (kg)	1.569	1.607	1,650	1.596
Dressed weight (%)	87.83	87.07	87.17	86.77
Eviscerated weight (%)	93.59	94.44	93.63	94.07
Liver (%)	2.74	2.39	2.65	2.67
Gizzards	1.87	1.99	2.03	2.15
Breast (%)	14.76	14.63	14.35	13.81
Thigh + Drumstick (%)	29.86	30.87	29.83	29.89
Abdomen fat (%)	1.88	1.98	2.38	2.02

Source: Chantsavang andWatcharangkul (1998)

2.6.3. Effect of DFM in Water on Layers

There is limited literature on the effects of adding probiotics in water on pullets, although they have the potential to improve general performance. Nonetheless, probiotics have been administered in layers drinking water with generally encouraging effects (Theodore, 1999).

The use of flavored probiotic (*Lactobacillus acidophilus*) administered through water for breeding birds has been shown to be effective. Birds tend to drink more water when the probiotic is flavored, this helps to reduce their vulnerability to stress related diseases (Theodore, 1999). Results of a research conducted by Chantsavang and

Watcharangkul (1998) on the effect of DFM administration in water and feed on egg quality of layer Quail are presented in Table 2.3.

Table 2.3 **Performance and Egg Qualities of Laying Japanese Quail as Affected by the Addition of DFM in Drinking Water and Feed**

DFM	Control	Water	Feed	Feed&Water
Body Weight gain (g)	59.72	60.30	60.83	57.72
Hen-day production (%)	87.07	79.36	86.96	81.09
Feed intake (g)	28.16	26.52	29.04	27.01
Egg weight (g)	10.67	10.02	10.47	10.64
Egg shell thickness (mm)	0.231	0.234	0.234	0.233

Source: Chantsavang and Watcharangkul (1998)

Chantsavang and Watcharangkul (1998) also reported that laying quails receiving DFM in water and in feed, recorded better average body weight gain than quails given DFM in both water and feed groups and the control. The average egg production values and average egg weight for the four respective groups did not show any significance ($P>0.05$). Feed intake and feed utilization were found to be similar among the treatment groups. Birds in the control group consumed 3.24 kg of feed to produce 100 eggs as compared to those fed diets that contained the DFM culture which consumed 3.36, 3.35 and 3.39 kg of feed, respectively to produce 100 eggs. Results of statistical analyses show that there were no significant differences among treatment groups for the production characteristics mentioned above and the addition of DFM did not influence the performance of the laying quails.

Significant influence of DFM supplementation was found in egg quality trait. Egg yolk colour score for the control group was 5.52 while scores for the DFM

supplemented groups were 6.32, 6.72 and 6.96. Eggshell thickness increased slightly at 8 weeks and 12 weeks of age for quails given DFM in water and/or in feed.

2.7. Effect of DFM in Feed on Layers and Broilers

Probiotics is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). DFM is one of several approaches that have been reported to have the potential to reduce enteric diseases in poultry and subsequent contamination of poultry products. It can also improve on the growth and health of broilers (Chapman, 1989; Patterson and Burkholder, 2003).

2.7.1 Effect of DFM on Health Status of Poultry

Probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Sacharomyces have potential effect on modulation of intestinal micro flora and pathogen inhabitation (Mountizouris *et al.*, 2007). The manipulation of gut micro flora via the administration of probiotics influences the development of the immune response (Luyer *et al*., 2005) which tends to reduce the incidence and duration of diseases (Kizerwetter and Binek, 2009).

Higgins *et al*. (2008) observed that oral administration of 10^6 or 10^8cfu of Lactobacilli-based probiotics significantly reduced *Salmonella enteritidis* from neonatal chicks within one hour, whereas lower dosage had no effect. DFM have been proposed to assist in the prevention of carcass contamination and improve the immune response in the chicken (Huang *et al.*, 2004). The positive effect can result either from

a direct nutritional effect of the probiotics or a health effect, with probiotics acting as bioregulators of the intestinal micro flora and reinforcing the host's natural defenses (Fuller, 1977; Fuller, 2001).

Sub-therapeutic antibiotics influence intestinal microbial population and activities by causing the intestinal pathogenic micro-flora to create resistance to useful micro-flora and assisting in the damage of digestive process. Anderson *et al.* (2001) and Gasson *et al.* (2004) showed that the administration of probiotic strain (*Lactobacillus johnsonii* F19785) to commercial fowls will confer protection (via competitive exclusion (CE)) against colonization of the fowl GI tract by *Clostridium perfringens* (and other potential pathogenic bacteria species). Twenty (20) day-old specific pathogen free chicks doses of single oral inoculums of 1×10^9 cfu. *Clostridium perfringens* strain and *E. coli* 078:K80 (EC34195, naf) and 24 hours later were challenged with *lactobacillus johnsonii*. A single oral dose of the CE strain was sufficient to suppress all aspects of colonization and persistence of *Clostridium perfringens* and *E. coli*.

Table: 2.4.**Evaluation of Different Concentrations of a Probiotic Culture for Reduction of *Salmonella Enteritidis* in Neonatal Chicks 24 h Post Treatment**

Group	Probiotic treatment (cfu/chicks)	*S.enteritidis* positive/total samples (%)
Control	0	17/20 (85) [a]
Treated	10^4	13/20 (65) [a]
Treated	10^6	3/20 (15) [b]
Treated	10^8	3/20 (15) [b]

[a,b]Different superscripts indicate significant differences between treatments
Source: Higgins *et al*. (2008)

Higgins *et al.* (2008) in an experiment to evaluate the effect of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella enteritidis*in neonatal broiler chicks observed a distinct effect due to the concentration of probiotic treatments administered (Table 2.4). The lowest concentration examined (10^4cfu/ chick) did not result in a significant reduction of *S. enteritidis.* However, both 10^6 and 10^8cfu/chick did result in a significant reduction of *S.enteritidis,* with only 15% of chicks remaining positive in the cecal tonsils. Remarkably, there was absolutely no improvement of effect following administration of 10^8cfu/chick, even though there was a 2-log increase in administered bacteria (Table 2.4). These data suggest that the effects of this culture are limited, in that an increase in the number of administered bacteria will not further reduce *Salmonella enteritidis* colonization.

2.7.2 Effect of DFM on Egg Quality

Probiotics (Lacto-Sacc) have been shown to be effective in diets fed to pullets and layers. The flock of pullets was fed probiotics from week 7 to 14, and the results showed a 42% and 28% average improvement in flock uniformity (Chapman, 1989). A study on the effect of the inclusion of DFM on young chickens for two years showed higher values on treated birds than the non-treated group in the following categories: average egg weight, eggshell strength, eggshell thickness, albumen height, Haugh units and yolk color (Hussain *et al*., 1994). Similarly Nahashon *et al.,* (1994) reported that, administration of probiotics significantly increased egg weight.

Horniakova and Busta (2006) reported that the use of probiotics, specifically *Enterococcus faecum*on layers at age 24weeks of age resulted in higher values for treated groups as compared to the control group which registered the lowest values.

This was confirmed by Kurtoglu *et al.* (2004) who observed higher values of egg weight for birds fed the diet that contained probiotics than the control birds, though results obtained were not statistically significant. Yoruk *et al*. (2004) observed no effect of dietary probiotics administration on feed intake and egg weight. Egg production for hens fed diet administered with probiotics was greater than for control hens. However no effect of dietary treatment was observed on egg quality. Mortality was also reduced in probiotics treated diets.

Hassanein and Soliman (2010) also observed that yeast culture (*Saccharomyces cerevisiae)* administration can enhance the productive performance of laying birds. The use of *Saccharomyes cerevisae* in the diet of layer hens of 70 weeks old resulted in an increased egg production of 83.4% and 80.6% for the two groups fed 0.4 and 0.8 probiotic against 74% for the control group due to nutrients utilization via inhibitory effect of yeast against pathogenic bacteria which may cause mild enteritis and mal-absorption of nutrients. Average egg weight was not affected but a slight improvement in egg shell thickness was observed in birds fed probiotics as shown in Table 2.5.

Table 2.5: **Effect of Feeding Different Yeast Levels on Laying Performance and Egg Components of 70 Weeks Layer Hens**

	Yeast level				
Item	**0.0%**	**0.4%**	**0.8 %**	**1.2**	**1.6%**
Egg production	74.0	83.4	80.6	74.9	74.6
Av. egg weight (g)	63.1	61.2	62.7	64.5	61.8
Egg mass (g egg/hen/day)	46.7	51.0	50.2	48.3	46.1
Egg component					
Egg yolk (%)	27.3	28.1	28.8	27.6	27.7
Egg albumin (%)	63.7	62.6	61.7	63.1	62.9
Egg shell (%)	9.00	9.33	9.45	9.39	9.39
Egg shell thickness (mm)	0.396^{b}	0.425^{ab}	0.426^{a}	0.416^{ab}	0.420^{ab}

a,b: Means with different superscripts are significantly different ($P<0.05$).
Source: Hassanein and Soliman (2010)

Yalcin *et al*. (2008) observed that yeast culture at the level of 2 g/kg of layer diets did not significantly affect feed intake, hen-day egg production and feed efficiency, or interior and exterior egg quality characteristics. However, body weight gain and egg weight were increased with yeast culture administration, whereas egg yolk cholesterol was reduced significantly in the groups fed the probiotics diets. In a research carried out by Bonsu *et al*. (2012), the hen-day production was numerically highest for birds fed the DFM supplemented diet (71.82%) followed by the combined diet (70.96%), basal diet (70.53%) and antibiotics diet (69.14%). Daneshyar *et al.* (2007) reported no increase in egg weight after testing probiotic (protexin) on breeder hens of 64-weeks-old for 10 weeks. Balevi *et al.* (2001) also reported a similar result when they recorded no significant increase in the egg weight.

Chantsavang and Watcharangkul (1998) in an experiment to assess the effect of DFM administration in diet on production performance and egg quality of layer birds, observed no significant differences among the treatment means with respect to egg production, feed per dozen eggs and mortality. However, consistently high egg production was observed in the period of study for layers that received DFM supplemented in feed. In egg quality traits, no significant differences were found in weight, percent yolk, Haugh unit, percent albumen, shell weight, shell thickness and specific gravity. However, consistently higher egg weight and lower shell weights were also observed in the studied periods for the groups given DFM. Results from Chantsavang and Watcharangkul (1998) are presented in Table 2.6.

Table 2.6 Layer Production Performance/Egg Quality as Affected by the Addition of DFM in Feed

Parameter	Control	0.5%DFM	1%DFM
Egg production (%)	67.73	75.83	71.9
Feed intake (g/bird/day)	96.54[b]	105.04[a]	99.21[b]
Egg weight (g)	58.21	58.98	60.25
Haugh unit	81.27	79.15	77.33
% Albumen	64.83	64.80	65.16
Shell thickness (mm)	0.41	0.41	0.41
Mortality (%)	0.00	2.38	0.00

Means in the same row with different superscript were significantly different at $p<0.05$

Source: Chantsavang and Watcharangkul (1998)

From the numerous studies on the effects of probiotics on egg production and quality, it has become much clearer that nutritionally adequate diets will indeed satisfactory

enhance egg production and egg weight of laying birds when the right doses or levels of probiotics have been given. It is also showed that yolk colour is not affected by the use of probiotics.

2.7.3. Effect of DFM on Weight Gain

Probiotics are the most effective growth promoters from studies by Baidya *et al.* (1993), because it has been observed that chickens fed with probiotics tend to have higher weights than chickens not fed probiotics (Mohan *et al*., 1996).

Ahmad (2006) in an experiment on the effect of probiotics on broiler growth reported that the growth pattern of treated birds showed an increase in weight gain relative to the control when given up to 1gram per 10 kg feed. Lee *et al.* (2006) also observed an improved performance of poultry and favorably lower gas production in broiler house when *Aspergillus oryzae* was used alone or in combination with *Lactobacillus* species.

Jin *et al.*, (1998b) reported that the addition of *L. acidophilus* 126 strains or mixture of 12 *Lactobacilli* to the basal diet of broilers increased significantly their body weight from 0-6weeks. Similarly, an increase in the body weight of chickens was observed after commercial probiotics was added to their diets (Kim *et al.*, 1998).

Sieo *et al*. (2005) in a study with transformed *Lactobacillus* strains, also reported that at 21days of age, body weight, body weight gain and feed conversion ratio of the broiler chickens were significantly improved over the control and the parental *Lactobacillus* strain (2.8 and 2.5% respectively).

Though, there have been many reports on the positive effect of probiotics on weight gain of broilers by some researchers like Patidar and Prajapati (1999); Ergun *et al.* (2000) think otherwise by stating that administration of probiotics has no effect on the performance of broiler chicks. A review of the weekly gains suggested that the beneficial effect from the probiotics are seen from the beginning of the fourth week onwards (Mohan *et al*., 1996). This gives an indication that beneficial effects of feeding probiotics for the first 3-weeks are perhaps seen in the health improvement and reduction in mortality of birds. O'Dea *et al*. (2006) observed no significant effect on weekly body weight gains of birds fed diets containing probiotics from 0-35 days. However, the body weight gain from 36-42 days of age was higher in the probiotics diet compared to the control diet. Also, Owings *et al*. (1990) observed significant ($P<0.05$) heavier weight of broilers at 36 days of age over the control when the diet was administered with *Streptococcus faecium* and further showed greater reduction in *E. coli.* The result of a research conducted by Jin *et al*. (1998a) on the growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures is presented in Table 2.7.

TABLE 2.7:**Body Weights and Feed to Gain Ratios of Broilers Fed Diets Without or With *Lactobacillus* Culture from 1 to 42 Days of Age**

	Weight Gain (g)		
Diet	**Id(g)**	**2Id(g)**	**42d(g)**
CS	41.8	645.9[b]	1,914[b]
CS+0.05%LC	41.4	671.8[b]	1,983[b]
CS+0.10%LC	41.4	681.0[a]	2,677[a]
CS+0.15%LC	41.5	647.9[b]	1,925[b]
SEM	1.51	20.9	52.5

[a–c]Means within columns with no common superscript differ significantly ($P<0.05$); ND, no data, LC - *Lactobacillus* culture, CS- corn-soybean, SEM -
Source: Jin *et al*. (1998b)

Results in Table 2.8 showed that, the treatment of feed with 0.10% LC (*Lactobacillus* culture) produced a significantly greater body weight (P<0.05) than the control or the treatments with 0.05 or 0.15% LC. A significant (P<0.05) increase in body weight was observed at 21 and 42 day of age in broilers fed the diets containing 0.10% LC but not 0.05 and 0.15% LC. In the experiment, the improvement of body weight was consistent in both the growing period (0 to 3 wk) and the finishing period (4 to 6 wk) (Jin *et al*., 1998b).

Table 2.8: Effect of Administering Probiotics and Antibiotics in Feed on the Growth Performance of Broilers from 4-8 Weeks.

Parameter	Treatment			COV
	BD	DFM	DFM+ANT	
Initial body weight (g)	555	555.63	555	26.12
Final body weight (g)	2302.38[b]	2571.25[a]	2654.08[a]	3.02
Mean feed intake (g)	4839.31[b]	4987.41[a]	5069.74[a]	1.69
Mean weight gain (g)	1762.87[b]	2002.9[a]	2004.95[a]	2.16
FCR	2.74[a]	2.49[b]	2.53[b]	2.84
Livability (%)	93.75	100	98.75	

Source: Bonsu *et al*.(2012)
COV-Coefficient of variation.
Values with different superscripts in row differ significantly (P<0.05)
BD-Basal Diet, DFM-Direct-fed microbial, ANT-Antibiotics, FCR-Feed conversion ratio. DFM+ANT-Direct-fed microbial plus Antibiotics

According to Bonsu *et al.* (2012), weight gain of chickens fed on the diet containing DFM, and DFM+ANT were significantly (P<0.05) higher than those on the basal diet (Table 2.8). Weight improvements in chickens on the diet containing DFM were observed in the 6th week. The overall body weight gain from day –old to 8th week also

indicated significant improvement for birds on DFM, and DFM+ANT diets. Although, broilers on combined (DFM+ANT) diet had the highest gain (2559.95 g), this did not differ markedly from those given DFM diet (2492.68 g).

From the literature it is apparent that the use of probiotics in broilers results in the improvement of their mean live body weight and body weight gain due to improvement in absorption of nutrient.

2.7.4. Effect of DFM on Feed Conversion Ratio

Feed conversion ratio as affected by probiotics is the subject of controversy. Some studies by Hamid *et al.* (1994) show that probiotics administration in feed of chickens improves the feed conversion ratio while others like Ahmad (2004) could not detect any difference in feed conversion ratio of broilers as compared to the control. Daneshyar *et al.* (2007) though observed an increase in feed conversion ratio on broiler breeder hens of 64 weeks of age fed probiotics (protexin supplemented diet), the increase was not statistically significant.

Aspergillus oryzae at 0.5, 0.7 and 1.0 percent in diet significantly affected feed conversion ratio but not weight gains and feed intake, of broiler chickens (Goh and Hwang, 1999). Toms fed DFM had similar 20-week body weights of 20.0 kg for the control and 20.1 kg for DFM treatment birds, but the DFM treatment birds had improved feed conversion ratio throughout the trial compared to the control birds because the DFM treated birds consumed less feed (Grimes and Russel, 2007).

In addition, Onifade *et al*. (1999) have shown that the use of *Saccharomyces cerevisiae* cell walls and the spore forming *Bacillus subtilis* was an alternative to the

use of antibiotics in broiler feed. They reported improvement in feed conversion ratio and weight gain of broilers fed the DFM as compared with antibiotic and control treatment groups. Kim *et al.* (2003) reported that feeding *Aspergillus oryzae* to broiler chickens significantly enhanced performance indices such as body weight gain and feed intake but failed to affect feed conversion ratio.

Efficiency of feed conversion values were significantly ($P<0.05$) better for chickens fed diets containing DFM and DFM+ANT than the basal diet (Table 2.8). The overall feed efficiency from day–old to 8th week (Table 2.8) also indicated significant improvement in chickens fed DFM and DFM+ANT supplemented diets than the basal diet. Although, broilers fed diet containing antibiotics were most efficient. Feed conversion ratio of both broilers and layers is positively affected by the administration of DFM in their diet or water due to the decrease feed consumption and the subsequent improvement of digestion, absorption and assimilation which result in better weight gain.

2.7.5. Effect of DFM on Carcass Characteristics

With regards to carcass parameters, it has been observed that dietary probiotic treatment may not have a significant influence on the dressing percentage and weight of internal organs (Owings *et al.*, 1990; Mohan *et al*., 1996; Mandal *et al*., 2000; Sieo *et al*., 2005). At the same time, the DFM treated birds with an increase in live body weight compared to the non treated birds, presented a decrease in the following measurement: offal weight, liver index, gizzard index, intestinal weight index, intestinal length index, kidneys index, and heart index. This indicates that DFM can work as a growth promoter without any associated risks.

From the result , dressing percentage, blood weight, liver weight, abdominal fat and intestine weight did not differ significantly (P>0.05) among dietary treatment means, the overall slaughter data showed that dietary DFM in broilers may not have a significant influence on the dressing percentage and weight of internal organs.

Awad *et al.* (2009) observed that the weight of spleen and thymus tended to be significantly greater (P<0.01) for birds fed probiotics supplemented diets. The relative weight of liver was also greater (P<0.05), however, no significant difference was indicated for dressing percentage. Abdominal fat has also been reduced by the administration of DFM. (Mohan *et al*., 1996; Jin *et al*., 1998b).

2.7.6. Effect of DFM on Blood Parameters

On serum or blood parameters, Mohan *et al*. (1996) observed that packed cell volume did not show any variation as a result of probiotics supplementation. However, there was a significant (P<0.05) reduction in haemoglobin content by the addition of probiotics resulting in a mean value of 7.9 g/dl compared to 9.2 g/dl in control birds. It was further indicated that prolong probiotics treatment may be potentially counterproductive from a nutritional point of view, as it could leave the host deficient in iron and folic acid. This is because probiotics can compete with the host for iron and folic acid. Blood or serum cholesterol levels have been shown to be significantly reduced by supplementation of diet with probiotics (Mohan *et al*., 1996; Jin *et al*., 1998).

Strompfova *et al.* (2005) demonstrated that administration of probiotics (AD1 strain) showed no significant differences in the red blood cell count, leucocyte count,

differential leucocyte counts, haematocrit, hemoglobin concentration and glutathione peroxidase, but there was a significant increase in the phagocytic activities of leucocytes in experimental birds after the application of AD1 strain.

According to Bonsu *et al*. (2012) the haemoglobin (HB) concentration of chickens fed DFM diet and the combined DFM and antibiotics diet were significantly lower than those fed on the basal diet (Table 2.9). The number of white blood cells (WBC) were also highest (P<0.05) for chickens given DFM in their diet followed by the combined DFM and antibiotics diet. The increased WBC count could be attributed to improvement in the immune system of the chickens brought about by improved stimulation of different subset of Th 2 cytokines induced by *Lactobacilli* (Christensen *et al*., 2002).

Table 2.9: Effect of Dietary Treatments on Blood Parameters of Broiler Chickens.

Parameter	Treatment				COV
	BD	DFM	ANT	DFM+ANT	
WBC $\times 10^9$/l	127.57^c	131.23^a	127.13^c	129.35^b	0.73
RBC $\times 10^{12}$/l	2.25	2.18	2.27	2.2	2.73
PCV %	27.13	26.69	27.09	26.92	1.42
HB g/dl	11.11^a	10.55^b	11.18^a	10.66^b	1.10
Serum cholesterol mg/dl	110.25^a	91.25^b	108.98^a	97.55^b	1.19

Values with different superscripts in the same row differ significantly (P<0.05).
WBC-white blood cell; RBC-red blood cell; PCV-packed cell volume; HB-haemoglobin
Bonsu *et al*. (2012)

2.8. Cost -Benefit Assessment of Using DFM in Poultry Diets

The use of DFM should ultimately be assessed and weighed against economic factors among others. Haddadin *et al*. (1996) observed numerous benefits of probiotics supplementation in layers. It was indicated that whether or not the economic benefits would justify the cost of supplementing the basal diet with high levels of *Lactobacillus acidophilus* remains an open question. It was also indicate that if other strains or rates of inoculation of *L. acidophilus* could achieve greater reduction in the cholesterol concentration of eggs and perhaps offer additional protection against the risk of infection of the eggs by Salmonella spp. (Watkins and Miller, 1983) then the expense of culture propagation might be acceptable. Mandal *et al*. (2000) observed that the economic returns per broiler were better for all diets containing probiotics.

According to Bonsu *et al*. (2012), Cost of production per bird was relatively highest for chickens fed the combined diet (DFM+ANT) GH¢2.84 (US$2.18) followed by the DFM diet GH¢2.67 (US$2.05) and the basal diet GH¢2.61 (US$2.01). Profit on returns per bird on the other hand was relatively higher for chickens fed the combined DFM and antibiotics diet GH¢3.29 (US$2.53) followed by DFM diet GH¢3.22 (US$2.48). It was therefore concluded that, in spite of the lower production cost, the least profit per bird of GH¢2.69 (US$2.07) was realized from chickens fed on the basal diet as a result of the reduced mortality for chickens fed diet containing DFM.

The use of DFM in poultry production is economical since its use in both layers and broilers greatly reduce infection of pathogenic bacteria and subsequently reduce the cost of purchasing coccidiostat, medications, antibiotics and mortality. DFM also decrease feed intake but increase growth and egg production due to improved

digestion, absorption, and assimilation. As a result, better and relatively higher economic returns of using DFM is achieved (Mandal *et al*., 2000 ; Bonsu *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Two experiments were carried out to assess the use of probiotics in water and feed on the performance of broiler and layer chickens. The first experiment was carried out at the Experimental farm of the University of Education, Winneba, Mampong Campus. The second was carried out at Raboam Farms in the Ejisu-Juaben Municipality. Though, both areas are found in the Ashanti Region of Ghana, yet they are located at different geographical areas.

3.2 Experiment 1

Effect of DFM in feed or water on growth performance of broiler chickens.

3.2.1Location of Experiment

Mampong is located 60 kilometers north of Kumasi in the Ashanti region. Geographically, the area is within the transitional zone lying between the guinea savanna in the north and the rain forest zone in the south. Mampong lies on latitude 07^{o}03' north and longitude 01^{o}24' west on an altitude of 457 meters above sea level. Yearly average rainfall is 516 mm while the daily temperature is between 28.5^{o}C and 31.7^{o}C (MSD, 2007). The humidity ranges between 70 – 80%.

3.2.2 Sources of RE3 and Feed Ingredients

The Direct- Fed Microbial used was RE3, a health and performance probiotic which contains *Lactobacilli* (1×10^{8} cfu/g), *Bacillus* (1×10^{12} cfu/g) and *Saccharomyces cerevisiae* (yeast, 1×10^{5} cfu/g) which indicated that the probiotic was a multi-strain

and their microbial population met the requirement as stated by Ewing and Cole (1994). The DFM used in the study was obtained from the Basic Environmental Systems and Technology Inc. (BEST), Canada. Upon receipt, the DFM was kept in a cold room at a temperature of 18^{o}C. Ingredients used in the feed formulation included maize, soya bean meal, wheat bran, tuna, Russian fish, oyster shell, salt and vitamin/mineral premix. These ingredients were purchased from accredited feed supplies in Kumasi.

3.2.3. Diet Formulation

The composition of the broiler experimental diet is presented in Table 3.1. The experimental diet was formulated to contain 203 crude protein kg^{-1} and a metabolizable energy content of 12.4 MJ

3.2.4 Experimental Design and Duration of the Experiment

One hundred and ninety two 4-week old hybrids Cobb broiler chicks were used for this experiment. The day old chicks were brought from Akate Farms in Kumasi. The birds were randomly allotted to four (4) treatments with three (3) replications for each treatment. Each replicate was made up of sixteen (16) chicks consisting of 8 females and 8 males with a mean weight of 643 g housed in a partitioned deep litter pens in a Completely Randomized Design (CRD). The floor space measured 1.2 m×1.8 m giving an area of 2.16 m^2 and affording a space per bird of 0.14 m^2.

Table 3.1: **Composition and Calculated Nutrient Analysis of Basal Broiler Experimental Diet**

Ingredients	**Quantity (g/kg)**
Maize	580
Wheat bran	145
Tuna fish meal	70
Russian fish meal	90
Soybean meal	80
Oyster shell	20
Vitamins/minerals premix	5
Dicalcium phosphate	5
Common salt	5
Total	1000
Calculated Analysis (gkg^{-1})	
Crude protein	203
Fibre	30.7
Ether extract	18.7
Ca	1.0
P (available)	6.2
Lysine	12.2
Methionine	4.8
Metabolizable energy ($MJkg^{-1}$)	12.4

Vitamin mineral premix provided the following per kg of diet: Fe 100mg, Mn 110mg, Cu 20mg, Zn 100mg, I 2mg, Se 0·2mg, Co 0·6mg, sanoquin 0·6mg, retinal 2000mg, cholecalciferol 25mg, α-tocopherol 23000mg, menadione 1·33mg, cobalamin 0·03mg, thiamin 0·83mg, riboflavin 2mg, folic acid 0·33mg, biotin 0·03mg, pantothenic acid 3·75mg, niacin 23·3mg, pyridoxine 1·33mg.

The four treatments were; treatment one (T_1), the control which contained no DFM in both feed and water. In treatment two (second control) (T_2), DFM was added to the feed at the rate of 1.5 ml per 1 kg feed with no DFM in their water. Treatment three (T_3) and treatment four (T_4) had no DFM in their diet, however, 1.5 ml and 2.5 ml of DFM per liter of water were added respectively. The DFM was measured with a

graduated syringe and added to their drinking water, while on the other hand the DFM was first mixed with about 50 g feed before mixing it to the total feed for the day. All the experimental birds were fed with the same basal diets which had a crude protein content of 203 g kg^{-1} and a metabolizable energy 12.4 MJ/kg. Light was provided throughout the night, Feed and water were also provided *ad libitum*. The basal diet used for experiment 1 is presented in Table 3.1. The experiment lasted for five (5) weeks.

3.2.5 Data Collection

Data were collected for the following parameters.

3.2.5.1Feed Consumption

Daily feed was weighed before being offered to the birds. The difference between feed supplied to a replicate and feed left-over at the end of each day was recorded as daily feed intake per replicate. These were added up at the end of each week to give weekly consumption values. The weekly consumption value was then divided by the number of birds to obtain the average weekly feed consumption.

3.2.5.2 Water Intake

Daily water was measured before being offered to the birds. Water intake was measured as difference between the amount of water given and the amount of water left over on daily basis per replicate. These were added up at the end of each week to give weekly water consumption values. The weekly consumption value was then divided by the number of birds to obtain weekly water consumption per bird per replicate.

3.2.5.3. Body Weight and Body Weight Gain

Birds were weighed at the start of the experiment and also at weekly intervals. Weight measured at the end of the previous week was deducted from that of the current week to obtain the weight gained for the week. Birds were weighed in batches using a box on a top-pan balance. The weekly body weight gain was then divided by the number of birds in a replicate to obtain average weekly body weight gain per bird per replicate.

3.2.5.4. Feed Conversion Ratio

Feed conversion ratio was calculated from feed consumed and weight gained for each replicate by dividing the weekly feed consumption value by the respective weight gains of the replicates for that week.

3.2.5.5. Carcass Characteristics

At the end of the feeding period, two birds from each replicate (a male and female) were selected at random, starved overnight to empty their crops, slaughtered, defeathered and eviscerated. The digestive tract was carefully removed and the carcass weighed. Carcass dressing percentage was calculated from the eviscerated weight and the live weight. The gizzard, liver, and intestines were excised, cleaned and weighed using an electronic balance. The fat deposits around the intestine and abdominal area were removed and weighed. Visual examination of the gizzard, liver and intestines was carried out to determine any abnormalities.

3.2.5.6. Haematological Studies

Blood samples were collected randomly from two birds from each replicate. Blood samples were collected (after feed withdrawal for 12 hrs) from the jugular vein with a

needle and syringe into anticoagulant (heparin) bottles and analyzed for total red blood cells (RBC), haemoglobin (HB), packed cell volume (PCV) and white blood cells (WBC) using a Haematological "SysnexKx- 2IN Auto Analyzer", and the biochemical factors with "FlexorJnr autoanalyzer".

3.2.5.7. Economics of Production

The economics of production was calculated for each treatment for broiler experiments. The assessments were done based on the cost of birds, feed and medication. Birds were sold based on their weight per kg per replicate. The mean cost of production per bird (cost of day old birds, DFM or medication, and feed) is then deducted from mean amount made from the sale of the birds.

3.2.6 Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA) using Genstat (2007). Least square difference test was used for testing differences among means. Detailed ANOVA is presented in the appendix.

3.3 Experiment 2

Effect of DFM in feed or water on performance of layer chickens.

3.3.1 Location of Experiment

Ejisu, on the other hand, has a semi-tropical climate with a yearly average rainfall of 1570 ± 344.9 mm, and the mean temperature varies little throughout the year. Maximum temperature of 32^{o}C is recorded in February or March while the minimum temperature of about 22^{o}C is recorded in May. Relative humidity varies from 75% in the afternoon to 90% in the mornings (Osafo, 1976).

3.3.2 Diet Formulation

The composition of the experimental basal diet is presented in Table 3.2

Table 3.2: **Composition and Calculated Nutrient Analysis of Layer Experimental Basal Diet**

Ingredients	**Quantity (g/kg)**
Maize	520
Wheat bran	200
Fish meal	100
Soybean meal	100
Oyster shell	70
Vitamin/mineral premix	2.5
Dicalcium phosphate	5
Common salt	2.5
Total	1000
Calculated Analysis (gkg^{-1})	
Crude protein	193
Fibre	34.9
Ether extract	35.8
Ca	8.15
P (available)	4.67
Lysine	9.1
Methionine	6.19
Metabolizable energy ($MJkg^{-1}$)	11.4

Vitamin mineral premix provided the following per kg of diet: Fe 100mg, Mn 110mg, Cu 20mg, Zn 100mg, I 2mg, Se 0·2mg, Co 0·6mg, sanoquin 0·6mg, retinal 2000mg, cholecalciferol 25mg, α-tocopherol 23000mg, menadione 1·33mg, cobalamin 0·03mg, thiamin 0·83mg, riboflavin 2mg, folic acid 0·33mg, biotin 0·03mg, pantothenic acid 3·75mg, niacin 23·3mg, pyridoxine 1·33mg.

3.3.3. Experimental Design

The laying chicken study was carried out on-farm in Raboam Farms at Ejisu, a medium scale farm. A total of three hundred (300) 30-week old layer Rhode Island

Red chickens were used for the study. Birds were housed in partitioned deep litter pens of dimension 2.0 m×2.5 m with each pen containing a wooden laying nest. The birds were randomly distributed into five groups of 60 birds per treatment (T_1, T_2, T_3, T_4, and T_5) with an average weight of 1514 g. The 60 birds in each treatment were further subdivided into three groups of 20 birds per replicate in a Completely Randomized Design (CRD). A normal layer basal diet was provided for all birds. However, differences were in the level of the RE3 administration via drinking water or in feed. Treatment one (T_1), which was the control, had no DFM in both feed and water, while treatment two (T_2) had no DFM in water but contained 1.5 ml DFM in 1kg feed. Treatment three (T_3), four (T_4) and five (T_5) contained only DFM (RE3) in only their drinking water at the rate of 1.0 ml, 1.5 ml and 2.0 ml per litre of drinking water respectively. Feed and water were provided *ad libitum*. The basal diet had a crude protein content of 193 g k^{-1} and a metabolizable energy of 11.4 MJ. The basal diet used for the experiment is presented in Table 3.2. The layer trial lasted for twelve (12) weeks.

3.3.4. Data collection

3.3.4.1Feed Consumption

Daily feed was weighed before being offered to the birds. Feed intake was measured as difference between the amount of feed given and the amount of feed left-over on weekly basis. The weekly consumption value was then divided by the number of birds to obtain a weekly feed consumption per bird per replicate.

3.3.4.2 Weight Gain

The weights of birds were determined twice at the beginning of the experiment and at the time of terminating the experiment. Weight gain was determined by the difference between the initial and final weight.

3.3.4.3 Feed Conversion Ratio

The monthly feed conversion ratio was estimated as the amount of feed consumed in relation to the number and weight (per kg) of eggs produced during that month.

3.3.4.4 Egg Production and Weight

The number of eggs laid per replicate will be recorded daily: from this, hen-day production and hen-housed production will be determined. Eggs from each replicate will be weighed weekly and average weight recorded.

3.3.4.5 Internal Egg Quality

Internal quality of the egg was determined every two weeks. Samples of (3) eggs from each replicate were opened every two weeks for the measurement of albumen height, width and shell thickness. The shell thickness measurements being taken at the equatorial plane of the egg after the shell membranes had been carefully removed using a tripod micrometer. The haugh unit scores were calculated using the albumen heights and the egg weights in the formula $100^{\log}{}_{10}$ (h-1.73$W^{0.37}$+ 7.6)

3.3.5 Mortality

Mortalities were recorded as they occurred and all dead birds were sent to the veterinary laboratory in Amakom for post-mortem examination to determine the cause of death.

3.3.6 Economics of Production

The economics of production was calculated for each treatment for layer experiments. Though the eggs were not sold, the total egg weight was calculated for each replicate using the number of egg laid and mean egg weight The cost assessments were done based on the cost of feed, medication or DFM.

3.3.7 Statistical Analysis

The data collected was subject to analysis of variance (ANOVA) using Genstat (2007). Least square difference test was used for testing differences among means. Detailed ANOVA is presented in appendix.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Experiment 1

The effect of DFM in feed or water on growth performance of broilers is presented in Table 4.1.

Table 4.1 **Effect of Probiotics in Water or Feed on the Growth Performance of Broilers from 4-9 Weeks of Age**

PARAMETERS	TREATMENTS				SEM
	T_1	T_2	T_3	T_4	
Initial body weight (g/bird)	643.7	645	641.7	639.9	11.29
Final body weight (g/bird)	3080	3116	3112	3079	36.8
Mean feed intake (g/bird)	5553	5548	5579	5477	81.5
Mean weight gain (g/bird)	2439	2475	2471	2448	35.3
Feed gain ratio	2.28	2.24	2.26	2.24	0.57
Livability (%)	100	100	100	100	0.00

T_1- (control) no DFM in feed and drinking water
T_2 - (control) 1.5ml DFM in feed but no DFM in drinking water
T_3- 1.5ml DFM in drinking water but no DFM in feed
T_4- 2.5ml DFM in drinking water but no DFM in feed

4.1.1 Feed Intake, Body Weight Gain and FCR

Feed intake was not influenced significantly ($P>0.05$) by the addition of DFM either in drinking water or feed in all the treatments. Average feed consumption per bird for the 5-week experimental period ranged from 5477 g to 5553 g with birds on 2.5 ml (T_4) DFM administered through water recording the lowest intake while birds on 1.5 ml (T_3) DFM in water recorded the highest intake (Table 4.1). This was as a result of late administration of DFM in bird's drinking water and feed.

Weight gain of all treated birds (T_2, T_3, and T_4) did not show much difference $P>0.05$ from the control, however, there was a slight improvement in the performance of birds provided DFM either in feed or water. The lowest value of weight gain was recorded for the control birds T_1 (2438) as compared to those birds on treatments T_2 (2475 g), T_3 (2471 g) and T_4 (2448 g). Yongzhen and Weijiong (1994) also recorded the least value of live body weight for the control group as compared to the treated group and attributed it to the improvement of nutrients absorption in birds fed probiotics.

Feed conversion ratio of DFM treated birds T_2, T_3 and T_4 were better than that of the control birds T_1. This is attributed to the reduction in feed intake and improvement in nutrient absorption and utilization resulting in better weight gain. From the study, it could be observed that, among the birds provided the DFM, T_2 and T_3 had similar weight gains. This could be due to the fact that birds on T_2 and T_3 had the same level of inclusion (1.5 ml) of DFM which consequently impacted the same effect on the birds, notwithstanding the fact that, DFM was incorporated into the feed for treatment 2 (T_2) and administered through the drinking water of birds in treatment 3 (T_3). T_2 showed numerically slight improvement over T_3 with decreased feed intake, increase weight gain and better FCR.

Treatment four, with the highest inclusion of DFM (2.5 ml per liter water), recorded the least feed intake and weight gain but registered a better feed conversion ratio. Birds on treatment T_4 recorded the least feed intake of 5,477 g which consequently affected their weight gain (2,448 g). This could be due to the high inclusion of DFM (2.5 ml) per liter in their drinking water. Ahmad (2004) confirmed this when the

growth rate of DFM treated birds increased as the DFM inclusion level was increased to 1.0 g per 10 kg feed but started to decline when the inclusion level was beyond 1.0 g. Therefore, the efficacy of probiotics depends on the administration level in bird's drinking water or feed (Romashko, 1999).

4.1.2 Carcass Characteristics

The effect of supplementation of DFM on carcass characteristics of broiler chicken is presented in Table 4.2.

Table 4.2 **Effect of DFM in Drinking Water or Feed on Carcass Parameters**

Parameters	TREATMENT			
	T_1	T_2	T_3	T_4
Life weight	3136	3042	3121	3105
Blood weight	4.3	4.2	3.8	4.0
Feather weight	5.1	4.9	5.3	5.0
Gizzard weight	1.8	1.4	1.6	1.5
Heart weight	0.49	0.47	0.44	0.39
Liver weight	1.5	1.6	1.7	1.6
Intestine weight	3.8	3.9	3.5	3.9
Abdominal fat weight	1.5	1.5	1.2	1.0
Dressing	81.35	81.83	82.17	81.17

The provision of DFM via water or feed of the broilers did not show significant ($P>0.05$) effects on the carcass dressing percentage, blood, liver, abdominal fat and intestine weight among the different treatment groups. Notwithstanding, birds in treatment 2 (T_2) and 3 (T_3) showed numerical improvement in dressing percentage as

treatment 3 (T_3) recorded the highest value of 82.17 followed by treatment 2 (T_2) with 81.83. Abdominal fat decreased as DFM level supplementation in water or feed increased from 1.5 ml inT_3 to 2.5 ml in T_4. Apparently, the lowest fat accumulation value was recorded for broilers in treatment 4 with 2.5 ml DFM supplemented in water (31.l g), followed by treatment 3 (36.8 g) and treatment 2 (44.7 g). Treatment 1 (control) recorded the highest value of abdominal fat (47.0 g). This is consistent with the results obtained by Chantsavang and Watcharangkul (1998) where no significant difference in the carcass quality of broilers given probiotics supplementation in drinking water or feed was observed. In a related study, Bonsu *et al*. (2012) also recorded no significant difference in carcass dressing percentage, blood weight, liver weight, abdominal fat and intestinal weight.

The carcass results obtained indicated that the internal organs of birds in all the treatment groups conformed to the normal recommended internal organ weights, it therefore implies that, DFM supplementation in water or feed has no adverse effect on the internal organs of chickens. However, it has the tendency to reduce abdominal fat deposition.

4.1.3 Blood parameters

Data on the impact of administration of DFM on blood characteristics of broiler chicken are presented in Table 4.3.

Table 4.3 **Effect of DFM in Drinking Water or Feed on Blood Parameters of Broiler Chickens**

PARAMETER	TREATMENT				SEM
	T_1	**T_2**	**T_3**	**T_4**	
WBC $X10^9$/L	234.5	243	241.2	236.8	3.88
RBC $X10^{12}$/L	2.5	2.5	2.6	2.5	0.075
PVC % (HCT)	30.93	30.47	31.02	32.07	1.21
HB g/dl	10.47	10.08	10.52	10.08	0.31
PLT	6.833^b	7.333^{ab}	7.333^{ab}	7.833^a	0.26

^{ab}Values with different superscripts in the same row differ significantly (P<0.05).
WBC-white blood cell; RBC-red blood cell; PCV-packed cell volume; HB-haemoglobin; PLT- platelet

Administration of Direct Fed Microbials (RE3) via drinking water did not have a significant (P>0.05) influence on the blood parameters including WBC, RBC, PCV and Hb. However the effect of DFM on blood platelet was significantly (P<0.05) influenced by DFM administration via drinking water. Birds fed the control diet obtained a significantly lower blood platelet value (6.833) as compared with that of treatment 4 (T_4) which had the highest value (7.833). This is consistent with the observation made by Strompfova *et al.* (2005) where no significant differences were detected in the red blood cell count, Hb concentration, leucocytes and glutathione peroxide, but noticed a significant increase in the phagocytic activities of leucocytes in experimental birds after applying DFM in their feed. The number of white blood cells (WBC) was least for chickens with no DFM supplementation in either water or feed (T_1). The increased WBC count for the DFM-treated birds could be attributed to improvement in the immune system of the chickens. (Christensen *et al*., 2002).

4.1.4 Economics of Production

Cost of production per bird as presented in Table 4.4 was highest for chickens given 2.5 ml DFM in their drinking water (T_2) (GH¢ 10.406) as a result of the high level of DFM used (2.5 ml/litre), followed by treatment 3 (T3) (GH¢ 10.366) with 1.5 ml DFM per litre drinking water, the control treatment (T_1) (GH¢ 10.325) with T_2 which contained 1.5 ml DFM in diet recording the least cost of production (GH¢ 10.142). Treatment 2 not only recorded the least cost but also obtained the highest income which resulted in the highest profit on returns per bird. This was followed by Treatment 3 with a relatively higher profit on returns (GH¢ 7.74) as compared with that of T_1 (GH¢ 7.6) and T_4 (GH¢ 7.56).

Table 4.4 **Cost- Benefits Assessment for Broiler Chickens (4-9 weeks)**

DESCRIPTION	T1	T2	T3	T4
Total feed intake for 4-9wks(Kg)	5.553	5.448	5.579	5.477
Per unit cost grower of feed (GH¢)	0.902	0.902	0.902	0.902
Total feed cost (GH¢)	5.734	5.730	5.757	5.665
Cost of drugs (GH¢)	0.3	-	-	-
Amount of DFM used (ml)	-	8.329	20.03	33.37
Per unit cost of DFM (GH¢)	0.015	0.015	0.015	0.015
Cost of DFM(GH¢)	-	0.125	0.300	0.501
Total cost (feed, drug & DFM) (GH¢)	6.034	5.855	6.057	6.166
Selling price per kg (GH¢)	5.9	5.9	5.9	5.9
Weight gain 4-9wks	2.438	2.475	2.471	2.448
Total income per bird (GH¢)	17.92	18.13	18.11	17.97
Profit (GH¢)	11.87	12.28	12.053	11.80

The high level of DFM inclusion in treatment 4 (2.5 ml) did not only increase the cost of production but also affected the performance of the birds negatively (Romashko, 1999). This accounted for the least profit returns accrued from their production. It is therefore more economical to rear broilers on DFM-supplementation in both feed and drinking water at the rate of 1.5 ml DFM per kg feed or litre drinking water.

4.2. Experiment 2

The impact of DFM supplementation in water and feed on growth and laying performance of layer birds (31-40weeks) are presented in Table 4.5

4.2.1 Feed intake, FCR, Egg production, HDP and HHP

The addition of probiotics to the feed or drinking water did not exert any significant influence ($P>0.05$) on feed intake. However, birds with 2.0 ml DFM administered via drinking water (T_5) consumed less feed (4752.3 g) compared to treatment one (T_1) the control birds with the highest consumption of 4860.9 g. Kim *et al.* (2002) stated that feed intake values were not statistically different among probiotics-fed groups and control. Though birds in treatment five (T_5) consumed the least feed, they recorded the best weight gain among all the other four treatments T_1, T_2, T_3, and T_4. Treatment five (T_5) gained 571.61 g with birds on control treatment (T_1) recording the least weight gain of (352.7 g). Although improvement in weight gain was observed in all the treated birds (T_2, T_3, T_4, and T_5), no significant difference was detected.

Table 4.5: **Effect of DFM in Feed and Water on Laying Performance (30-40 Weeks of Age)**

PARAMETER	TREATMENT					
	T1	T2	T3	T4	T5	SEM
Initial Body Weight (g/bird)	1488.30	1495.30	1514.30	1508.20	1526.70	28.33
Final Body weight (g/bird)	1841.00	1898.07	1922.75	2029.71	2098.31	42.6
Total Feed Intake (g/bird)	4860.9	4771.1	4768.0	4801.1	4752.3	854.7
Feed Gain Ratio	2.38	2.27	2.19	2.23	2.11	-
HHP(%)	43.0	42.7	44.3	45.3	46.7	3.54
HDP(%)	47.33	46.8	47.9	50.4	50.8	-
Mean Egg Weight	61.55	63.98	63.9	61.79	62.78	3.62
1^{s}(X-large) %	66.67	83.4	75.0	66.65	88.33	-
2nd (medium) %	33.33	16.6	25.0	33.35	33.67	-
Egg/ Albumen Height	9.36	9.34	9.90	9.68	9.82	0.397
Haugh Unit	93.12	94.73	95.13	94.35	94.29	1.027
Shell Thickness (mm)	0.29	0.31	0.30	0.31	0.32	0.024
Livability (%)	92.7	90	85	88.33	96.67	1.07

T_1 – (control) no DFM in feed and drinking water

T_2 - (control) 1.5ml DFM in feed but no DFM in drinking water
T_3- 1.0ml DFM in drinking water but no DFM in feed
T_4- 1.5ml DFM in drinking water but no DFM in feed
T_5- 2.0ml DFM in drinking water but no DFM in feed

Feed conversion ratio among all treatments (T_1, T_2, T_3, T_4 andT_5) did not show any significance ($P>0.05$). However, there was improvement in the FCR of water-treated birds (T_3, T_4 and T_5) as compared to birds fed DFM diet (T_2) and control birds (T_1). Treatments T_5, T_4 and T_3 recorded 2.11, 2.23, and 2.19 respectively; while 2.38 and 2.27 were respectively recorded for $T_1$and T_2. In a related study, Yalcin *et al.* (2008) observed no significant effect on feed intake, and feed conversion ratio. The water treated birds showed an improvement in egg production. The highest hen-housed egg

production percentage was for treatment T_5 (46.7%) with the highest level of DFM (2.0 ml), while T_2 recorded the least value of 42.7%

Mean egg weight was not significantly influenced by supplementing DFM in layer birds' water or feed, however DFM treated birds laid the largest eggs. All DFM treated birds (T_2, T_3, T_4 and T_5) had improved egg weights as compared to the control. Treatment 2, 3, 4 and 5 had 63.98 g, 63.96 g, 61.79 g and 62.78 g respectively compared to the control T_1 (61.55).This is in agreement with findings of Yoruk *et al.* (2004) and Kurtoglu *et al*. (2004). No significant effect of dietary probiotics supplementation on egg weight was recorded, however higher values of egg weight for birds fed probiotics were observed as compared to control birds.

Layers fed the DFM supplemented diet T_2 and those given 2.0 ml DFM via the drinking water T5 produced the highest number of extra large eggs (grade 1) 83.4% and 88.33% respectively than the other treatments (T_1, T_3 and T_4). This observation indicated that the use of DFM at the rate of 1.5 ml in feed and 2.0 ml in drinking water in layer birds resulted in a shift from smaller to larger eggs. The improved mean egg weight and size could be attributed to improved intestinal condition, nutrient retention and utilization (Nahashon *et al*., 1994).

Average egg production did not indicate significant ($P>0.05$) difference among all the treatments (T_1, T_2, T_3, T_4 and T_5). The hen-day production was numerically highest for birds given 2.0 ml DFM in water (50.8%) followed by birds on 1.5 ml DFM in water (50.4%), 1.0 ml DFM in water (47.9%) control (47.33%) and birds on 1.5 ml DFM in feed (46.8%). The low hen-day and hen housed values for all the treatments

(T_1, T_2, T_3, T_4 and T_5) was as a result of chronic respiratory disease (CRD) outbreak on the farm (*E. coli* infection). The 2.0 ml DFM administered via the drinking water of the birds in treatment five (T_5) increased the resistance of the bird's ability to colonize pathogenic species of the bacteria and also stimulated the bird's immune response.

From the hen-day results, values obtained were not different for birds in all treatments from week 30 to 36. The hen-day percentage of T_1 and T_2 started decreasing significantly relative to T_3, T_4 and T_5 (DFM in water treated birds). Though the results of T_5 was below performance, it registered the highest hen-day performances in week 36, 37, 39 and 40 compared to the other treatments, with T_1 (control with no DFM) recording the lowest value for the 5-week period (Table 4.6 and Figure.4.1). The improved performance of T_5 over birds in T_2, T_3 and T_4 was because of the high DFM inclusion level (2.0 ml) which enabled the good bacteria (DFM) compete against the colonization of the E. coli resulting in a better performance because the efficacy of probiotics depend on the administration level (Mountzouris *et al*., 2007). This is in agreement with the work of Higgins *et al*. (2008) where oral administration of 10^6 or 10^8cfu of Lactobacilli based probiotic significantly reduced *salmonella enteritidis* infection but lower dosage of the probiotic had no effect on the bacteria.

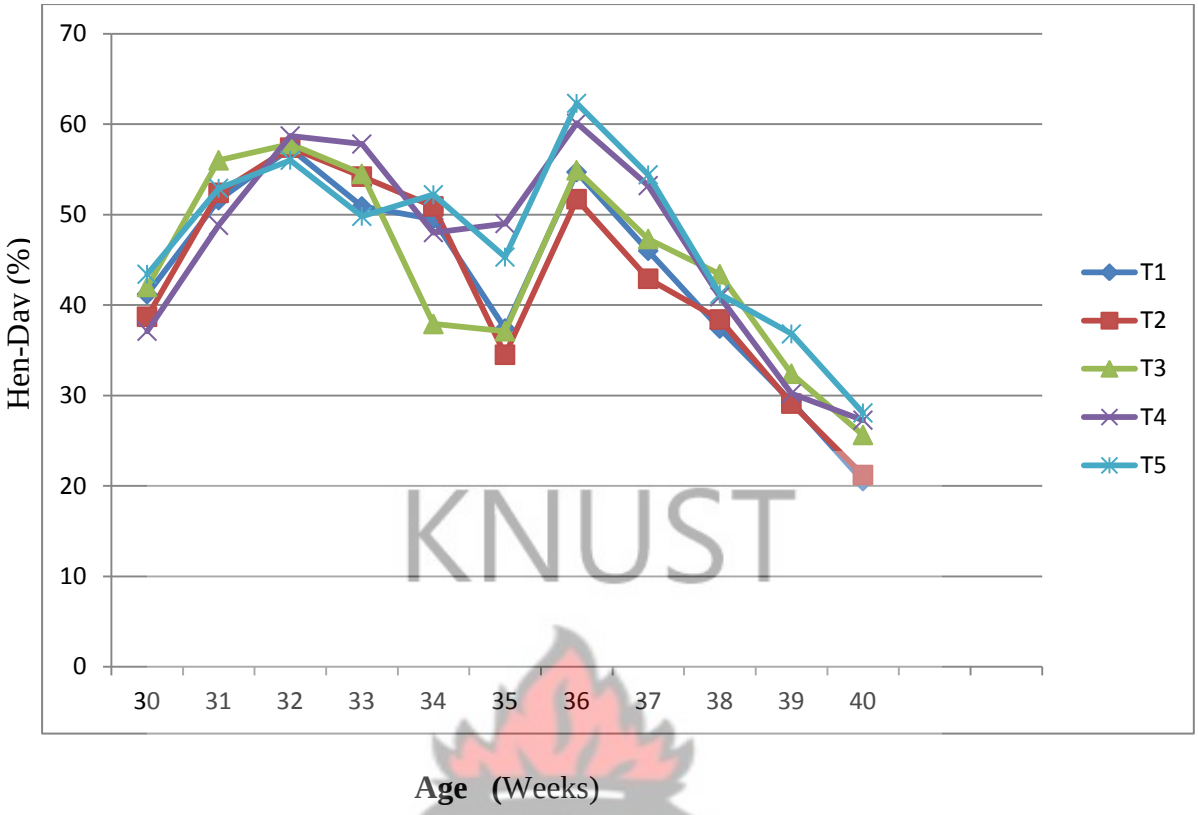

Figure 4.1 Hen-Day Production Curve of Layers Given DFM in Their Feed or Water**.**

Table 4.6: **Effect of DFM in Feed or Water on Weekly Hen-Day Percentage (30-40 Weeks of Age)**

	TREATMENT					
weeks	T_1	T_2	T_3	T_4	T_5	SEM
30	41.2	38.7	42.0	37.1	43.4	6.92
31	51.6	52.4	56.0	48.8	52.9	7.24
32	57.3	57.4	57.8	58.7	56.0	5.75
33	50.9	54.2	54.5	57.8	49.8	7.49
34	49.5	50.9	37.9	48.0	52.2	7.73
35	37.4^{ab}	34.5^{a}	37.1^{ab}	49.0^{a}	45.3^{ab}	5.43
36	54.7	51.7	54.9	60.1	62.3	5.58
37	46.0^{ab}	42.9^{b}	47.3^{ab}	53.2^{a}	54.4^{a}	3.88
38	37.4	38.4	43.4	40.9	41.2	4.17
39	29.3	29.1	32.4	30.2	36.8	3.50
40	20.53^{b}	21.2^{b}	25.63^{ab}	27.27^{a}	28.07^{a}	2.36

[ab]Values with different superscripts in row differ significantly (P<0.05)

Values obtained for the hen-housed production (HHP), which uses the number of hens present in the facility at the start of egg production as a constant denominator, can be used to derive a combined estimate of mortality and egg production that is a more accurate reflection of economic viability of flock performance. Although HHP showed no significant effect among dietary treatments, birds on treatment 5 recorded the highest value numerically as compared to the other treatments and were 8.6% better for chickens given DFM supplementation in water compared to the control birds. The improved hen-housed percentage for birds given 2.0 ml DFM in water was attributed to the least occurrence of mortality.

4.2.2 Egg quality

The results obtained for egg quality showed that there were no significant differences in the shell thickness, haugh unit and albumen height among all the treatments (T_1, T_2, T_3, T_4 and T_5). However, numerical improvement was observed in the shell thickness of T_5 and T_2 other than T_3, T_4 and control. Treatment 5 which contained the highest DFM level (2.0 ml) recorded the thickest shell (0.32 mm) followed by layers on dietary treatment with 1.5 ml DFM inclusion level (0.31 mm) in feed. All the other three treatments recorded 0.30 mm egg shell thickness each. Nahashon *et al*. (1994, 1996) in a work done with laying hens, observed that the addition of *Lactobacillus*-based DFM to the diet improved N, Ca and P retention. This probably accounted for the improvement observed in shell thickness of T_5. The result for egg quantity is in agreement with studies conducted by Daneshyar *et al.* (2010). Similar values for egg quality on 64- week-old broiler breeder birds were recorded and were attributed to the age of the birds.

The values obtained for egg albumen height did not differ significantly (P>0.05) among all treatments. However, there was improvement in the albumen height for birds whose drinking water was supplemented with DFM. Values obtained for haugh unit also did not differ for all the treatments. This indicates that the DFM supplementation in water or feed did not have an effect on the haugh unit of layers.

Lack of significant influence of DFM on hen performance may be due to the fact that the DFM was not supplemented early in the life of the birds (31weeks) because *lactobacilli* become established in the gut of most species of animals soon after birth (Naqi *et al.*, 1984)

4.2.3 Economics of Production

The cost of production per bird (feed, DFM, drugs) was high (GH¢ 3.955) for treatment five (T_5) due to the high DFM inclusion level of 2.0 ml, yet it recorded the highest value for total egg weight (2256.9). Treatment two (T_2), on the other hand, recorded the least cost of GH¢3.397 because the amount of DFM used depended on the feed intake, which was less as compared to those on the water treatment. Total egg weight for T_2 was 2100.5 g and was least among all the other treated birds, (T_3, T_4, T_5), with 2174.5 g, 2152.1 g and 2256.9 g respectively. The control group, on the other hand, showed a relatively high cost but recorded the least total egg weight (2034.2). Though eggs and birds were not sold and the work lasted for only 10 weeks, with the information provided in Table 4.7 (total cost, total egg weight, egg laid and mortality recorded), it can be concluded that rearing layers with added DFM in water is economical.

Table 4.7: **Cost -Benefit Assessment for Layer Chickens (30-40 weeks of Age)**

DESCRIPTION	T_1	T_2	T_3	T_4	T_5
Total feed intake31-41weeks	4860.9	4771.1	4768.0	4801.1	4752.3
Unit cost of feed	0.6895	0.6895	0.6895	0.6895	0.6895
Total feed cost	3.3516	3.2897	3.2875	3.3104	3.2767
Cost of drugs	0.4	-	-	-	-
Total water intake	22.88	23.95	22.17	22.95	22.58
Amount of DFM used	-	7.16	22.17	34.42	45.17
Per unit cost of DFM (mm)	-	0.015	0.015	0.015	0.015
Cost of DFM	-	0.107	0.333	0.516	0.678
Total cost (feed, drug & DFM)	3.752	3.397	3.621	3.826	3.955
Egg laid	33.05	32.83	34.03	34.83	35.95
Mean Egg weight	61.55	63.98	63.9	61.79	62.78
Total egg mass	2034.2	2100.5	2174.5	2152.1	2256.9

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. The results of these studies show that DFM administered via drinking water can be used in broilers without any deleterious effects on their general performance or carcass characteristics. It can render the production more efficient and economical.
2. Results obtained suggest that DFM administered in water can be used for layers without any adverse effect. DFM inclusion in layer drinking water at level 2.0 ml/litre renders layers more efficient, improves egg weight and size better than control.
3. DFM administered via drinking water of broilers and layers can be used in hot humid environments (Ghana) and also serve as a good alternative to the use of Direct-Fed Microbials (DFM) administered in feed. DFM administered via drinking water is therefore recommended for use in broiler and layer birds.

5.2 Recommendations

1. Further research work involving supplementation of DFM in water from day old to market weight for broilers and to end of lay for layers needs to be carried out to ascertain the long term effect of DFM administered via drinking water in both layers and broilers. This is because to obtain maximum result, probiotics must be administered when birds are newly hatched and the intestinal tract is colonized by a broad spectrum of micro-organism at that early age.

2. The administration of DFM via water intermittently, instead of daily mix, is also suggested to assess its economical and production benefits since intermittent use of DFM means less use of DFM and reduced cost of production.

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APPENDICES

Appendix 1: Egg quality for the experimental period

	T1	T2	T3	T4	T5	SEM
Egg weight(g)	61.55	62.23	61.89	60.37	62.01	3.62
Haugh	93.12	94.73	95.13	94.35	94.29	2.722
Albumen height(mm)	8.776	9.118	9.182	8.962	9.020	0.680
Shell thickness(mm)	0.3001	0.3064	0.2984	0.2992	03117	0.024

Appendix 2: Egg weight for the experimental period (g)

WEEKS	T1	T2	T3	T4	T5
34	62.76	63.80	60.28	59.91	62.98
36	61.10	60.60	62.10	61.10	62.40
38	61.95	63.98	63.96	61.76	62.78
40	60.42	60.55	61.23	58.72	59.92

Appendix 3: Mortality for the experimental period

WEEKS	T1	T2	T3	T4	T5
30 – 40	1	1	1	2	1

Appendix 4: Percent hen day production over the experimental period

WEEKS	T1	T2	T3	T4	T5	SEM
30	41.2	38.7	42.0	37.1	43.4	6.92
31	51.6	52.4	56.0	48.8	52.9	7.24
32	57.3	57.4	57.8	58.7	56.0	5.75
33	50.9	54.2	54.5	57.8	49.8	7.49
34	49.5	50.9	37.9	48.0	52.2	7.73
35	37.4^{ab}	34.5^{a}	37.1^{ab}	49.0^{a}	45.3^{ab}	5.43
36	54.7	51.7	54.9	60.1	62.3	5.58
37	46.0^{ab}	42.9^{b}	47.3^{ab}	53.2^{a}	54.4^{a}	3.88
38	37.4	38.4	43.4	40.9	41.2	4.17
39	29.3	29.1	32.4	30.2	36.8	3.50
40	20.53^{b}	21.20^{b}	25.63^{ab}	27.27^{a}	28.07^{a}	2.36

Appendix 5: ANOVA Table for performance parameters (broilers)

Table 1: Average daily Gain

Variate: ADG

Source of variation	DF	Sum of Squares	m.s.	v.r.	F pr.
REP stratum	2	2.874	1.437	1.00	
TREATMENT	3	2.260	0.753	0.52	0.682
Residual	6	8.636	1.439		
Total	11	13.771			

Table 2: Average daily feed intake

Variate: DI

Source of variatio	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	23.139	11.570	1.51	
TREATMENT	3	13.181	4.394	0.57	0.654
Residual	6	46.072	7.679		
Total	11	82.393			

Table 3: Feed conversion efficiency

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	4985.	2492.	0.51	
TREATMENT	3	3133.	1044.	0.21	0.883
Residual	6	29226.	4871.		
Total	11	37343.			

Table 4: Initial weight

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	160.8	80.4	0.42	
TREATMENT	3	44.6	14.9	0.08	0.970
Residual	6	1147.0	191.2		
Total	11	1352.4			

Table 5: Total feed intake

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	29988.	14994.	1.51	
TREATMENT	3	17083.	5694.	0.57	0.654
Residual	6	59710.	9952.		
Total	11	106781.			

Table 6: Total weight gain

Variate: T_W_G

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	3725.	1863.	1.00	
TREATMENT	3	2929.	976.	0.52	0.682
Residual	6	11193.	1865.		
Total	11	17847.			

Table 7: Final body weight

Variate: final_wt

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	4570.	2285.	1.12	
TREATMENT	3	3663	1221.	0.60	0.638
Residual	6	12186.	2031.		
Total	11	20418.			

Table 8: Mortality

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.733	0.867	0.51	
TREATMENT	4	8.400	2.100	1.24	0.369
Residual	8	13.600	1.700		
Total	14	23.733			

Appendix 6: ANOVA Tables for blood Parameters (broilers)

Table 1:Heamoglobin

Variate: Hb

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	1.1587	0.5794	4.03	
TREATMENT	3	0.5040	0.1680	1.17	0.397
Residual	6	0.8629	0.1438		
Total	11	2.5256			

Table 2: Haematocrit

Variate: HCT

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	0.900	0.450	0.20	
TREATMENT	3	4.106	1.369	0.62	0.628
Residual	6	13.266	2.211		
Total	11	18.272			

Table 3: Platelet

Variate: PLT

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	2.0417	1.0208	9.80	
TREATMENT	3	1.5000	0.5000	4.80	0.049
Residual	6	0.6250	0.1042		
Total	11	4.1667			

Table 4: Red blood cells

Variate: RBC

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	87.78	43.89	0.97	
TREATMENT	3	131.71	43.90	0.97	0.464
Residual	6	270.31	45.05		
Total	11	489.80			

Table 5: White blood cell

Variate: WBC

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	13.62	6.81	0.30	
TREATMENT	3	136.73	45.58	2.02	0.212
Residual	6	135.21	22.53		
Total	11	285.56			

Appendix 7: ANOVA Table for carcass characteristics (broilers)

Table 1: Defeathered weight

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
REP stratum	2	62494.	31247	2.74	
TREATMENT	3	11209	3736.	0.33	0.806
Residual	6	68320	11387.		
Total	11	142023			

Table 2: Dressing percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.500	0.750	0.25	
TREATMENT	3	1.896	0.632	0.21	0.883
Residual	6	17.667	2.944		
Total	11	21.062			

Table 3: Fat accumulation Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.2	0.6	0.00	
TREATMENT	3	476.4	158.8	0.78	0.546
Residual	6	1217.8	203.0		
Total	11	1695.4			

Table 4: Gizzard weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	180.38	90.19	2.77	
TREATMENT	3	235.73	78.58	2.41	0.165
Residual	6	195.46	32.58		
Total	11	611.56			

Table 5: Heart weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.375	1.188	0.50	
TREATMENT	3	15.896	5.299	2.22	0.186
Residual	6	14.292	2.382		
Total	11	32.562			

Table 6: Intestine weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.3	1.6	0.01	
TREATMENT	3	193.2	64.4	0.49	0.705
Residual	6	796.7	132.8		
Total	11	993.2			

Table7: Liver weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	29.29	14.65	0.47	
TREATMENT	3	57.50	19.17	0.61	0.632
Residual	6	188.38	31.40		
Total	11	275.17			

Table 8: Live weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	81896.	40948		
TREATMENT	3	15405.	5135	0.33	0.803
Residual	6	92754.	15459.		
Total	11	190055.			

Table 9: Slaughter weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	57751.	28876.	2.05	
TREATMENT	3	15297	5099.	0.36	0.783
Residual	6	84571.	14095.		
Total	11	157619.			

Appendix 8: ANOV Table for performance parameters (layers)

Table 1: Hen house

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3040	1520.	0.83	
TREATMENT	4	3293.	823.	0.45	0.770
Residual	8	14627	1828.		
Total	14	20960.			

Table 2: Total feed intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.249E+07	3.624E+07	0.33	
TREATMENT	4	4.566E+08	1.142E+08	1.04	0.443
Residual	8	8.767E+08	1.096E+08		
Total	14	1.406E+09			

Table 3: Initial body weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3677.	1838.	1.53	
TREATMENT	4	2777.	694.	0.58	0.688
Residual	8	9634.	1204		
Total	14	16087			

Table 4: Egg albumen height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.7809	0.3904	1.65	
TREATMENT	4	0.8113	0.2028	0.86	0.527
Residual	8	1.8881	0.2360		
Total	14	3.4803			

Table 5: Shell thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0002401	0.0001201	0.15	
TREATMENT	4	0.0010697	0.0002674	0.33	0.847
Residual	8	0.0063879	0.0007985		
Total	14	0.0076977			

Table 7: Haugh unit

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.994	0.497	0.31	
TREATMENT	4	6.792	1.698	1.07	0.430
Residual	8	12.662	1.583		
Total	14	20.447			

Table 8: Mean egg weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	6.451	3.225	1.01	
TREATMENT	4	6.453	1.613	0.51	0.734
Residual	8	25.547	3.193		
Total	14	38.451			

Table 9: Feed intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.249E+07	3.624E+07	0.33	
TREATMENT	4	4.566E+08	1.142E+08	1.04	0.443
Residual	8	8.767E+08	1.096E+08		
Total	14	1.406E+09			

Appendix 9: ANOVA Table for hen day (wk. 30-40)

Table 1: Hen day wk 30

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	376.71	188.35	2.62	
TREATMENT	4	76.61	19.15	0.27	0.891
Residual	8	574.87	71.86		
Total	14	1028.19			

Table 2: Hen day wk 31

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	393.47	196.73	2.50	
TREATMENT	4	79.54	19.88	0.25	0.900
Residual	8	629.57	78.70		
Total	14	1102.57			

Table 3: Hen day wk 32

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	34.15	17.07	0.34	
TREATMENT	4	12.10	3.03	0.06	0.992
Residual	8	397.16	49.65		
Total	14	443.41			

Table 4: Hen day wk 33

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	122.93	61.47	0.73	
TREATMENT	4	122.84	30.71	0.36	0.827
Residual	8	673.66	84.21		
Total	14	919.44			

Table 5: Hen day wk 34

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	113.13	56.56	0.63	
TREATMENT	4	388.39	97.10	1.08	0.426
Residual	8	717.25	89.66		
Total	14	1218.77			

Table 6: Hen day wk 35

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	194.79	97.39	2.21	
TREATMENT	4	458.15	114.54	2.59	0.117
Residual	8	353.24	44.16		
Total	14	1006.18			

Table 7: Hen day wk 36

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.33	0.16	0.00	
TREATMENT	4	223.98	56.00	1.20	0.382
Residual	8	374.12	46.77		
Total	14	598.44			

Table 7: Hen day wk 37

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	313.11	156.55	6.93	
TREATMENT	4	286.84	71.71	3.17	0.077
Residual	8	180.79	22.60		
Total	14	780.73			

Table 8: Hen day wk 38

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	244.42	122.21	4.68	
TREATMENT	4	68.64	17.16	0.66	0.639
Residual	8	208.96	26.12		
Total	14	522.02			

Table 9: Hen day wk 39

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	64.83	32.41	1.76	
TREATMENT	4	122.43	30.61	1.66	0.250
Residual	8	147.13	18.39		
Total	14	334.38			

Table 10: Hen day wk 40

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	45.292	22.646	2.73	
TREATMENT	4	144.829	36.207	4.36	0.037
Residual	8	66.415	8.302		
Total	14	256.536			

Appendix 10: ANOVA Table for layer Mortality (wk. 30-43)

Table 1: Mortality of layers (wk. 30-40)

Source	d.f	s.s	m.s	v.r	F pr.
Model	4	0.26666667	0.06666667	0.20	0.9327
Error	10	0.33333333	0.33333333		
Corrected Total	14	3.60			

Table 2: Mortality of layers (wk.41-43)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Model	4	11.33333333	2.83333333	2.36	0.1233
Error	10	12.00000000	1.20000000		
Corrected Total	14	23.33333333			