EFFICACY OF PROBIOTICS IN KENYA ON GROWTH, FEED INTAKE, EFFICIENCY IN BROILERS AND IMMUNE RESPONSE AND THEIR ANTIBIOBIC PROPERTIES

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this thesis is my original work and has not been presented in this or any other University for the award of a degree.

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Recommendation

This thesis has been presented with our approval as the university supervisors for examination according to Egerton regulations.

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DEDICATION

This work is dedicated to the Almighty God, my husband, parents and friends.

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ABSTRACT

This study was aimed at assessing the extent to which probiotics are used in the poultry feed industry in Kenya and their efficacies in broiler diets. In experiment 1, two surveys involving 100 Agro Vets and 36 Poultry farmers were carried out. 16 products were being sold as probiotics in Agro Vets in Kiambu County. The actual trade names of the probiotics are coded as Products 1 to 16 (see Appendix 3). It was found that 74.4% of the farmers used probiotics. The most commonly used by farmers were Products 1, 2, 4 and 7 at 23.1 %, 12.8 % 20.5 % and 15.4 % respectively. In the second experiment, 307, day-old broiler chicks were randomly assigned to 5 dietary treatments and fed for 42 days broken into two phases (starter phase, 0-21 days: finisher phase 22-42 days). These diets were; Diet 1 (Control with no probiotic), Diet 2 (with Product 1), Diet 3 (with Product 4), Diet 4 (with Product 7) and Diet 5 (with Product 2). During the finisher phase, the 5 diets were identified as diets 6-10 where the only differences were the levels of CP being 21.9% and 19.8% for starter and finisher diets respectively. The data were analyzed using GLM procedures of SAS version 9.00. The LSD method at a level of ($P<0.05$) was used to separate means. The results showed that probiotics had no effect ($P>0.05$) on daily gain, feed intake and feed efficiency during both the starter and finisher phases except that Product 2 depressed growth. In this experiment, blood samples were collected to measure the effects of probiotics on antibody response to Infectious Bursal Disease virus and the results showed no significant effect ($P=0.6868$) associated with probiotics. Additionally, the antibiotic properties of the probiotics were tested using the disk diffusion test by measuring the inhibitory effects on *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Candida albicans* bacterial cultures. Two of the probiotics (Products 4 and 7) showed inhibitory effects on the cultures indicating that they either produced antibiotic compounds or that antibiotics had been added to these products. It is concluded that probiotics are used in Kenya although their inclusion in this experiment had no effect to performance and immune responses. However, two of the probiotics tested had antibiotic properties and research should be carried out to establish the origin of the antibiotic properties. Furthermore, more in depth studies should be undertaken to not only establish the micro-organisms in the probiotics and their effects on immune response while examining the mucosal, cellular and humoral immunities under stressful conditions.

Key words: probiotics, growth feed efficiency, broilers, antibody response

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LIST OF ABBREVIATIONS

AGP	Antibiotic Growth Promoters
BW	Body Weight
CAFO	Concentrated Animal Feeding Operations
CRD	Complete Randomized Design
FCR	Feed Conversion Ratio
FOS	Fructo- Oligosaccharides
GDP	Gross Domestic Product
GIT	Gastrointestinal Tract
GLM	General Linear Models
IBD	Infectious bursal disease
KALRO	Kenya Agricultural Livestock and Research Organization
LSD	Least Significant Difference
MOS	Mannan-Oligosaccharide
ND	Newcastle Disease
NGP	Natural Growth Promoters
SAS	Statistical Analysis Systems
VRE	Vancomycin-resistant enterococci

CHAPTER ONE
INTRODUCTION

1.1 Background information

Domestic animals have become important in their contribution to food production especially animal protein, hence making the livestock sector one of the fastest growing agricultural sectors worldwide. Livestock products account for about 30 percent of the global value of agriculture and 19 percent of the value of the food production, and provide 43 percent of the protein and 16 percent of the energy consumed in human diets (Gamsworthy and Wiseman, 2008).

In the Kenyan economy, the agricultural sector accounts for approximately 22% of the country's Gross Domestic Product (GDP) with the livestock sector contributing 12% of the GDP and 42% of agricultural GDP (Ministry of Livestock and Fisheries Development, 2008). This mainly comprises of dairy products, meat, eggs, hides, skins and wool from cows, sheep, goats and poultry.

Over the years, livestock production has evolved significantly from integrated farming to intensive systems of production, with respect to both large number of animals and feeding systems (Udo *et al.,* 2011). The use of antibiotic growth promoters (AGP), developed concurrently with the intensification of the livestock industry. Large amounts of antibiotics have been used to control diseases, improve performances and increase production in livestock, despite their poor living conditions.

The sub-therapeutic use of antibiotics as growth promoters in animal feed evoked global concern of the increasing prevalence of antimicrobial-resistant bacteria, associated with human and animal diseases. This led to the ultimate prohibition on the use of antibiotics as growth promoters in animal feed in January 1, 2006 by the European Union (European Union Commission, 2005), followed by other parts of the world (Yegani *et al.*, 2008). Since 2010, the Kenyan Government has prohibited the use of chloramphenicol and nitro furans in food producing animals. The possibility of antibiotics ceasing to be used as growth stimulants for farm animals and the concern on the side-effects of their use as therapeutic agents has led to an increase in research on the alternative supplements to AGP and strategies for food-producing animals (Dong *et al*., 2007).

Currently natural growth promoters such as, essential oils and plant extracts, spices, probiotics, prebiotics, synbiotics, enzymes, toxic binders, organic acids, oligosaccharide and

phytogenics, have been recognized and proposed as antibiotic alternatives in farm animal nutrition.

1.2 Statement of the problem

As a result of the prohibition in use of antibiotics, Kenyan poultry farmers have resulted to using Natural Growth Promoters (NGP) such as probiotics and prebiotics as effective and safe alternatives to AGPs. At present, there are a number of NGPs available in the market. However, there is limited information regarding the availability and usage of probiotics in the Kenyan feed industry and their efficacy in terms of performance and immune response in broilers.

1.3 Objectives

1.3.1 Main objective

To contribute to improved poultry production through use of probiotics in broiler diets.

1.3.2 Specific objectives

a) To determine the extent to which probiotics are used in the Kenyan poultry feed industry.
b) To determine the efficacy of probiotics on broiler performance.
c) To determine the effect of probiotics on antibody production against infectious bursal disease.
d) To evaluate antibiotic properties of common probiotics used in the study.

1.4 Hypotheses

a) There is no significant difference in performance between chicks supplemented with or without probiotics.
b) There is no significant difference in performance within chicks supplemented with different types of probiotics.
c) There is no significant difference in antibody production between chicks supplemented with or without probiotics
d) The probiotics used in the study do not have antibiotics and or antibiotic properties.

1.5 Justification

Probiotics are live microorganisms that are non-pathogenic and non-toxic in nature and when administered through the digestive route are favorable or beneficial to the host's health. Numerous probiotic products are on the market, while some products clearly have potential,

for others their efficacacies are not conclusive. There is therefore a need to determine their efficacy in terms of performance and immune response in broilers with specific reference to Kenya.

1.6 The scope and limitations of the study

The survey on the use of probiotics was carried out in Kiambu County. The feeding trial using unsexed broiler chicken was carried out in the Kenya Agricultural Research and Livestock Organization (KARLO) Centre in Naivasha under controlled conditions. The probiotics used were not sourced directly from manufacturers but rather from Agro-vet retail shops and hence their shelf life was undetermined. The bacterial composition of the probiotics was not determined and only the information on the label was used. These limitations might have had an effect on the outputs of the present study

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CHAPTER TWO
LITERATURE REVIEW

2.1. Introduction

Worldwide urban populations are expected to grow in proportion from the current 50 percent to 69 percent by 2050 (UNDP, 2009). Increased urbanization and changes in lifestyle has altered the patterns of food consumption and increased the demand for livestock products, which has been driven by various factors like; product availability, product type, increasing incomes in developing countries and population growth (Delgado, 2005). The urban population in Kenya has constantly increased from 285, 000 in 1948 to 12 million in 2009 and continues to grow (National Council for Population and Development, 2012). This presents a challenge to the livestock industry which has been forced to adapt and expand to satisfy the expectations of society. Production not only requires increased quantity of livestock products, but also improved quality, as a wider variety and higher quality products are desired in many countries or regions.

Population growth has become a major driving force to the rapid changes in livestock production systems (Thornton, 2010) and resulted in intensification of livestock production, a response to the increasing demand for livestock products. Intensive livestock farming has increased food production at a low cost per unit produced but has led to an increase in stress that animals are subjected to, and has resulted to a decrease in hygiene and sanitation, decreased productivity and immune function and emergence of infections and diseases (Udo *et al.,* 2011).

The use of antibiotics to improve growth and feed efficiency has developed concurrently with the intensification of the livestock industry worldwide. Antibiotics are used as therapeutic agents to treat bacterial diseases in intensive farming systems and also to counteract the adverse consequences of stress responses.

2.2. The use of antibiotics in the livestock industry.

The livestock industry has undergone a dramatic change that began around 1950 worldwide. This is as a result of the increasing demand for livestock products which are a major source of protein. What was an extensive industry became extremely intensive with units increased in animal concentration, both physically and numerically. In the United States, farmers maintain poultry in large flocks in one location, concentrated animal feeding operations (CAFOs), where about 10,000 to 20,000 broilers are typically raised in one house and some

operations have as many as a million laying hens in one location. With such a concentration of broilers it is essential to control diseases hence prevent disastrous losses (Thomas *et al.*, 2015). Evidence on antibiotic use in farm animals indicates that these medicines are used primarily (90%) as standard disease preventions and treatment (Kariuki *et al.*, 2013).

It has been proposed that the use of antibiotics as a feed additive was an integral part of this revolution in animal-production technology for therapeutic treatment, disease prophylaxis and growth promotion. A review paper by Sinovec and Radmila (2005) outlined some of the salient features of Antibiotic Growth Promoters (AGPs) in poultry production. They modify the intestinal micro flora and help to improve bird's performance and health status. They also have inhibitory effect on enzymes released by microorganisms and also on enzymes involved in microbial metabolism. AGPs have been proven to reduce the growth-depressing metabolites produced by microorganisms.

Addition of antibiotics to feed increases the amino acid levels in the gut and improves nitrogen balance and absorption of feed nutrients because of thinning of intestinal wall. They also have been proven to increase egg production and hatchability in layers and reduce stress and mortality in chicks by boosting body defense. They reduce the damage caused by dietary fluctuations and destroy the harmful bacteria, keeping and minimize the adverse effects of dietary changes. Antibiotics prevent exponential multiplication of common pathogenic bacteria (*E. coli, Salmonella spp., Streptococcus spp and Hemophilus*) hence reducing incidences of non-specific diarrhea or enteritis of chicken and reduce the microbial use of nutrients.

Global consumption of antimicrobials in food animal production was estimated at 63,151 (±1,560) tons in 2010. Two thirds (66%) of the global increase in antimicrobial consumption is due to the growing number of animals raised for food production while the remaining third (34%) is as a result use of intensive farming systems for livestock production. By 2010, the five top countries in terms of antibiotic consumption for animal feeding were China (23%), the United States (13%), Brazil (9%), India (3%), and Germany (3%) (Thomas *et al.*, 2015).

Table 2.1 Table showing the use of AGPs in China.

Antibiotic Class	Poultry (kg)	Swine (kg)
Tetracycline	613,120	16,336,823
Penicillin	61,312	1,737,362
Macrolides	164,985	3,209,370
Coccidiostats*	3,407,220	-
Arsenicals*	2,879,624	556,716
Sulfonamides	-	4,457,557
Aminoglycosides	-	129,537

* Many arsenical compounds function as coccidiostats. (Krishnasamy *et al.*, 2015).

In South Africa, a study revealed an extensive use of antimicrobials in poultry production with tylosin being used at 42.4% of the total volume sold, tetracycline at 16.7%, sulphonamides at 12.4%, penicillin at 10.7% and cephalosporin, which belong to the class of antimicrobials known as beta-lactams (Eagar *et al.*, 2012). In many developing countries, majority of the antibiotics used in poultry are for treatment of infections and also to counteract the adverse consequences of stress responses.

In Kenya, Wanjiru (2014) reported that antibiotics were widely used in poultry production in Kiambu County (Table 2.2) and were readily accessible to farmers. It was further reported that farmers administered antibiotics without the assistance of professional veterinary personnel.

Table 2.2 Summary of the response to different classes of antibiotics used in poultry production in Kikuyu and Gatundu North sub-counties, Kiambu County.

Class of antibiotics	Broilers			Layers		
	Kikuyu District	Gatundu North District	Overall	Kikuyu District	Gatundu North District	Overall
	N	N	N	N	N	N
Tetracycline	59 (73.8%)	30 (50.8%)	89 (64%)	56 (53.8%)	53 (51%)	109 (52.4%)
Sulfonamides	19 (23.8%)	16 (27.1%)	35 (25.2%)	41 (39.4%)	39 (37.5%)	80 (38.4%)
Quinolones	2 (2.5%)	9 (15.35%)	11 (7.9%)	3 (2.9%)	11 (10.6%)	14 (6.7%)
Nitro furans	0	4 (6.8%)	4 (2.9%)	4 (3.8%)	1 (1%)	5 (2.4%)
Total	80	59	139	104	104	208

N, the total number of responses given by poultry producers (Wanjiru, 2014).

2.2.1. Emergence of antibiotic resistant bacteria

Apart from positive effects, there were negative and harmful effects of AGPs that developed with time such as production of resistant strains of bacteria. This occurred when an antibiotic was continuously used in animal feed over a period of time and began to eliminate the sensitive bacterial strains hence selecting those variants with unusual traits that can resist it. These resistant bacteria then multiplied and became the predominant micro-organism in the gut population.

Resistant bacteria hinder antibiotic treatment by interfering with their mode of action via a range of effectors' mechanisms including synthesis of inactivating enzymes, alteration of the specific configuration of target sites and inhibition or changes in membrane transport systems to remove the antibiotic (Apata, 2009). Poultry products and meat are common reservoir of antibiotic residues which cause resistances in pathogenic bacteria strains and also in commensal bacteria inhabiting humans (Lukasova and Sustackova, 2003). The emergence and spread of resistant bacterial strains like *Campylobacter* sp, *Escherichia coli* and *Enterococcus* sp. from poultry products to consumers put humans at risk to new strains of bacteria that resist antibiotic treatment and hence certain essential life-saving antimicrobials

are becoming less effective. There are fewer alternatives available to treat the diseases for which these antimicrobials are required.

A study done by Fallon *et al*. (2003) on antibiotic susceptibility of *Campylobacter jejuni* and *Campylobacter coli*, showed that of the 78 *Campylobacter jejuni* isolates, the highest level of resistance was recorded to ampicillin (35.9%), tetracycline (20.5%) naladixic acid (20.5%), ciprofloxacin (17.9%), erythromycin (10.5%), streptomycin (2.5%) and kanamycin (1.2%). Chloramphenicol was found to be active against all the *Campylobacter jejuni* strains and 46.2% of these strains were sensitive to all the eight antibiotics agents studied. Collignon (2003) reported that use of the antibiotic avoparcin as a growth promoter in livestock production in Europe resulted in the development of vancomycin-resistant enterococci (VRE) and the colonization of a significant percentage of the human population via the food chain. Similarly, Waste from poultry raised in industrial chicken houses was found to contain bacteria with antibiotic resistant genes (Cardinale, *et al.,* 2005).

This was associated with the presence of growth promoters in the feeds. In Kenya, the bacterial infections that contribute most to human disease are often those in which resistance is most evident. Examples are multidrug resistant enteric bacterial pathogens such as typhoid, diarrhoeagenic *Escherichia coli* and invasive non- typhi salmonella, penicillin resistant Streptococcus Pneumoniae, vancomycin resistant enterococci, methicillin resistant Staphylococcus Aureus and multidrug resistant Mycobacterium tuberculosis (Global Antibiotic Resistance Partnership, 2011). A study carried out on "Comparative study of anti-biotic resistance profiles among enteric bacteria in broiler and traditional chicken" in Kericho County showed that the preference of resistance was recorded in *E.coli* isolated from broiler and indigenous chicken. It also stated that the source of antibiotic resistance was from the immediate environment of the chicken as a result of misuse of antibiotics during treatment and control of diseases (Mutsami, 2011).

However, the increasing risk of prevalence of antimicrobial-resistant bacteria in both humans and livestock, linked to the use of antibiotics in animal production, led to the ban of the use of antibiotics as growth promoters in animal feed in 2006 by the European Union. The possibility of antibiotics ceasing to be used as growth stimulants for farm animals and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumer and manufacturer are looking for alternatives.

2.3. Probiotics as alternatives to antibiotics

Probiotics have stood out as the alternatives that are able to maintain high productivity and to be economically feasible, as well as not being harmful to human and animal health, thereby complying with the requirements of consumers and foreign markets. Probiotics were defined as "a preparation of viable microorganisms that is consumed by humans or other animals with the aim of inducing beneficial effects by qualitatively influencing their gut microflora and/or modifying their immune status (Fuller, 2004). According to the currently adopted definition by (FAO/WHO, 2009) probiotics are "live microorganisms which when administered in adequate amounts, confer a health benefit on the host".

In the wild state the animal obtains its gut flora from its immediate environment which is heavily contaminated with bacteria from the mother. Prior to hatch or birth, the gastrointestinal (GI) tract of poultry and swine is sterile (Kelly and King, 2001). Bacteria from the environment, the mother (in case of mammals), and the diet begin to colonize the GI tract almost immediately. The final indigenous gut microflora which stabilizes in the gut is a very complex collection of about 10^{14} micro-organisms consisting of 400 different types of bacteria. The composition of the flora is determined by the host and microbial factors and not only do the successful ones have to run the gauntlet of the antimicrobial chemicals present in the gut, but they also have to avoid the effects of peristalsis which tends to flush out bacteria with the food. This can be done either by immobilizing themselves by attaching to the gut wall, or by growing at a rate which is faster than the rate of removal by peristalsis.

The survival of probiotic organisms in the gut depends on their possessing colonization factors which enable them to resist the antibacterial mechanisms (chemical and physical) which operate in the gut. The stable flora which develops in the intestine helps the animal to resist infections, particularly in the gastrointestinal tract. The protective flora which establishes itself in the gut is very stable, but it can be influenced by some dietary and environmental factors which include: excessive hygiene, antibiotic therapy and stress.

These conditions, where the balance of the gut microflora is adversely affected, are all situations in which probiotics are of potential value. The delivery of large numbers into the lower gut may be achieved either by feeding large numbers of viable cells continuously (e.g. as with yoghurt where the bacteria are non-intestinal and do not grow in the gut) or by restricted dosing with an intestinal strain which will colonize the gut and become self-replicating.

However, the composition of this protective flora can be altered by dietary and environmental influences, making the host animal susceptible to disease and/or reducing its efficiency of food utilization. Probiotics are selected to presumably withstand the gastro-intestinal environment and adhere to the intestinal epithelium.

2.3.1. Classification of micro-organisms used as probiotics.

a) Bacterial and non-bacterial probiotics: Most of the micro-organisms used are bacteria and examples of bacterial probiotics are several species of *Lactobacillus* (Mookiah *et al.,* 2014), *Bifidobacterium* (Salehimanesh *et al.,* 2016), *Bacillus* (Rahman *et al.,* 2013), and *Enterococcus* (Sarangi *et al.,* 2016). Non-bacterial (yeast or fungal) probiotics include *Aspergillus oryzae*, *Candida pintolopesii* (Daskiran *et al.,* 2012), *Saccharomyces bourlardii*, (Rahman *et al.,* 2013) and *Saccharomyces cerevisiae* (Yousefi and Karkoodi, 2007).

b) Spore forming and Non-spore forming probiotics: Although non-spore forming *Lactobacillus* and *Bifidobacterium* strains predominated initially, spore forming bacteria include; *Bacillus subtilis* and *Bacillus amyloliquefaciens* (Chen *et al.,* 2005, Ahmed *et al.,* 2014).

c) Multi-species (or multi-strain) probiotics and Single-species (or single-strain) probiotics: The microbial composition of probiotic products ranges from a single strain to multi-strain or species compositions. Examples of multi-species probiotics are Khaksefidi and Rahimi (2005) who used a probiotic containing similar proportions of six strains of variable organisms namely *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis sps.* Micro guard (contains various species of *Lactobacillus, Bacillus, Streptococcus, Bifidobacterium* and *Saccharomyces* (Rahman *et al.,* 2013). Timmerman *et al.* (2006) who used a multispecies (MSPB) probiotic preparation of a combination of 6 strains: *Lactobacillus acidophilus* W55, *Lactobacillus salivarius* W57, *Lactobacillus casei* W56, *Lactobacillus plantarum* W59, *Lactococcus lactis* W58, and *Enterococcus faecium* W54 and a chicken-specific (CSPB) probiotic preparation of *Lactobacillus* strains in fluid form.

d) Allochthonous probiotics and Autochthonous probiotics: The micro-organisms used as probiotics which are normally not present in the GIT of animals are referred to as allochthonous (yeasts), while the micro-organisms normally present as indigenous

inhabitants of the GIT are referred to as autochthonous probiotics (*Lactobacillus* and *Bifidobacterium*) (Timmerman *et al.*, 2006).

Probiotics have been reported to work through various modes like; suppression of viable count of bacteria populations by production of antibacterial compounds and competition for nutrients and adhesion sites which suppress growth of the bacteria colonies along the gut. Another mode of action is the alteration of microbial metabolism by increasing or decreasing the enzyme activity in the gut. Probiotics also stimulate the immunity of the host by increasing the antibody levels and macrophage activity.

A good probiotic should be a strain which is capable of exerting a beneficial effect in the form of growth promotion or increased resistance to disease. It should be non-pathogenic and non-toxic and be present as viable cells, preferably in large numbers, although the minimum effective dose has not yet been determined. It should also be capable of surviving and metabolizing in the gut environment, e.g. resistant to low pH and organic acids. Finally, it should be stable and capable of remaining viable for long periods under storage and field conditions (Patterson and Burkholder, 2003).

2.3.2. Effects of probiotics on performance in livestock.

Several studies on probiotics have been carried out and have shown positive results on the effects of probiotics on feed intake and feed conversion efficiency in the livestock industry. Eman *et al.* (2014) observed that the inclusion of probiotics in broiler diets enhanced immune status and improved humoral immune response against NDV and IBDV as well as treatment of *E. coli* infection. An experimental study on African Catfish showed that probiotics are essential for enhancing fish growth, feed utilization, muscle structure and resistance to pathogens (Abdel Hamid *et al.*, 2009).

In Goats, Chiofalo *et al.* (2004) observed that supplementation of the probiotic 'Bios' in Maltese goats resulted in better growth performances testified by a higher BW, by a higher anamorphosis index, which resulted from a greater development of the respiratory and gastro-intestinal apparatus, and a higher body proportion index, which resulted from a better development of the skeletal structure in a longitudinal direction. These are important characteristics for milking producing goat breeds. In pigs, Chen *et al.* (2005) and Hancox *et al.* (2015) observed that continuous probiotic administration could have significant effects on production. However, Heo *et al.* (2013) observed that while some reports have indicated that

supplementation of probiotics improves performance in suckling pigs, weanling pigs, growers and finishers, others have not shown any significant difference.

For pigs, it is suggested that the effects of probiotics appear to be more consistent and positive in piglets rather than in growing finishing pigs since the digestive tract of the piglets is still developing. However, probiotics were less effective for growing and finishing pigs, which already have a balanced population of microorganisms (McDonald *et al.,* 2010). Inconsistencies on the performance of probiotics in various studies have been observed.

Estienne *et al.* (2005) reported that there was little value to routinely administering a probiotic (lactobacillus and streptococcus) to neonatal pigs with regards to pre- weaning performance. In contrast, Silva *et al.* (2010) concluded that the use of probiotics in the diet of sows in late gestation and during lactation, associated with the use of probiotics in the diet of piglets after weaning is effective in maintaining animal performance, the histo-physiological conditions of the gastrointestinal tract and the incidence of diarrhea during the nursery phase. A recent study carried out by Yang *et al.* (2015) indicated that Lactic acid bacteria which include; Lactobacillus species, Bifidobacterium spp and Bacillus spp in pigs have potential as alternatives to in- feed antibiotics. Anna *et al.* (2010), in their study on the effect of probiotics on the morphological characteristics of the small intestinal mucosa in pigs, concluded that probiotics have no adverse effect on mucosal epithelial cells having found no significant differences between the experimental groups.

In layers, a study carried out by Balevi *et al.* (2001) showed that there was no significant difference between the control and the groups receiving 250 and 750 ppm probiotic on feed intake, FCE, damaged egg ratio, the egg yield, egg weight, specific gravity, and peripheral immune. A study carried out by Mahdavi *et al.* (2005) showed that the inclusion of probiotic caused no significant decrease in FCE and had no significant effect on egg production and egg weight. It also showed that probiotic inclusion did not influence the egg weight, shell hardness and shell thickness significantly. Similar results were observed by Chen and Chen (2003) in a different study. The reasons for the inconsistencies in animal performance are unknown. The summary of the effect on performance of various strains of probiotics in poultry, and other livestock species from various studies is shown in (Table 2.3) and (Table 2.4);

Table 2.3 Summary of studies showing the effect on performance of various strains of probiotics in poultry.

Author	Species	Quality of Diet CP (%)	Effect on Performance	Strains of Probiotic
(Khaksefidi and Rahimi, 2005)	Broilers	21.5	Positive	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis sps.*
(Rahman *et al*., 2013)	Broilers	21.0	Positive	*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Streptococcus faecium*, *Bifidobacterium bifidus*, *Bacillus subtilis*, *Bacillus Licheniformis*, *Bacillus Megaterum*, *Bacillus Mesentricus*, *Bacillus polymyxa* and *Saccharomyces bourlrdii.*
(Ashayerizadeh *et al*., 2011)	Broilers	20.48	Positive	*Lactobacillus acidophilus*, *Bifidobacterium thermophilum* and *Enterococcus faecium.*
(Palamidi *et al*., 2016)	Broilers	21.0	Positive	*Lactobacillus reuteri DSM 16350*, *Enterococcus faecium DSM16211*, *Bifidobacterium animalis DSM 16284*, *Pediococcus acidilactici DSM 16210* and *Lactobacillus salivarius DSM 16351*
(Zahra *et al*., 2013)	Broilers	21.6	Positive	*Streptococcus and Bifidobacterium.*
(Fernandes *et al*., 2014)	Broilers	22.0	No Effect	*Lactobacillus acidophilus* and *Saccharomyces cerevisae*
(Hung *et al*., 2012)	Broiler	21.6	Negative	*Bacillus coagulans ATCC 7050*
(Yin-bo *et a*l., 2014)	Broilers	22.2	Positive	*Bacillus subtitles*, *Rhodopseudomonas palustris*, *Candida utilis* and

				Lactobacillus acidophilus
(Zhi-gang *et al*., 2014)	Broilers	21.14	Positive	*Bacillus subtilis, Lactobacillus acidophilus* and *Bacillus licheniformis.*
(Khondokar *et al*., 2016)	Broilers	21.5	No Effect	*Lactobacillus sp.*
(Andrew and Irene , 2008)	Cockerels	21.3	No Effect	*Lactobacillus acidophilus, Saccharomyces cerevisae* and *Saccharomyces boulardii.*
(Wondmeneh *et al*., 2011)	Indigenous chicken	21.0	No Effect	*Lactobacillus sp Saccharomyces cerevisae* and *Saccharomyces boulardii.*
(Toghyani *et al*., 2015)	Broilers	21.0	Positive	Not specified
(Salehimanesh *et al*., 2016)	Broilers	22.0	No Effect	*Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium thermophilum* and *Enterococcus faecium.*
(Mahdavi *et al*., 2005)	Layers	16	Negative	*Bacillus subtilis (CH201)* and *Bacillus licheniformis (CH200)*
(Mookiah *et al*., 2014)	Broilers	22.0	Negative	*L. reuteri C 1, C 10 and C 16; L. gallinarum I 16 and I 26, L. brevis I 12, I 23, I 25, I 218* and *I 211* and *L. salivarius I 24*
(Pelicano *et al*., 2004)	Broilers	22.0	No Effect	*Bacillus subtilis, Bacillus licheniformis, Saccharomyces cerevisae, Lactobacillus reuteri* and *Lactobacillus johnsonii*.
(Daskiran *et al*., 2012)	Broilers	23.1	No Effect	*Lactobacillus plantarum, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacte614 rium bifidum, Streptococcus salivarius ssp. thermophilus, Enterococcus faecium, Aspergillus oryzae* and *Candida Pintolepesii.*
(Cao *et al*., 2013)	Broilers	20.9	Positive	*Enterococcus faecium*

(Behrouz *et al*., 2012)	Broilers	21.5	No Effect	*Lactobacillus plantarum, Streptococcus salivarius ssp. thermophilus, Enterococcus faecium* and *Bifidobacterium thermophilum.*
(Timmerman *et al*., 2006)	Broilers	22.0	No Effect	*Lactobacillus bifermentans, Lactobacillus sanfranciscensis, Lactobacillus reuteri*, and *Lactobacillus fermentum.*
(Fajardo *et al*., 2012)	Broilers	21	Negative	*Lactococcus lactis CECT 539 and Lactobacillus casei CECT 4043.*
(Sarangi *et al*., 2016)	Broilers	22.4	No Effect	*Lactobacillus, Streptococcus, Enterococcus, Bifidobacterium, Aspergillus, Candida* and *Saccharomyces.*
(Chen and Chen, 2003)	Layers	17.3	Negative	*Bacillus subtilis, Lactobacillus acidophilus* and *Bacillus licheniformis*
(Balevi *et al.*, 2009)	Layers	16.1	Negative	*Lactobacillus plantarun, Lactobacillus delbruecki subsp. Bulgaricus, Candida pintolopesii Lactobacillus acidophilus, Lactobacillus rhamnosus, Aspergillus oryza , Bifidobacterium bifidum, Streptococcus salivarus subsp. Thermophilus and Enterococcus faecium*
(Hayirli *et al.*, 2005)	Layers	16.2	Negative	*Enterococcus faecium.*
(Yousefi and Karkoodi, 2007)	Layers	13.8	No Effect	*Saccharomyces cerevisae*

Table 2.4 Summary of studies showing the effect on performance of various strains of probiotics in different livestock species.

Author	**Species**	**Quality of Diet CP (%)**	**Effect on performance**	**Strains of Probiotic**

(Chiofalo *et al.,* 2004)	Maltese Goats	18.6	Positive	*Lactobacillus acidophilus, Lactobacillus salivarius* and *Lactobacillus reuteri.*
(Chen *et al.,* 2005)	Pigs	19.0	Positive	*Lactobacillus acidophilus, Bacillus subtilis* and *Saccharomyces cerevisae.*
(Anna *et al.,* 2010)	Pigs	16.0	No Effect	*Bacillus toyoi strain*
(Hancox *et al.,* 2015)	Pigs	20.0	Positive	*Saccharomyces cerevisiae var. boulardii*
(Herfel *et al.,* 2013)	Pigs	20	Negative	*Bifidobacterium longum (AH1206).*
(Guo *et al.,* 2006)	Pigs	20.3	Negative	*Bacillus subtilis MA139.*

2.3.3. Use of probiotics in livestock production in Africa

Currently in Africa, probiotics are being adopted to solve the problems associated with the withdrawal of antibiotics in livestock production. Several studies have proven the benefits of probiotics. Olatoye *et al*. (2014) confirmed that the use of the yeast probiotic (Antox®) improves performance and inhibits the colonization of ceacal salmonella in broilers. Similar results were reported by Owosibo *et al*. (2013) who also concluded that inclusion of probiotics in broiler diets can elevate the serum cholesterol value.

Results on Kenyan indigenous chicken showed that supplementation with a probiotic, Mola plus, which contained; *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp.* and *Escherichia coli,* in drinking water significantly improved the weight gain (Atela *et al*., 2015). Khobondo *et al*. (2015) in a separate study concluded that supplementation of 5 ml of Mola plus poultry microbes in 1000 ml of water is recommended to maximize beneficial effects in chicken and higher concentrations do not always result in better performance.

2.3.4. The manufacture of probiotics

Fermentation

Probiotic micro-organisms are generally produced by either batch or continuous fermentation a fermentation process with species- and strain-specific temperature and pH. In batch fermentation, all of the substrate (sterilized) and the inoculum are mixed together in the fermenter at the beginning and kept at the optimum temperature for the growth of the probiotic. For spore-forming bacteria like, *Bacillus subtilis,* vegetative cells are induced to sporulate, generally by limiting nutrient availability, before harvesting. Batch fermentation has been preferred because it is less costly than continuous fermentation (Muller *et al.*, 2009). After fermentation the bacterial and yeast cells are usually dried for ease of transport and storage thus avoiding any need for specialized facilities for storage and transport of liquid inoculants or frozen cells.

Drying

Probiotic micro-organisms are generally dried by freeze drying or spray drying (Muller *et al.*, 2009). Freeze drying involves a two-step process of freezing and drying where by the bacteria are first frozen by using liquid nitrogen or dry ice, or refrigerated at -20°C and then dried under high vacuum to reduce the moisture level to 4% or below. With spray drying**, f**ine droplets of probiotic culture, atomized by spraying through a heated nozzle, are sprayed into the drying chamber against hot air.

Storage

Probiotic microorganisms are highly sensitive to environmental stresses like; pH, oxygen and heat levels hence there is need to maintain their viability during manufacturing, storage and handling, and quality control is needed to ensure this so as to increase their efficacy. Recent technologies have been used to increase the resistance of the probiotic microorganisms to the adverse gut environmental and gut conditions and one of the proposed methods is microencapsulation. It is defined as a technology of packaging solids, liquids or gaseous material in miniature, sealed capsules that can release their contents at controlled rates under the influence of specific conditions like heat, solvation, diffusion and pressure (Kailasapathy and Masondole, 2005). It may also be designed to open in specific areas of the digestive system of the host.

2.3.5. Possible reasons for inconsistencies in response to performance

Probiotics are to become a major and viable alternative to Antibiotic Growth Promoters and the factors causing variations can be controlled, more consistent and viable results can be obtained. Several reasons have resulted to this variability (Fuller, 2006).

i. Classification of microorganisms

It is important that the buyer of the probiotics is confident that what is on the label of the product corresponds with its composition. Weese and Martin (2011) noted that some products contain incorrect information on their labels and this is mainly on the probiotic microorganisms. It is hence important to consider the variation of bacteria species and their classification during manufacture. This is because species with similar names may differ in terms of structure, biochemical and metabolic activities hence produce variations in results in the host.

ii. Viability

Poor viability accounts for some of the negative results obtained. The viability of a probiotic may be as a result of sub-dorminant microorganisms, which may suppress the growth of the others especially in multi-strained probiotics. Shelf life is also another important aspect since it involves proper storage conditions like humidity, temperature, light and pressure which vary worldwide.

iii. Production conditions

Probiotic microorganisms are highly sensitive to environmental stresses like; pH, oxygen and heat levels hence there is need to maintain their viability during manufacturing. The source of the inoculum should also be kept constant to prevent variations.

iv. Method of administration

The minimum dosage of administering probiotics is yet to be determined and this makes it essential. Various studies have used single, multiple and continuous dosing and this has definitely produced varying results. The mode of administration may also cause variations since while some probiotics are administered directly into the mouth as tablets, capsules or powder, others are through water. Addition in water has its disadvantages due to osmotic shock or the bactericidal effect of chlorine in case of tap water.

v. State of the gut

The survival of probiotic organisms in the gut depends on their possessing colonization factors which enable them to resist the antibacterial mechanisms (chemical and physical) which operate in the gut. The protective flora which establishes itself in the gut is very stable, but it can be influenced by some dietary and environmental factors which include: excessive hygiene, antibiotic therapy and stress, making the host animal susceptible to disease and/or reducing its efficiency of food utilization.

There are other alternatives to antibiotics after probiotics;

2.4. Prebiotics

They are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and altering the composition and metabolism of the gut microbiota (Das *et al.*, 2012). A prebiotic essentially improves a chicken's gastrointestinal health by providing a nutrient source for one or more beneficial bacteria currently residing within the animal.

These bacteria populate the gut filling any niches were pathogenic bacteria may attempt to take hold. Prebiotics function by lowering the gut pH through lactic acid production, inhibiting colonization of pathogens, modifying metabolic activity of normal intestinal flora, and stimulation of the immune system. Fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) are the two most common prebiotics available for use in poultry (Alloui *et al.*, 2013). The effects of prebiotic supplementation are summarized in Table 2.5.

Table 2.5 Summary of studies showing the types of prebiotics and their effects on the gut microbiota and immune system of poultry.

Type of prebiotics	Biological activities	References
FOS or MOS	Decreased populations of C. perfringens and E. coli in the gut. Increased the population of *Bifidobacterium* in the small intestine and colon. Increased the population and diversity of lactobacilli in the ileum. Affected the heterophil: Lymphocyte ratio and basophil levels	(Kim *et al.,* 2011)
FOS	Provided nutrients for the growth of beneficial bacteria in the gut.	(Alloui *et al.,* 2013)
Inulin	Increased bifidobacterium counts and decreased *E. coli* counts in caecal contents.	(Nabizadeh *et al.,* 2012)
GOS	Increased *Bifidobacterium* spp. and decreased *Campylobacter* spp. in the faecal samples.	(Baffoni *et al.,* 2012)
IMO	Increased the caecal populations of *lactobacilli* and *bifidobacteria* and decreased the caecal *E. coli.*	(Mookiah *et al.,* 2014)
FOS and MO	Increased serum concentration of IgA	(Kim *et al.,* 2009)
Commercial prebiotic	Increased serum concentration of IgA and IgM, and enhanced systemic immune capacity in chickens	(Vidanarachchi *et al.*, 2013)
FOS	Enhanced the IgM and IgG antibody titres in plasma	(Janardhana *et al.,* 2009)
Prebiotic-based MOS and β-glucan	Increased the relative weight of spleen, decreased the heterophil-to-lymphocyte ratio and increased antibody titres against *S. enteritidis*	(Sadeghi *et al.,* 2013)

Source: Sugiharto, (2016).

2.5. Synbiotics

When probiotics and prebiotics are combined in a form of synergism, they form synbiotics. (Huyghebaert *et al*., 2011). This is because they are thought to act together, since a probiotic, without its prebiotic food, does not survive well in the digestive system. Synbiotics

beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract.

They are designed not only to present beneficial microorganisms populations, but also to promote proliferation of autochthonous-specific strains (*Lactobacillus* and *Bifidobacterium*) in the intestinal tract (Gourbeyre *et al*., 2011). Table 2.7 summarizes some of the effects synbiotics in poultry.

Table 2.6 Examples of probiotics, prebiotics and synbiotics used as animal feed additives.

Probiotic	**Prebiotic**	**Synbiotic**
Lactobacillus sps.	Inulin	*Lactobacilli* + inulin
Bifidobacterium sps.	Galactooligosaccharides (GOS)	*Bifidobacteria* + FOS
Saccharomyces sps.	Fructo-oligosaccharides (FOS)	*Lactobacilli* + FOS
Streptococcus sps.	Lactulose	*Bifidobacteria, Lactobacilli* + inulin
Bacillus coagulans	Lactitol	*Bifidobacteria, Lactobacilli* + FOS
Propionibacterium	Cereals fibres	*Lactobacilli* + lactitol
Bacillus coagulans	Xylooligosaccharides	*Bifidobacteria* + GOS
Enterococcus faecium	Isomaltooligosaccharides	

Source: Hamasalim, (2016).

Table 2.7 Summary of studies showing the types of synbiotics and their effects on the gut microbiota and immune system of poultry.

Types of synbiotics	Biological activity	References
Commercial synbiotics (Biomin Imbo)	Increased the LAB population and reduced *E. coli* and total coliform populations in the intestine	(Dibaji *et al.,* 2014)
Bifidobacterium-based synbiotic product	Reduced C. jejuni concentration in poultry faeces.	(Baffoni *et al.,* 2012)
Synbiotic (S. cerevisiae plus MOS)	Reduced the number of *E. coli* in the small intestinal and caecal digesta.	(Abdel-Raheem *et al.,* 2012)
Synbiotic (11 Lactobacillus strains plus IMO)	Increased the caecal populations of *lactobacilli* and *bifidobacteria* and decreased the caecal *E. coli.*	(Mookiah *et al.,* 2014)
Synbiotic (E. faecum plus FOS)	Reduced the intestinal colonization by *C. Perfringens.*	(El-Ghany, 2010)
Commercial synbiotic (Biomin Imbo)	Increased antibody production	(Hassanpour *et al.,* 2013)
Synbiotic (combination of Lactobacillus, Bifidobacterium and oligosaccharides derived from yeast cell wall)	Improved the antibody response to NDV and infectious bronchitis virus (IBV) vaccines.	(El-Sissi and Mohamed, 2011)
Synbiotic (L. lactic plus raffinose family oligosaccharides)	Stimulated the expression of IL-6 and IFN-c during in vitro culturing of chicken lymphocytes	(Slawinska *et al.,* 2012)

Source: Sugiharto, (2016).

2.6. Organic acids

Organic acids are any organic carboxylic acid of the general structure R-COOH. The organic acid with anti-microbial activity are short chain acids (C1-C7), which are either simple

mono-carboxylic acids such as formic, acetic, propionic and butyric acids or are carboxylic acids bearing hydroxyl group such as lactic, malic, tartaric and citric acid.

Organic acids have been shown to exhibit beneficial effects on the intestinal health and performance of broilers by reducing microbial competition with the host for nutrients and endogenous nitrogen losses. Organic acids can freely diffuse through the semi-permeable membrane of the bacteria into the cell cytoplasm, where they dissociate and suppress bacterial cell enzymes and nutrient transport systems (Huyghebaert *et al*., 2011). They also lower the incidence of subclinical infections and secretion of immune mediators, and reduce the production of ammonia and other growth-depressing microbial metabolites (Adil *et al*., 2010).

2.7. Exogenous enzymes

They are special proteins that catalyze or accelerate the rate of specific chemical reactions in which the enzyme activity may be dependent on the substrate in a random manner or it may be through very specific sites on substrates such as fat, protein, or carbohydrates. They include b-glucanase, xylanase, amylase, a-galactosidase, protease, lipase and phytase. Cereals such as wheat, barley and rye are incorporated into animal feeds to provide a major source of energy.

However, much of the energy remains unavailable to monogastrics due to the presence varying levels of different anti-nutritive factors (e.g., non-starch polysaccharides (NSP) and protease inhibitors) that can impede normal digestion and absorption processes of nutrients in the digestive tract (Yegani and Korver, 2013). Most of selected carbohydrases (enzymes) will break down NSP, releasing nutrients (energy and protein), as well as reducing the viscosity of the gut contents.

2.8. Phytobiotics

Phytobiotics are plant-derived natural bioactive compounds that can be added to the feed to improve the performance and well-being of animals and to improve the quality of food derived from the animals fed these products (Windisch *et al*., 2008). The active compounds of phytobiotics are mostly secondary plant constituents, such as terpenoids (mono- and sesquiterpenes, steroids, etc.), glycosides, alkaloids (alcohols, aldehydes, ketones, esters,

ethers, lactones, etc.) and phenolics (tannins). Based on the biological origin, formulation, chemical description and purity, phytobiotics are classified as:

a) Herbs (product from flowering, non-woody and non-persistent plants).

b) Botanicals (entire or processed parts of a plant, e.g., root, leaves, bark).

c) Essential oils (hydro distilled extracts of volatile plant compounds).

d) Oleoresins (extracts based on non-aqueous solvents) (Yang *et al*., 2009).

The mechanisms by which the phytobiotics exert their benefits on the gut remain unclear, but possible mechanisms have been shown as:

a) Modulating the cellular membrane of microbes leading to membrane disruption of the pathogens.

b) Increasing the hydrophobicity of the microbial species which may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes.

c) Stimulating the growth of favourable bacteria such as *lactobacilli* and *bifidobacteria*
in the gut.

d) Acting as an immunostimulatory substance and protecting the intestinal tissue from microbial attack (Vidanarachchi *et al*., 2005).

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CHAPTER THREE

Experiment 1. The use of probiotics in poultry production in Kenya (Kiambu County)

Abstract

In this study, two surveys were conducted in Kiambu County, Kenya. 100 agro vets were sampled in 9 out of 12 sub-counties of Kiambu County namely: Thika, Juja, Kiambu, Gatundu North, Gatundu South, Kikuyu, Kabete, Ruiru and Limuru. Data was collected using semi-structured questionnaires which were administered so as to identify the products sold in the Kenyan market as probiotics. The second part was carried out in 3 sub-counties of Kiambu County; Thika, Kiambu and Kabete among 36 poultry farmers who use probiotics. They were grouped into two production systems; household and commercial and catered for broiler, layers and indigenous chicken production. The data was collected using a semi-structured questionnaires so as to obtain information on probiotic use in layer/broiler production and its effect on performance. The data collected was analyzed using Statistical Package for Social Sciences (SPSS) version 22 descriptive statistics.

The results of the agro vet survey showed that, a total of 15 types of probiotics were found in the market and the most common probiotics brands were Product 1, Product 2, Product 3 and Product 4. The results from farmers' survey showed that 74.4% of the poultry farmers were using probiotics as feed additives and the most common brands were; Product 1, Product 7, Product 2 and Product 4.

In conclusion, the Kenyan farmer in Kiambu has accepted to use probiotics in poultry production and is reporting positive feedback on productivity. Therefore there is need to test the effectiveness of common probiotics used in Kenya in broiler production in a controlled experiment.

Key words: probiotics, performance, feed additives, farmers, broiler.

3.1. Introduction

The worldwide increase in population growth and urbanization has resulted in an increase in demand of livestock products which has become a major driving force to the rapid changes in livestock production systems (Thornton, 2010). Poultry is one of the world's fastest growing sources of meat, representing nearly a quarter of all the meat produced. In

Kenya, the poultry industry is growing fast due to the rise in demand for poultry products. According to the 2009 census, the country had a total of 31.8 million broilers out of which 25.7 million were indigenous and 6.1 million of commercial type. In Kenya's Kiambu County, there is a relatively high number of chicken constituting 8.5% of the total poultry population (Kenya National Bureau of Statistics, 2009). Due to the high demand for poultry products there has been intensification of livestock production (Udo *et al*., 2011).

The use of antibiotics to improve growth and feed efficiency has developed concurrently with the intensification of the livestock industry worldwide. The widespread use of antibiotics is a major risk factor due to an increase in the occurrence of bacterial resistant strains where bacteria species displayed variable levels of resistance to antibiotics. At the global level, the increasing prevalence of antimicrobial-resistant bacteria is a public health risk of concern and has led to the ultimate prohibition to the use of antibiotics as growth promoters in animal feed with effect from January 1, 2006 by the European Union (European Union Commission, 2005), which was followed by many other countries worldwide.

In recent years, probiotics have received much attention as they are considered natural alternatives to antibiotics due to their effect on growth promotion and immune response among various animal species. According to the currently adopted definition by FAO/WHO (2009); probiotics are "live microorganisms which when administered in adequate amounts, confer a health benefit on the host".

In Kenya, the concept of use of probiotics is becoming more comprehensible especially among commercial poultry farmers who wish to improve their flock productivity. The use of probiotics is becoming increasingly common in order to avoid antibiotics in poultry production. Therefore, the purpose of this study was to assess the extent to which probiotics are used in Kiambu County of Kenya and their perception by farmers.

3.2. Materials and methods

3.2.1. Study area

This study was carried out in Kiambu County, Kenya. Kiambu County consists of 12 sub-counties namely; Limuru, Githunguri, Lari, Gatundu North, Gatundu South, Kikuyu, Kabete, Kiambaa, Kiambu, Ruiru, Juja and Thika. The economy of Kiambu County is dominated by smallholder agriculture which employs about 75 percent of the population

(Okello *et al*., 2010). Some of the major economic activities include livestock production (dairy, sheep, goats, pigs and poultry), crop production (coffee, tea, and horticulture), small and large scale businesses and real estate development.

3.2.2. Surveys

Two surveys were carried out between January and May, 2016. The first survey involved Agro Veterinary stockists using a predesigned questionnaire (Appendix I). The sample size was arrived at using the method of Cochran (1963) as indicated below;

$$\mathbf{n} = \frac{pqz^2}{\mathbf{e}^2}$$

Where n= sample size, z= confidence level (α=0.01), p= proportion of the population containing the variables of interest, q= 1-p, and e is the allowable (or desired) error because the proportion of the population is not known. In this case, p, q, z and e were assumed to be, 0.1, 0.9, 1.96 and 0.059 respectively.

$$\mathbf{n= 0.1\times 0.9\times (1.96)^2/(0.059)^2}$$

$$\mathbf{= 99.32}$$

Using the above formula, 100 agro vets were surveyed in the County.

Part 1: Survey involving agro veterinary stockist

The study included a survey which was carried out in agro vets in 9 out of 12 sub-counties of Kiambu County namely: Thika, Juja, Kiambu, Gatundu North, Gatundu South, Kikuyu, Kabete, Ruiru and Limuru. Cross-section primary data were used in this study. The primary data was collected on agro vets using a semi-structured questionnaire. The questionnaires were administered to the owners of the agro vets with the aim being to evaluate which products were sold in the market as probiotics.

Part 2: Survey involving the farmers

This included a survey which was carried out in 3 sub-counties of Kiambu County among poultry farmers who use probiotics and were grouped into two production systems; household and commercial, in order to take into account of the unique attributes of the systems with respect to probiotic use. The three sub counties were; Thika, Kiambu and

Kabete and it catered for broiler, layers and indigenous chicken production. A total of 36 farmers were sampled.

The primary data were collected on farmers using a semi-structured questionnaire**.** The questionnaires were administered with an aim of obtaining information on probiotic use in layer/broiler production from the farmers and its effect on performance.

3.3. Data analysis

All the questionnaire data from the Agro Veterinary stockiest was captured in Microsoft Excel and analyzed in Statistical Package for Social Sciences (SPSS) version 22 descriptive statistics.

3.4. Results

3.4.1. Part 1: Distribution of probiotics

In the 100 agro-vets, 168 probiotics were found because many agro-vets stocked more than one probiotic. The results of this survey showed that, a total of 15 types of probiotics were found in the market in Kiambu County as shown in Table 3.1. The most common probiotics were Product 1 (25.6%), Product 2 (14.3%), Product 3 (13.7%) and Product 4 (11.3%) and were found to be the most popular brands in this market. Most of the products were locally produced, but a few like Product 10, Product 14 and Product 12 were imported.

Table 3.1 Types of probiotics used in livestock production in Kiambu County and the frequency of occurrence.

Product	Frequency	Percentage
Product 1	43	25.6
Product 2	24	14.3
Product 3	23	13.7
Product 4	19	11.3
Product 5	18	10.7
Product 6	16	9.5
Product 7	6	3.6
Product 8	4	2.4
Product 9	4	2.4
Product 10	3	1.8
Product 11	2	1.2
Product 12	2	1.2
Product 13	2	1.2
Product 14	1	0.6
Product 15	1	0.6
Total	168	100.0

Descriptions of products in the market

Using the product labels, each of the probiotics found in the market was described.

1. Product 1

It is a commercially available yeast culture for both broiler and layer chicken containing *Sacchromyce cerevisiae* and is fortified with vitamins, amino acids and minerals. It is a product of Champion distributor Ltd, Kenya. It is sold in powder form for Ksh 250 per Kilo gram (Kg).

2. Product 2

It is a commercially available yeast culture containing *Sacchromyce cerevisiae* and is fortified with vitamins, amino acids and minerals. It is sold in powder form for Ksh 750 per Kg.

3. Product 3

It is a complex solution of various beneficial microorganisms found naturally and used in food manufacturing. They provide chelated minerals, anti-oxidants, enzymes, vitamins, organic acids, lactic bacteria, yeast and prototrophic bacteria to poultry. It is sold in liquid form at Ksh 250 per liter.

4. Product 4

It *is a* commercially available probiotic containing a mixed microbial culture of selected species of microorganisms such as lactic acid bacteria, yeasts and photosynthetic bacteria which avail essential amino acids, energy, minerals and enzymes when used in poultry production. It is a product of J.V Enterprises, Kenya. It is sold in liquid form at Ksh 250 per liter.

5. Product 5

This is a commercially yeast culture containing Sacchromyce cerevisiae which provides amino acids, energy, minerals and enzymes when used in poultry production and is a product of Vetpro Ltd, Kenya. It is sold in powder form for Ksh 200 per Kg.

6. Product 6

It is a commercially yeast culture containing Sacchromyce cerevisiae which provides energy, minerals, amino acids and enzymes when used in poultry production and is a product Afri Vet Ltd, Kenya. It is sold in powder form for Ksh 120 per Kg.

7. Product 7

It is a commercially available probiotic containing a mixed microbial culture of selected species of microorganisms such as lactic acid bacteria, yeasts, photosynthetic bacteria and actinomycetes and can be used both for soil composting and also as a feed additive to animals. It is sold in liquid form at KSh 300 per liter.

8. Product 9

This is a commercially available probiotic containing a mixed microbial culture of selected species of microorganisms such as lactic acid bacteria, yeasts and photosynthetic bacteria which avail essential amino acids, energy, minerals and enzymes when used in poultry production. It is sold in liquid form at KSh 250 per liter.

9. Product 8

This is a commercially available probiotic containing a mixed microbial culture of selected species of microorganisms such as lactic acid bacteria, yeasts, photosynthetic bacteria and actinomycetes and can be used both for soil composting and also as a feed additive to animals. It is a product of Organic Africa Ltd, Kenya. It is sold in liquid form at Ksh 200 per liter.

10. Product 10

This is a commercially available monoculture yeast probiotics containing *Sacchromyce cerevisiae*. It is a product of Montajat Vet Pharmaceutical Co.Ltd (India). It is sold in liquid form at Ksh 2000 per liter.

11. Product 13

It is a yeast probiotic manufactured by Venkys Ltd (India) and avails essential amino acids, energy, minerals and enzymes when used in poultry production. It is sold in liquid form at Ksh 1500 per liter.

12. Product 11

This is a complex solution of various beneficial microorganisms found naturally and used in food manufacturing which provide chelated minerals, anti-oxidants, enzymes, vitamins, organic acids, lactic bacteria, yeast and prototrophic bacteria when used in poultry production. It is manufactured by Rhonjas Enterprises, Kenya. It is sold in liquid form at Ksh 250 per liter.

13. Product 12

It is a probiotic containing live yeast cells (*Sacchromyce cerevisiae*) and bacteria (*Lactobacillus sporogenes) and fortified with enzymes like phytase, cellulase, Xylanase and pectinase. It is also rich in carbohydrates, vitamins, minerals and UGF (Unknown Growth Factors) and is a product of Bremer Pharma GMBH (Germany).* It is sold in powder form at Ksh 1200 per Kg.

14. Product 14

This is a commercially available yeast probiotic containing *Sacchromyce cerevisiae* which avails essential amino acids, energy, minerals and enzymes when used in poultry production. It is manufactured by Venkys Ltd (India). It is sold in liquid form at Ksh 1500 per liter.

15. Product 15

It is a commercial yeast based probiotic containing *Sacchromyce cerevisiae* and avails enzymes like cellulase, amylase, arabinase pectinase, protease, lipase, Xylanase, beta glucanase and alpha galactosidase. It is produced by (Wockhardt Limited) wockhardt towers, bandra-kurla complex, bandra (E) Mumbai- 400 051 and sold in powder form at Ksh 300 per Kg.

3.4.2. Form

The products were sold either as a powder or in liquid forms as indicated in Table 3.2.

Table 3.2 Proportion of the form in which the probiotics were packaged

	Frequency	Percent	Product
Liquid	63	37.5	Product 10, Product 8, Product 14, Product 13, Product 7, Product 3, Product 11, Product 4 and Product 9.
Powder	105	62.5	Product 12, Product 15, Product 2, Product 5, Product 6 and Product 1
Total	168	100.0	

3.4.3. Targeted livestock

Most products (92.2%) were targeted to both broiler and layer production. They included; Product 10, Product 14, Product 15, Product 2, Product 13, Product 7, Product 5, Product 6, Product 3, Product 11, Product 9, Product 4 and Product 1. Some products (6.0%) were multipurpose and could be used as probiotics and also for making organic compost.

Probiotics such as Product 7 and Product 8 are used for composting which is the process of segregating organic waste back to the soil. They are applied straight to the soil as innoculants and function to exert beneficial effects on soil quality by fermenting the organic matter hence removing all the problems of pathogenic bacteria, greenhouse gasses and bad odor from the process. They are also used for silage and septic tank treatments (http://www.livingsoil.co.uk). However, Product 12 (1.8%) was the only probiotic for cattle (Table 3.3).

Table 3.3 Proportion of probiotics administered to various types of livestock in Kiambu County.

Livestock	Frequency	Percent
Cattle	3	1.8
Poultry	155	92.3
Poultry /Organic Compost	10	6.0
Total	168	100.0

3.4.4. Type: Yeast based or live microbes?

The classification of the probiotics was based on the active ingredient indicated on the label inserts. According to the study results, 64.3% of the products were yeast based probiotics and contained the *Saccharomyces cervisiae spp*. They included; Product 10, Product 14, Product 15, Product 2, Product 13, Product 5, Product 6 and Product 1. 35.7% of the products had live microbes which mainly was the *Lactobacillus spp* and included; Product 8, Product 12, Product 7, Product 3, Product 11, Product 9 and Product 4 (Table 3.4).

Table 3.4 Classification of the probiotics found in Kiambu County.

Class	Frequency	Percent
Yeast based	108	64.3
Live Microbes	60	35.7
Total	168	100.0

3.5. Results

3.5.1. Part 2: Farmers' feedback on the benefits of probiotics

The majority farmers in the county were commercial poultry producers (92.3%) and their flock sizes ranged from 50- 800 for all types of poultry. Broiler poultry farmers had the largest flock size which ranged from 300- 600 chicken, followed by layers poultry farmers whose flock ranged from 200-400 and indigenous poultry farmers had the smallest flock size that ranged from 50-200. The farmers who kept poultry for household purpose were about (7.7%) and their flock size ranged from 10-50 chicken which were mainly indigenous chicken. These results are shown in Table 3.5.

Table 3.5 The type of poultry production systems practiced by farmers in Kiambu County

Type	Frequency	Percent
Household	3	7.7
Commercial	36	92.3
Total	39	100.0

48.7% of the farmers in the county kept indigenous chicken and 35.9% of the farmers kept broiler chicken. 15.4% of the farmers kept layers as shown in Table 3.6.

Table 3.6 Distribution of respondents by the type of poultry in Kiambu County

Poultry type	Frequency	Percent
Broiler	14	35.9
Layers	6	15.4
Local	19	48.7
Total	39	100.0

The probiotics used

About 74.4% of the poultry farmers were using probiotics as feed additives while 25.6% were not. According to the study, probiotics were mainly being used by the commercial poultry farmers while the household poultry farmers did not use probiotics. They had other alternatives which included antibiotics, poultry supplements and medicinal plants and trees such as *Aloe vera*, red pepper, desmodium and *Aloe kendogensis* (Table 3.7).

Most of the respondents surveyed in this study administered the probiotics in the early growth stages of the chicken (day 1- 4 weeks) to allegedly increase their appetite hence boost their growth rate. All the poultry farmers who used probiotics gave a positive feedback on its performance in poultry.

Table 3.7 Farmers' response on the use of probiotics in poultry production in Kiambu County.

Response	Frequency	Percent
Yes	29	74.4
No	10	25.6
Total	39	100.0

The results of the common probiotics used are shown in Table 3.8.

Table 3.8 Summary of the probiotics used by poultry farmers in Kiambu County.

Probiotics	Frequency	Percent
Product 1	9	23.1
Product 4	8	20.5
Product 7	6	15.4
Product 2	5	12.8
Product 16	1	2.6
N/A	10	25.6
Total	39	100.0

Note: N/A represents the number of non-respondents

Product 1 for both broilers and layers was widely used among the poultry farmers at 23.1%. It is yeast based, containing *Saccharomyces cerevisiae* and is in powder form. The farmers administered it in poultry feed at 10 grams per 70 kg bag.

Product 4 followed at 20.5 %. It was administered to the chicken through water at a dosage of 2 ml for 1 litre of water daily for one week especially for day old chicks and thereafter 5 ml for 1 litre of water twice a week which contains live microbes, *Lactobacillus*, and is in liquid form. Product 7 was at 15.4% and contains live microorganisms. It was administered through water at a dosage of 2 ml for 1 litre of water daily for one week especially for day old chicks and thereafter 5 ml for 1 litre of water twice a week.

Product 7 was also used for making compost by farmers. 12.8% of the farmers used Product 2 which is in powder form. It contains yeast and was administered through the feed at 10 grams for 70 kg bag. Product 16, which is for layers, was at 2.6% and is in powder form. It was administered through the feed at 10 grams for 70 kg bag. Despite Product 1 being the most readily available probiotic, most respondents preferred the liquid form probiotics like Product 4 and product 7 due to the ease of administration to the poultry.

Source of information

Most poultry farmers obtained the information about probiotics from agro vets (28.2%), while others got the information from the livestock extension officers assigned in their sub counties (25.6%). This is because agro-vets were readily accessible on average being, less than 4 km away from farmers' homesteads in both production systems. Animal extension officers were also, on average, about 4 km away and occasionally organize seminars and meetings with the farmers to teach them on various things.

Approximately 12.8% of the poultry farmers obtained the information from the lead farmers in their sub counties or groups, who normally are the leaders for their groups. The farmers who received information from the livestock officers were about 5.1% and 2.6% got the information form other sources like; livestock field days and shows, agricultural magazines, seminars and books (Table 3.9).

All the respondents interviewed had some form of agricultural training either through organized seminars and workshops or through the small groups that they formed from where they received training from extension officers.

Table 3.9 The sources of information on probiotics use in poultry production in Kiambu County.

Source of information	Frequency	Percentage
Agro vet	11	28.2
Extension Officer	10	25.6
Lead Farmer	5	12.8
Veterinary	2	5.1
Other sources	1	2.6
N/A	10	25.6
Total	39	100.0

Note: N/A represents the number of non-respondents.

The average weight of mature broilers at six weeks was 1.4 kg and were sold at Ksh 380 (wholesale price) and 400 (consumer price). For farmers rearing laying hens, they sold an average of 4 trays a week while for farmers rearing indigenous chicken, they sold their chicken every three months with an average weight of 3 kg at Ksh 500.

3.6. Discussion

Probiotic is a culture of living microorganisms that is used as functional ingredients to manipulate and maintain good health by controlling gut micro flora and increasing digestive enzyme activity. Charalampopolus and Rastall (2009) stated that the species currently being used in the preparations of probiotics are mostly of two bacterial genera: *Bifidobacterium and Lactobacillus. These include*; *Lactobacillus bulgaricus, Lactobacillus sporogenes, Lactobacillus salivarius, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus lactis, and Lactobacillus plantaru.*

The probiotic microorganisms can only be effective if they are capable of exerting a beneficial effect in the form of growth promotion or increased resistance to disease and also be non-pathogenic and non-toxic. They should also be present as viable cells, preferably in large numbers and capable of surviving and metabolizing in the gut environment, e.g. resistant to low pH and organic acids.

Probiotics act in various ways like an antagonistic action towards pathogen bacteria through; production of antibacterial compounds, modification of gut pH. Another mode of action is competitive exclusion which is the colonization ability and adhering competition in the intestinal mucous membranes hence preventing the adhesion and invasion of pathogens. They also act through competition for nutrients with the pathogens hence inhibiting their colonization in the gut. They alter the microbial metabolism by increase or decrease of enzyme activity and stimulate immunity by increasing antibody levels and macrophage activity.

Probiotics have been used in a wide range of animal species. In poultry, probiotics are used as natural growth promoters and as alternatives to antibiotics and have shown various benefits in the performance and immunity of the broilers by increase of growth rate and decrease in colonization by pathogens in the gut (Mohamed *et al*., 2013). Probiotics have also been used in cattle health and productivity mainly to reduce incidences of diarrhea in calves, prevent ruminal acidosis through pH stabilization, to control the growth of pathogens in the rumen and also to balance the gut microbiota (Yutaka *et al*., 2015).

The data collected in the survey carried out in Kiambu showed an increase in awareness and use of probiotics among Kenyan farmers. There were 15 brands of probiotics and they were used for poultry, dairy cattle and compost making. After using the probiotics, poultry farmers noted an increase in feed intake among the chicken after administering probiotics and hence faster growth rate.

These results tally with a report by Samanya and Yamauchi (2002) who indicated that the broilers fed on probiotics had a tendency to display pronounced intestinal histological changes such as prominent villi height which increase the rate of absorption of available nutrients hence resulting in greater growth performance and increase in body weight gain. Other studies have shown that supplementation of broilers with probiotics increased the villus height: crypt depth ratio in the ileum significantly (Ghahri *et al*., 2013).

The farmers also indicated low mortality rates among the young chicks and good health among their flock after using probiotics. These results were similar to those of Talebi *et al*. (2008) who stated that, probiotics feeding also have been reported to improve antibody titres against viral diseases like Newcastle Disease (ND) and Infectious Bursal Disease (IBD). Lee *et al*. (2010) stated that probiotics reduced the clinical signs of avian coccidiosis in a set experiment and increases various parameters of immunity in broiler

chicken. Kabir *et al. (2004)* reported significantly higher antibody production in experimental broilers as compared to control ones. They also demonstrated that the differences in the weight of spleen and bursa of probiotics and conventional fed broilers were attributed to different level of antibody production in response to Sheep Red Blood Cells (SRBC).

In layers, farmers noted reduction in stress factor especially during peak production, production of strong egg shells and maintainace of optimum egg production. Radu-Rusu *et al*. (2010) stated that the use of probiotics would enhance the performance of layers especially during the stress periods that is; at the early stages of life and immediately prior to and after the move from pullet house to layer house. Zarei *et al*. (2011) reported an increase in egg mass and weight and egg shell weight and thickness on feeding laying hens with diets supplemented with some commercial probiotics, prebiotics and synbiotics. Use of probiotics in layers have been reported to improve fertility, egg quality and reduce yolk cholesterol concentration without affecting yolk weight (Chen *et al*., 2005).

Poultry farmers surveyed in the present study reported the production of dry litter by the chicken on administering probiotics and reduction of bad odor in the pens. Probiotics have been found useful in reducing ammonia production in litter and faecal water contents by their antagonistic action towards ammonifying bacteria and reducing urease activity (Patterson and Burkholder, 2003). This has also been confirmed by Chen *et al*. (2003) who concluded that supplementing broiler with the lactobacilli type probiotics Ecozyme reduced the environmental ammonia and volatile organic compound levels. It also reduced the pH and moisture content of the excreta.

Recently, there has been a great increase in the productivity of indigenous chicken in Kenya, which has caused a shift in consumer preference from broiler to indigenous chicken products since they are considered tasty and safe as they are produced naturally without growth hormones (Wachira, 2003). Another reason is due to the high production cost of broiler chicken rearing in terms of feed and disease control in comparison to rearing indigenous chicken. There has also been an increase in campaign for indigenous chicken rearing from the Ministry of Livestock where they are encouraging poultry farmers to venture into indigenous chicken rearing commercially by offering seminars and support to the common interest groups of farmers in various sub counties (Sibitali, 2013). This trend was also observed in Kiambu County during the survey.

3.7. Conclusions

There are many types of probiotics available in the market and the common probiotics being used are Product 1, Product 7, Product 2 and Product 4.

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CHAPTER FOUR

Experiment 2. Effects of common probiotics on broiler chicken performance in Kenya

Abstract

In this study, three hundred and seven, day-old broiler chicks were randomly assigned to dietary treatments containing different probiotics; Control diet, Diet 2 (Product 1), Diet 3 (Control + Product 4), Diet 4 (Control + Product 7) and Diet 5 (Product 2). Weekly body weight gain was determined and daily feed intake recorded. Feed conversion ratio was determined as the ratio of feed intake to body weight gain. Data was analyzed using Statistical Analysis Systems. Data for the starter (day old-21), finisher (22-42 days) and overall (day old-42) were analyzed to determine performance across the dietary treatments.

The mortality of broilers during the experiment was low (2.28 %). Dietary probiotics had no significant effect on daily weight gain which ranged from 19.05 to 22.59 g/d, feed intake (34.35 to 37.67 g/d) and feed conversion ratio (1.61 to 1.84) in broilers during starter phase. Similarly, the performance of broilers was not significantly affected by probiotic during the finisher phase except for Product 2 which depressed growth (28.44 vs. 47.38 g/d for control diet). The results of the study showed that addition of common probiotics to broiler diets was not beneficial to broilers performance while some depressed growth.

Key words: weight gain, treatments, probiotics, performance, feed intake, broilers.

4.1. Introduction

Poultry production has shown the highest increase in intensity compared to other livestock, with high growth rate and feed efficiency being the two main targets to ensure high production over the years (Yegani and Korver, 2008). The use of antibiotics increased concurrently with the intensification of the livestock industry to improve animal welfare and obtain economic benefits in terms of improved animal performance and reduced medical costs. However, there was an increasing risk of prevalence of antimicrobial-resistant bacteria in both humans and livestock as a result.

The ban on antibiotic use as growth stimulants for farm animals, and concerns regarding the side-effects from their use as therapeutic agents, has produced a climate in which both the consumer and manufacturer are looking for alternatives to antibiotics. Consequently, there has been a shift in consumer preference from broiler meat to indigenous chicken products

since they are considered tasty and safe as they are produced naturally without growth hormones (Wachira, 2003). With the increasing demand for quality animal products, as well as a vast awareness about the effects of these products on human health in Kenya, animal production systems have not only been focusing on increased production, but also on their effects on the environment and health of the consumers.

Probiotics have stood out as alternatives with the ability to maintain high productivity and to be economically feasible, as well as safe to human and animal health, thereby meeting the requirements of consumers and foreign markets. Palamidi *et al.,* (2016) concluded that probiotics have the potential to replace antibiotics as growth promoters since dietary inclusion of probiotics positively enhanced broiler performance in a similar manner to avilamycin supplementation. Results on Kenyan indigenous chicken suggested that supplementation with probiotics (Product 3) in drinking water significantly improved weight gain (Atela *et al*., 2015).

The results of a survey conducted among poultry farmers and Agro Veterinary stockiest in Kiambu County of Kenya, indicated that farmers use probiotics in poultry production and reported positive feedback on productivity. The most common feed additives sold as probiotics were; Product 1, Product 7, Product 2 and Product 4. The objective of this experiment was to evaluate the effects of probiotics on broiler performance.

4.2. Materials and methods

4.2.1. Study site

The study was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) station in Naivasha, Nakuru County. The area lies at an altitude of 2,086 m above sea level, with an annual mean range temperature of 17 to 28^0C and annual rainfall of <700-1400 mm/month.

4.2.2. Broilers and experimental facility

Three hundred and seven, day-old Cobb 700 broiler chicks (unsexed), were randomly allocated into 48 experimental cages, each having 6 or 7 chicks. The initial weight of the chicks was taken on arrival from the hatchery and randomly placed into individual experimental cages which measured 1.5 m^2 and equipped with a bell drinker and a feed trough. The chicks were housed in a clean, well-ventilated room, with a wood shaving floor that had been previously disinfected.

Temperatures were maintained at 32°C using infrared bulbs in the first week and then eventually heat was provided only at night since the usual ambient temperature during that season ranged between 25 to 28 ºC during the day. Vaccination against New Castle disease (NCD) was given at days 7 and 21 while that against Infectious Bursal Disease (Gumboro) was given at days 14 and 28 via drinking water as per the hatchery/ breeder recommendations. The feeding trial lasted for six weeks (42 days). The first experimental phase was the growing phase (which was from day old to 21) followed by finishing phase (from day 22 to 42).

4.2.3. Dietary treatments

The chicks were randomly assigned to the five dietary treatments which were; Diet 1 (Control), Diet 2 (Product 1 as a powder), Diet 3 (Control diet + Product 4 added in water), Diet 4 (Control diet + Product 7added in water) and Diet 5 (Product 2 as a powder). There were 10 replicates for three treatments (Control, Product 4 and Product 7) and 9 replicates for two treatments (Product 1 and Product 2). The chicks were fed on starter diet from day 1 to 21 and finisher diet from day 22 to 42 and all the diets were formulated to meet the NRC (1994) requirements.

The composition of the diets used in this experiment are shown in Tables 4.1 and 4.2 below. Product 1 and Product 2, which are in powder form, were added in the feed in accordance to the manufacturer's specifications while Product 4 and Product 7 were added into drinking water at the rate of 5 ml of microbes/1 litre once a day at 0900hrs. The diets and water were provided ad-libitum. No antibiotics were used during the entire experimental period.

4.2.4. Data collection

Weekly body weight gain measurements for each dietary treatment were determined by calculating the difference in weight between two consecutive weighing. Feed intake was monitored and recorded daily, and in the course of the whole experiment for each treatment, by carefully collecting, sieving out non-feed material and weighing all the leftover feed on the feeding trough which was subtracted from the initial feed offered. Feed conversion ratio (FCR) was determined as the ratio between feed intake and body weight gain as shown below;

FCR= Feed Intake (g/d)/ Body weight gain (g/d).

4.2.5. Statistical analysis

The experimental design was a Complete Randomized Design (CRD) and the model is; $Y_{ij=}\mu + t_i + \varepsilon_{ij.}$ Where Y_{ij} is the overall observation of the i^{th} treatment and j^{th} observation, μ is the overall mean, t_i is the treatment effect and ε_{ij} is the random error term.The data was analyzed using the General Linear Models (GLM) procedure of SAS version 9.00. Least Significant Difference (LSD) method at a level of ($P<0.05$) was used to separate significant means.

Table 4.1 Composition and nutrient content of starter (day 1 to 21) basal diets for broiler chicks (%)

Ingredient					
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Maize	61.60	60	61.60	61.60	61.45
Soybean meal	21.95	22	21.95	21.95	21.97
Fishmeal	12	12.15	12	12	12
Oil	2	2	2	2	2
Dicalcium Phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	0.45	0.45	0.45	0.45	0.45
Vitamin /Trace Mineral premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30
Probiotics					
None (Control)	-	-	-	-	-
Product 1	-	1.40	-	-	-
Product 4	-	-	+	-	-
Product 7	-	-	-	+	-
Product 2	-	-	-	-	0.13
Total	**100**	**100**	**100**	**100**	**100**
Calculated Analysis					
Calculated Crude Protein (%)	21.90	21.90	21.90	21.90	21.90
Metabolizable Energy (MJ/kg)	15.72	15.50	15.72	15.72	15.72
Lysine (%)	1.30	1.30	1.30	1.30	1.30
Methionine + Cysteine (%)	0.80	0.80	0.80	0.80	0.80

+ (added in water at 5 ml per 1 litre)

**Composition of vitamin/ trace mineral premix per kg diet:*

Vitamin A (8x 103IU); Vitamin D3 (2.0 IU); Vitamin E (10.0 IU); Vitamin K3 (1.5 mg); Vitamin B2 (2x10mg); Vitamin B12 (0.5mg); Folic acid (0.6mg); Nicotinic acid (5 mg); Calcium panthotenate (4mg); Choline (0.078mg); Trace elements: Mg (5x10mg); Zn (5x10mg); Cu (2.5mg); Co (0.5mg); I (2mg); Se (0.2mg). Antioxidants: Butylated hydroxytoluene (0.625mg); Carrier: Calcium carbonate q.s.p (0.25kg).

Table 4.2 Composition and nutrient content of finisher (day 22 to 42) basal diets for broiler chicks (%)

Ingredients					
	Diet 6	**Diet 7**	**Diet 8**	**Diet 9**	**Diet 10**
Maize	66.35	64.70	66.35	66.35	66.20
Soybean meal	19.20	19.45	19.20	19.20	19.22
Fishmeal	10	10	10	10	10
Oil	2	2	2	2	2
Dicalcium Phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	0.45	0.45	0.45	0.45	0.45
Vitamin /Trace mineral premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30
Probiotics					
None (Control)	-	-	-	-	-
Product 1	-	1.40	-	-	-
Product 4	-	-	+	-	-
Product 7	-	-	-	+	-
Product 2	-	-	-	-	0.13
Total	**100**	**100**	**100**	**100**	**100**
Calculated Analysis					
Calculated Crude Protein (%)	19.80	19.80	19.80	19.80	19.80
Metabolizable Energy (MJ/kg)	15.51	15.49	15.51	15.51	15.62
Lysine (%)	1.10	1.10	1.10	1.10	1.10
Methionine + Cysteine (%)	0.70	0.70	0.70	0.70	0.70

+ (added in water at 5 ml per 1 litre)

**Composition of vitamin/ trace mineral premix per kg diet:*

Vitamin A (8x 103IU); Vitamin D3 (2.0 IU); Vitamin E (10.0 IU); Vitamin K3 (1.5 mg); Vitamin B2 (2x10mg); Vitamin B12 (0.5mg); Folic acid (0.6mg); Nicotinic acid (5 mg);

Calcium panthotenate (4mg); Choline (0.078mg); Trace elements: Mg (5x10mg); Zn (5x10mg); Cu (2.5mg); Co (0.5mg); I (2mg); Se (0.2mg). Antioxidants: Butylated hydroxytoluene (0.625mg); Carrier: Calcium carbonate q.s.p (0.25kg).

4.3. Results

4.3.1. Mortality

The total mortality was generally low at 7 out of 307 broilers (2.28%) as shown in Table 4.3. The experimental group with the highest mortality rate was that containing Product 4which occurred within 2 weeks hence, its dosage was reduced from 5 ml per 1 litre of water daily to once a week to prevent further mortality. The diets containing Product 1 was second with two mortalities which occurred in the 4th week. The diets containing Product 7 had only one mortality which also occurred in the 4th week. There were no mortalities reported in the control diets and those containing Product 2.

Table 4.3 Summary of the mortality rate of broilers throughout the experimental period

Treatment	**Mortalities**	**(%)**
Control	0	0
Product 1	2	3.33
Control + Product 4	4	6.67
Control + Product 7	1	1.67
Product 2	0	0
Total	**7**	**2.28**

4.3.2. Broiler performance

The results on the effects of probiotics on the growth rate, feed intake and feed conversion during both the starter and finisher period are shown in Tables 4.4 – 4.6.

Table 4.4 Effects of treatments on average daily gain (ADG)

Growth rate	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	P value
ADGs	22.59 ± 0.91	22.16 ± 0.96	19.05 ± 0.91	20.43 ± 0.91	20.32 ± 0.96	0.0594
ADGf	47.38^{ba} ± 2.31	41.38^{b} ± 2.44	47.83^{ba} ± 2.31	48.75^{a} ± 2.31	28.44^{c} ± 2.44	0.0001
ADGo	34.98^{a} ± 1.44	31.77^{a} ± 1.51	33.44^{a} ± 1.44	34.59^{a} ± 1.44	24.65^{b} ± 1.51	0.0001

Where; Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets

[a, b, c] Means in the same row with different superscripts differ significantly (*P< 0.05*), The results are reported as Mean ± SEM (standard error of means), ADGs, Average daily gain starter, ADGf, Average daily gain finisher, ADGo, Average daily gain overall

The initial body weight of the day old chicks ranged from 39.8 to 45.5 g. The results indicate that the addition of probiotics had no significant (*P>0.05*) effect on the daily weight gain in the chicks during the starter phase (day 1-21). Daily weight gain in the finisher stage tended to increase significantly (*P< 0.05*) in the experimental groups and especially in diets 8 and 9 in comparison to the control. However, the diets 7 and 10 depressed the growth of the chicks in the finisher phase. Overall, diet 10 depressed growth rate of the broilers (Table 4.4).

Table 4.5 Effects of treatments on average daily feed intake (ADFI)

Feed Intake	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	P value
ADFIs	37.67± 1.49	35.68 ± 1.57	34.49 ± 1.49	35.54 ± 1.49	34.35 ± 1.57	0.5439
ADFIf	118.16^{a} ± 3.55	104.09^{b} ± 3.74	111.91^{ba} ± 3.55	110.67^{ba} ± 3.55	86.10^{c} ± 3.74	0.0001
ADFIo	72.78^{a} ± 2.44	65.88^{a} ± 2.57	68.18^{a} ± 2.44	69.14^{a} ± 2.44	56.54^{b} ± 2.57	0.0001

Where; Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets

$^{a, b, c}$ Means in the same row with different superscripts differ significantly (*P< 0.05*), The results are reported as Mean ± SEM (standard error of means), ADFIs, Average daily feed intake starter, ADFIf, Average daily feed intake finisher, ADFIo, Average daily feed intake overall

The results representing feed intake are shown in Table 4.5. Feed intake of broilers did not differ significantly *(P>0.05*) between the dietary treatment groups in the starter phase (day 1-21). However, diets containing Product 1 and Product 2 depressed daily feed intake during the 22-42 days finisher period (P<0.05). During the entire period of experiment, the diets containing Product 2 suppressed the feed intake by 22.3% (P<0.05) compared to the control diet.

Table 4.6 Effects of treatments on feed conversion ratio (FCR)

Feed Conversion	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	P value
FCRs	1.67 ± 0.06	1.61 ± 0.06	1.84 ± 0.06	1.75 ± 0.06	1.70 ± 0.06	0.1185
FCRf	2.52^{b} ± 0.15	2.57^{b} ± 0.16	2.45^{b} ± 0.15	2.29^{b} ± 0.15	3.04^{a} ± 0.16	0.0227
FCRo	2.09 ± 0.09	2.09 ± 0.09	2.07 ± 0.09	2.02 ± 0.09	2.33 ± 0.09	0.1544

Where; Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets.

[a, b, c] Means in the same row with different superscripts differ significantly (*P< 0.05*), The results are reported as Mean ± SEM (standard error of means), FCRs, Feed conversion ratio starter, FCRf, Feed conversion ratio finisher, FCRo, Feed conversion ratio overall

In the present experiment, there was no significant differences in FCR (*P>0.05*) among the treatments during the starter phase and overall period. However, in the finisher phase the feed efficiency was poorer (P<0.05) for the diet containing Product 2 (Table 4.6).

4.4. Discussion

Poultry farmers in Kiambu, Kenya ordinarily purchase feed additives which are marketed as probiotics from Agro-veterinary shops. Farmers reported that probiotics confer a positive effect on growth and chickens hardly get sick. In an earlier experiment, 15 products were found in the Kenyan market which were sold as probiotics. The most common of these were, Product 1, EM 1, Product 2 and Product 4. The price of probiotic which is adequate for either one tonne of feed or for 1000 litres of water ranged from Ksh 750 to 3500.

The responses to feeding diets containing probiotics on the performance of broiler chickens have been mixed with a majority of studies reporting positive responses. There are however other studies which have reported either no effect or negative responses (Table 4.7). In the current experiment, Product 4 was found to be deleterious to the performance of broilers in that the broilers started to die in the first one week of the experiment. The dosage offered in water was reduced from the recommended once a day to once a week. A postmortem carried out on the broilers that died showed that there was no feed found in the crop. An examination of the product showed that it contained a lot of sugar due to the presence of molasses. It is speculated that the broilers died of starvation perhaps because they eat to meet their energy needs. This energy need could have been met with the high sugar content of the probiotic. In their study, Toghyani *et al*. (2015) observed that the use of molasses kefir depressed daily weight gain and feed intake in broilers.

Furthermore, probiotics were of no benefit at all in the starter phase (1-21 days). The performance of the broilers supplemented with probiotics was as good as that of the broilers in control group. Similar results were obtained by Fernandes *et al*. (2014) who showed that broilers fed on probiotic, prebiotic, synbiotic and organic acids in the starter period were similar in weight gain to those in control group. Pelicano *et al*. (2004) also observed that there were no differences in weight gain for broilers receiving probiotics and control group in the starter phase.

During the finisher period (22-42 days), broilers fed diets containing Product 2 performed poorer those broilers on the other diets. Broilers offered diets contained Product 1 also tended to perform poorly. These two probiotics suppressed the feed intake and feed efficiency. Pelicano *et al*. (2004) reported similar results in their study in that a lower feed intake ($p<0.05$) was observed. This was associated with poor feed conversion resulting to lower

weight gain in the broilers in the finisher phase. The reasons for the poorer performance are not apparent.

Negative effects could also occur when high levels of probiotics are administered to chickens. A study carried out by Mahdavi *et al*. (2005) showed that using probiotics at levels of the 1000 and 2000 gr ton^{-1} in dietary treatments caused serious damages to absorptive area of digestive system. This caused a reduction in feed intake and negatively affected the FCR of the broilers since probiotic supplementation at these levels had almost damaged the apical cells significantly ($P<0.05$). In the current experiment a dose response of Product 2 on broiler performance was not tested.

There are many reports in the literature showing that probiotics have a positive effect on growth and feed efficiency (Table 7). Examples of the studies by Yin-bo Li *et al*. (2014) and Rahman *et al*. (2013) showed that administration of probiotics in diets of broilers displayed a growth-promoting effect and significantly improved the daily weight gain and feed efficiency. However, in the current experiment, broilers receiving probiotics did not perform better than the control ($P>0.05$). There are also other studies where probiotics have not shown any positive effect on performance (Table 7). Examples include the report by Wondmeneh *et al*. (2011) who concluded that supplementation of probiotic EM.1 had no significant effect on weight gain, mortality and FCR in the Fayoumi and Horro chicken breeds. Similar results were also observed by Andrew and Irene (2008) who concluded that probiotics generally have no effect on the growth performance of two strains of cockerels.

Growth performance and FCR obtained in broilers that were fed on a diet supplemented with a probiotic, "primalac", did not significantly improve compared with the control group (Ashayerizadeh *et al*., 2011). It is possible that the rearing environment for the broilers in this experiment presented a low stress situation where all factors of management were handled well, hence presenting a low challenge. Landy and Kavyani (2013) demonstrated that supplementation with the probiotic "primalac" to broilers reared under heat stress conditions had a favorable effect on performance, immune responses and cecal microflora. The other possible reasons for the lack of consistent results are low or variable viability of microbial cultures, strain differences in cultures selected, dose level and frequency of product feeding, antimicrobial and feed ingredient interactions which reduce/neutralize viable colonies before feeding, and composition of diet. It is therefore important to control the factors causing the variations for more consistent results (Fuller, 2006).

Conclusion

Overall, the results demonstrate that the probiotics sold in the Kenyan market were of no value on the performance of broilers under current experimental conditions. In fact some of them caused a negative effect of reduced feed intake and growth rate of the broilers especially in the finisher phase (22-42 days). Further research should be carried out to establish the circumstances under which probiotics in Kenya can positively impact on the performance of broilers. Additionally, more in depth analysis is required to establish the micro-organisms involved.

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CHAPTER FIVE

Experiment 3. Effect of common probiotics on the antibody production of broilers when vaccinated against infectious bursal disease

Abstract

Infectious bursal disease (Gumboro disease) is a viral infection, affecting the immune system of poultry. It is characterized by the destruction of the lymphoid organs, especially the bursa of Fabricius, where B lymphocytes mature and differentiate. The study tested the effects of common probiotics on antibody response to Infectious bursal disease.

Blood samples were collected from the wing vein of one chick per replicate on days 13 and 35. Enzyme-linked immuno-sorbent assays were used to determine antibody titres of the chicken against Infectious bursal disease where by a total of 34 samples were analyzed. The data was analyzed using the SAS version 9.00. Least Significant Difference (LSD) method at a level of ($P<0.05$) was used to separate significant treatment means. The results indicated that the addition of probiotics had no significant ($P= 0.6868$) effect on the antibody response to Infectious Bursal Disease after vaccination.

Key words: probiotics, infectious bursal disease, enzyme-linked immuno-sorbent assays, antibody response.

5.1. Introduction

The agricultural sector is the backbone of Kenya's economy and the major means of livelihood for most of the rural population. Livestock production has been identified as a useful development tool in Kenya and particularly poultry production, which is one of the fastest growing industries due to the increasing demand for poultry products (FAO, 2016). Diseases have been identified as major constraints in the development of the poultry industry in many developing countries thus causing a huge loss to farmers. In Kenya the main viral diseases of economic importance are Marek's disease, Newcastle disease and Infectious bursal disease (Nyaga, 2007).

Infectious bursal disease (IBD) has been a great challenge to the poultry industry in Kenya, causing a major setback to productivity and profitability in the industry hence rendering this investment risky (Musa *et al.*, 2010). Mutinda *et al*. (2016), concluded that IBD outbreaks

occurring in Kenya, cause high mortality rates in all chicken flock types; broilers, layers and indigenous chicken and are more severe in indigenous chicken. They also observed that outbreaks occur in vaccinated as well as in unvaccinated chicken flocks of all types.

Infectious bursal disease (Gumboro disease) is a viral infection, affecting the immune system of poultry. The disease is highly contagious and is caused by a Birnavirus of serotype 1 of the genus *Avibirnavirus* in the family *Birnaviridae* (Van den Berg *et al*., 2000). The affected broilers are listless and depressed, pale, ruffled feathers, huddling producing watery white diarrhea. Post-mortem lesions include dehydration of the muscles with numerous haemorrhages, enlargement and discoloration of the kidneys, with urates in the tubules.

In broilers that die at the peak of the disease outbreak, the bursa is enlarged and turgid with a pale yellow discoloration (OIE *Terrestrial Manual*, 2008). Early IBD infection result in permanent immunosuppression without mortality which is economically important due to increased susceptibility to secondary infections especially in the respiratory tract. In broilers this form of the disease results in poor performance with low weight gains and poor feed conversion while in layers low egg production. New cases of IBD have a mortality rate of about 5 to10% and can be as high as 60% depending on the pathogenicity of the strain involved. Highly pathogenic strains are called "very virulent" IBD (vvIBD) resulting in high mortality. Probiotics have been proven to increase the antibody titre against Infectious Bursal Disease in broilers (Landy and Kavyani, 2013).

The objective of the current experiment was to evaluate the effect of common probiotics in Kenya on the antibody responses to Infectious bursal disease vaccination in broilers.

5.2. Materials and methods

5.2.1. Vaccination

Vaccination was carried out according to the routine broiler vaccination programme. The broilers were vaccinated with Newcastle disease Lentogenic (B1 Strain) and Avian Infectious Bronchitis (Massachusetts H120 strain) live vaccine at day 7 and 21 via drinking water. They were vaccinated against IBD with Hipragumboro CH/80 Infectious Bursa Disease cloned live vaccine at day 14 and 28 via drinking water as per the manufacturer's recommendations.

5.2.2. Dietary treatments

The chicks were randomly assigned to the five dietary treatments which were; were; Diet 1 (Control with no probiotic), Diet 2 (with Product 1), Diet 3 (with Product 4), Diet 4 (with Product 7) and Diet 5 (with Product 2). The chicks were fed on starter diet from day 1 to 21 and finisher diet from day 22 to 42.

The probiotics (Product 1 and Product 2) ,which are in powder form, were added in the feed in accordance to the manufacturer's specifications while probiotics (Product 4 and Product 7), which were in liquid form ,were added into drinking water at 5 ml of microbes/1 litre once a day at 0900hrs. The diets and water were provided ad-libitum without any inclusion of antibiotics during the whole experimental period.

5.2.3. Sampling

Blood samples to determine serum titers of antibodies against Gumboro were collected from the wing vein of one chick per replicate on days 13 and 35. Enzyme-linked immunosorbent assays (IDEXX Laboratories, B.V. Netherlands) were used to determine antibody titres of the chicken against Infectious bursal disease. A total of 34 samples were analyzed for antibody production against Gumboro disease after vaccination.

5.3. Statistical analysis

5.3.1. Calculation of results

The presence or absence of antibody to IBD was determined by relating the A (650) value of the unknown to the Positive Control mean. The Positive Control was standardized and represented significant antibody levels to IBD in chicken serum. The relative level of antibody in the sample was determined by calculating the sample to positive (S/P) ratio. The following equations of calculation provided in ELISA kit were used for the calculation of antibody titre (IDEXX Laboratories, B.V. Netherlands. IBD).

a) Negative control mean ($\bar{NC}$)

$$\bar{NC} = \frac{\text{Well A1 (650)} + \text{Well A2 (650)}}{2}$$

b) Positive Control Mean ($PC\bar{x}$)

$$PC\bar{x} = \frac{\text{Well A3 (650) + Well A4 (650)}}{2}$$

c) S/P Ratio

$$S/P = \frac{\text{Sample Mean} - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$$

d) Titres were expressed as Log_{10} values using the following equation which relates S/P at a 1:500 dilution to an endpoint titer;

$$\text{Log10 Titer} = 1.09 (\log10\ S/P) + 3.36$$

The data was analyzed using the General Linear Models (GLM) procedure of SAS version 9.00, 2007. Least Significant Difference (LSD) method at a level of ($P<0.05$) was used to separate treatment means.

5.4. Results

5.4.1. Infectious bursal disease antibody titres

The results indicated that the addition of probiotics had no significant ($P= 0.6868$) effect on the antibody response to Infectious Bursal Disease after vaccination as shown in Table 5. 1.

Table 5.1 Antibody responses against infectious bursal disease (expressed as Log10 titre) of broilers fed on the dietary treatments.

Treatment	Log $_{10}$ Titre mean value
Diet 1	$2.61^{a} \pm 0.11$
Diet 2	$2.61^{a} \pm 0.11$
Diet 3	$2.67^{a} \pm 0.11$
Diet 4	$2.77^{a} \pm 0.11$
Diet 5	$2.85^{a} \pm 0.11$

[a, b, c] Means in the same column with different superscripts differ significantly ($P< 0.05$).

The results are reported as Mean ± SEM (standard error of means).

5.5. Discussion

According to this study, the addition of probiotics had no significant effect on the antibody responses to infectious bursal disease vaccination. The results agree with Balevi *et al.* (2009) who reported that probiotic supplementation did not affect specific antibody synthesis to Newcastle Disease vaccine antigen administered via drinking water. Similar results were observed in another study using the probiotic *B. longum* PCB133 in turkeys, where there was no significant immune response to NDV antibody production (Seifert *et al*., 2011). On the contrary, a study by Eman *et al*. (2014) showed that, the use of probiotic routinely in broiler diets improves the immune status and humeral immune response against New Castle Disease (ND) and Infectious Bursal Disease as well as treatment of *E. coli* infection in chicks.

So far, studies dealing with probiotic effects on vaccination efficiency on antibody production in poultry has shown mixed results This may be due to the effect of various factors like, low or variable viability of microbial cultures, strain differences in cultures selected, dose level and frequency of product feeding, antimicrobial and feed ingredient interactions which reduce/neutralize viable colonies before feeding, composition of diet, effects of age and strain-host interactions.

The exact mechanisms of stimulation of immune response by probiotics have not been fully explained but several studies have shown that they may stimulate different subsets of immune system cells. A study on oral administration of probiotics in broilers has shown significant

effects on both the systemic and mucosa-associated immune responses, resulting in disease prevention (Dallout *et al*., 2003). According to the results of our study, it is uncertain if probiotics stimulate mucosal, cellular or humoral immunity response in broilers since we only focused on systemic immunity. Therefore, further studies on immunity response should be considered to focus on experimental designs examining mucosal, cellular and humoral immunities.

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CHAPTER SIX

Experiment 4. Antimicrobial susceptibility test of common probiotics in Kenya on bacterial cultures.

Abstract

The disk diffusion test was used to test the inhibitory effect of 16 probiotic treatment replicates A, B, C and D; Product 1, Product 4, Product 7 and Product 2 respectively, on the following pure bacteria cultures; *Escherichia coli***,** *Staphylococcus aureus, Bacillus cereus* and *Candida albicans.* The test showed that two of the probiotics; Product 4 and Product 7 had inhibitory effect while the other two; Product 1 and Product 2 had no inhibitory effect on the bacteria cultures. In conclusion, Product 4 and Product 7 showed positive results in both experiments but it is unknown whether these products contain antibiotics or the probiotic strains in the products have antimicrobial effect.

Key words: *Staphylococcus aureus, Escherichia coli,* probiotic, *Candida albicans,* bacteria culture, *Bacillus cereus, a*ntimicrobial effect.

6.1. Introduction

Probiotics have been reported to stimulate the immunity of the host through suppression of viable count of bacteria populations by production of antimicrobial compounds. Antimicrobial effects of probiotic species like; *Lactobacillus GG*, *L. casei*, *B. bifidum* and *S. thermophilus* are formed by producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins (Quwehand and Vesterlund, 2004).

The aim of this study was to determine whether the products sold in the market as probiotics are antibiotics or have any antimicrobial effect on some common bacterial pathogens. The disk diffusion test was used to test the inhibitory effect of the probiotic treatments A, B, C and D; Product 1, Product 4, Product 7 and Product 2 respectively, on the following pure bacteria cultures; *Escherichia coli***,** *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans.*

6.2. Disk diffusion test

The disk diffusion test is a simple procedure for screening substances to determine if they have significant antibiotic activity and its reagents include:

Müeller-Hinton agar medium

Müeller-Hinton agar is considered to be the best for routine susceptibility testing of no fastidious bacteria for reasons such as; it shows acceptable batch-to-batch reproducibility for susceptibility testing. It is also low in sulphonamide, trimethoprim, and tetracycline inhibitors and gives satisfactory growth of most no fastidious pathogens.

Müeller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath. It was then cooled and poured into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. The agar medium was allowed to cool to room temperature, stored in a refrigerator (2 to 8°C) and were ready for use (Lalitha, 2004).

The probiotic solutions

For probiotic samples A (Product 1) and D (Product 2) which were in powder form, 1 gram of each treatment was accurately weighed and dissolved in 9ml of dilute water for 1 hour to yield the required concentration, using sterile glassware. For samples B (Product 4) and C (Product 7) which were in liquid form, 5 ml of each treatment was measured and used in their concentrated form. The 4 probiotic samples were aliquoted in 5 ml volume solutions.

Dried filter paper discs

Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter, which were placed in a Petri dish and sterilized in a hot air oven.

Commercial anti-microbial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. The containers were refrigerated at 8°C or below in a no frost-free freezer until needed. The unopened disc containers were removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening to minimize the amount of condensation that occurs when warm air contacts cold disks.

6.3. Materials and methods

6.3.1. Control disc plates

6.3.1.1. Antibiotic treated disc plates

A standard disc plate showing the inhibitory effect of 8 antibiotics; Tetracycline (TE), Streptomycin (S), Kanamycin (K) Gentamycin (GEN), Sulphamethoxazole (SX), Co-Trimoxazole (COT), Chloramphenicol (C) and Ampicilin (AMP) on *Staphylococcus aureus* was used as shown in Plate 6.1.

6.3.1.2. Sterilized distilled water treated disc plates

Two disc plates were used as a control where only sterilized distilled water was used and the bacterial cultures used were *E. coli* and *Staph. Aureus* as shown in Plate 6.2.

6.3.2. Probiotics treated disc plates

16 disc plates had 4 probiotic treatments with 4 bacteria cultures of *E. coli, Staph. Aureus, Bacillus cereus* and *Candida albicans.* The dried surface of a Müeller-Hinton agar plate was inoculated with the bacteria cultures by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. After that, the lids were placed and the plates allowed to dry for 2-5 minutes.

The sterilized filter papers discs (Whatmann No. 1) were saturated with the probiotic solutions for 1 hour in a dispenser. Using sterile forceps, the discs were then transferred on to the surface of each plate. The lids were replaced and the plates inverted. The plates were then labelled at the bottom and incubated at 38°C for 2 days (Hudzicki, 2009).

6.4. Results

After incubation, the plates were examined for zones of inhibition, which are the areas wherein there is a prominent reduction of 80% growth.

Product 1 (Sample A) and Product 2 (Sample D) showed no inhibitory effect on the growth of any microorganism cultures; *E. coli, Staph. Aureus, Bacillus cereus* and *Candida albicans* as shown in plates (6.3 and 6.4) and (6.9 and 6.10) respectively. Product 4 (Sample B) and

Product 7 (Sample C) showed inhibitory effects on all the cultured microorganisms as shown in plates (6.5 and 6.6) and (6.7 and 6.8) respectively. All plate photos are at Appendix 6

6.5. Discussion

Bacillus cereus is a Gram-positive, aerobic or facultative anaerobic, catalase positive and spore-forming microorganism capable of causing foodborne diseases like diarrheal and emetic in humans (Patel *et al*., 2009). The symptoms of *B. cereus* diarrheal type food poisoning include abdominal pain, watery diarrhea, rectal tenesmus, moderate nausea that may accompany diarrhea, seldom vomiting and no fever. The signs of *B. cereus* emetic type food poisoning include nausea, vomiting, abdominal cramps and/or diarrhea. The genus *Bacillus* is very diverse and is divided into six subgroups based on spore morphology. *B. cereus* is classified in the *Bacillus subtilis* group and closely related to *B. anthracis, B. mycoides* and *B.thuringiensis*. *B. cereus* and *B. anthracis* are both recognized as pathogens (Griffiths and Schraft, 2002).

The genus *Staphylococcus* is composed of Gram-positive bacteria with diameters of 0.5-1.5 μm, characterized by individual cocci that divide in more than one plane to form grape-like clusters. It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci. *S. aureus* causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicemia. This bacterium is also one of the most common species in nosocomial infections. In addition, *S. aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Winn Washington, 2006).

Belonging to the family of Enterobacteriaceae, *E. coli* is a coccobacillus Gram negative (2-3 x 0.6 μm), nonspore- forming and able to grow in aerobic and non-aerobic condition. It can have a capsule and most strains are motile and have peritrichous flagella. It causes food poisoning in humans and colibacillosis in poultry especially layers. Colibacillosis causes elevated morbidity and mortality leading to economic losses on a farm especially around the peak of egg production and throughout the late lay period. Indeed, *E. coli* is a common inhabitant in the intestinal tracts of poultry at concentrations up to 106/g. Coliform bacteria

can be found in litter and fecal matter, and dust in poultry houses may contain 105—106 *E.coli*/g.

This bacteria persist for long periods, particularly under dry conditions and its presence in drinking water is an indication of fecal contamination. Stress acts as a predisposing factor for the manifestation of colibacillosis in broilers be it, infectious, physical, toxic, and/ or nutritional and is characterized by the presence of exudations in the peritoneal (abdominal) cavity including serum, fibrin, and inflammatory cells (pus). Fibrin, a white to yellow material, is the product of the inflammatory response in the chicken and can be seen covering the surfaces of multiple organs including the oviduct, ovary, intestine, air sacs, heart, lungs, and liver.

Colibacillosis is a common cause of sporadic death in both layers and breeders, but can cause sudden increased mortality levels in a flock. Inflammation of the oviduct (salpingitis) caused by *E. coli* infection results in decreased egg production and sporadic mortality, and it is one of the most common causes of mortality in commercial layer and breeder chickens (Nolan, 2013). Colibacillosis in neonatal chicks can also be a consequence of poor chick quality and sanitation in the hatchery, leading to early chick mortality and this is commonly due to egg transmission of pathogenic *E. coli*. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from which they hatched, suggesting rapid spread after hatching. The most important source of egg infection seems thus to be fecal contamination of the egg surface with subsequent penetration of the shell and membranes.

Candida species are eukaryotic opportunistic pathogens that reside on the mucosa of the gastrointestinal tract as well as the mouth and oesophagus (Kim and Sudbery, 2011). They are major human fungal pathogens that cause both mucosal and deep tissue infections. They also cause candidiasis in humans, which results from an overgrowth of the fungus. Candida albicans is a type of yeast that belongs to the Family *saccharomycetaceae* and Genus *candida*. Over a period of time, pathogens have developed a resistance to antibiotics that were commonly used to treat them hence making them more virulent and expensive to treat. For example, resistance to quinolones has been reported in a variety of important bacterial pathogens, including *E. coli, K. pneumoniae* and other enteric organisms; *P. aeruginosa*; *Chlamydia trachomatis, Mycoplasma pneumoniae*; *Campylobacter jejuni, B. cepacia*; *S. maltophilia*, *N. gonorrhoeae, S. aureus, Enterococcus faecium* and *S. pneumoniae* (Lalitha, 2004).

The ability of Candida species to form drug-resistant biofilms has been considered an important factor in their contribution to human diseases (Sardi *et al*., 2013). 80% of all *S. aureus* strains were resistant to penicillin since 1960 and to treat infections caused by penicillin-resistant *S. aureus*, a semi-synthetic antibiotic methicillin, which is derived from penicillin, but resistant to β-lactamase inactivation, was introduced in 1959 (Deurenberg and Stobberingh, 2008). However, in 1961 there were reports from the United Kingdom that *S. aureus* isolates had acquired resistance to methicillin, MRSA (methicillin resistant *S. aureus*) and they were soon recovered from other European countries, and later from Japan, Australia, United States, and now, MRSA is a leading cause of nosocomial infections worldwide emerging as a community-associated pathogen (Boyce, 2005; Chambers and Deleo, 2009). It is due to these reasons that alternatives like probiotics have been studied as natural and effective means of controlling these pathogens.

It is essential for probiotic strains to show antagonism against pathogenic bacteria through antimicrobial substance production or competitive exclusion, to have an impact on the digestive system flora. Research has shown that different species produce different antimicrobial substances like: *Lactobacillus reuterii* produce a low molecular weight antimicrobial substance called reuterin and the subspecies of *Lactococcus lactis* produce a class I bacteriocin, known as nisin A. *Enterococcus feacalis* DS16 produces a class I bacteriocin cytolysin and *Lactobacillus plantarum* produces a class II bacteriocins plantaricin S. Lastly, *Lactobacillus acidophilus* produces a class III bacteriocin acidophilucin A (Quwehand and Vesterlund, 2004).

An assessment on the presence of antimicrobial effects among the probiotics isolated from different bio yoghurts; *Lactobacillus* sp., *Streptococcus* sp. and *Bifidobacterium* sp against some common bacterial pathogens; *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*. The results showed the presence of antimicrobial effects among the probiotics that were isolated from bio yoghurts (Hami, 2011). Results from another study showed that the growth of pathogenic bacteria, *Staphylococcus aureus*, was inhibited in the peritoneal cavity of animals treated with *Lactobacillus acidophilus* and *Saccharomyces boulardii*, whereas such inhibition of the bacterial growth was not observed in the control group (Ali *et al*., 2014). Duncan (2017) concluded that viable *S. cerevisiae* cells not only physically inhibited the *C. albicans* colonization of epithelia, but also directly inhibited the elaboration of several key pathogenicity factors.

However, results from a different study showed that treatment of *E. coli* with probiotic suspension was not effective on inhibition of the plasmid carrying hypothetical ampicillin resistant gene (Naderi *et al*., 2014). Despite the variability in results, probiotics still provide the best alternative for prevention and treatment of various pathogenic microorganisms without causing harmful side effects to both animals and humans.

6.6. Conclusion

The Antimicrobial Susceptibility Test of the common probiotics proved that two of the probiotics; Product 4 and Product 7 had antimicrobial effect. Product 1 and Product 2 had no inhibitory effect on the growth of the bacteria cultures of *Escherichia coli***,** *Staphylococcus aureus, Bacillus cereus* and *Candida albicans*. It is unknown whether antibiotics were actually added to these products or the probiotic strains in the products have antimicrobial effects on bacterial pathogens. Therefore, further studies are recommended to determine the ingredients and contents of the probiotics sold in Kenyan market to verify this.

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CHAPTER SEVEN

GENERAL DISCUSSION

7.1. Use of antibiotics in livestock production

It has been proposed that the use of antibiotics as feed additives was an integral part of the revolution in animal-production technology for therapeutic treatment, disease prophylaxis and the promotion of growth. A review paper by Sinovec and Radmila (2005) outlined some of the salient features of Antibiotic Growth Promoters (AGPs) in poultry production. AGPs modify the intestinal micro flora and help to improve bird's performance and health status. They also have inhibitory effect on enzymes released by microorganisms and also on enzymes involved in microbial metabolism. Addition of antibiotics to feed increases the amino acid levels in the gut and improves nitrogen balance and absorption of feed nutrients because of thinning of intestinal wall. They also have been proven to increase egg production and hatchability in layers and reduce stress and mortality in chicks by boosting body defense. They reduce the damage caused by dietary fluctuations and destroy the harmful bacteria, keeping and minimize the adverse effects of dietary changes.

Antibiotics prevent exponential multiplication of common pathogenic bacteria (E. coli, Salmonella spp., Streptococcus spp and Hemophilus) hence reducing incidences of non-specific diarrhea or enteritis of chicken. The emergence and spread of antibiotic resistant bacterial strains such as *Campylobacter* sp, *Escherichia coli* and *Enterococcus* sp. from poultry products to consumers put humans at risk to new strains of bacteria that and hence certain essential life-saving antimicrobials are becoming less effective. There are fewer alternatives available to treat the diseases for which these antimicrobials are required. However, the increasing risk of prevalence of antimicrobial-resistant bacteria in both humans and livestock, linked to the use of antibiotics in animal production, led to the ban of antibiotics as growth promoters in animal feed in 2006 by the European Union. The possibility of antibiotics ceasing to be used as growth stimulants for farm animals and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumers and feed manufacturers are looking for alternatives.

7.2. Probiotics as an alternative to antibiotics

Probiotics have stood out as the alternatives that are able to maintain high productivity and to be economically feasible, as well as not being harmful to human and animal health, thereby

complying with the requirements of consumers. According to the currently adopted definition by (FAO/WHO, 2009) probiotics are “live microorganisms which when administered in adequate amounts, confer a health benefit on the host”.

Probiotics have been reported to work through various modes such as; suppression of viable count of bacteria populations by production of antibacterial compounds and competition for nutrients and adhesion sites which suppress growth of the bacteria colonies along the gut. Another mode of action is the alteration of microbial metabolism by increasing or decreasing the enzyme activity in the gut. Probiotics also stimulate the immunity of the host by increasing the antibody levels and macrophage activity (Patterson and Burkholder, 2003).

In poultry production, probiotics have been proven to lower the number of bacterial pathogens like *S. enteritidis*, *C. perfringens*, coliform and Campylobacter in the gut (Khaksefidi and Rahimi, 2005). They also enriched the diversity of Lactobacillus flora in jejunum and caecum by increasing the abundance and prevalence of Lactobacillus spp. inhabiting the intestine. Probiotics has been reported to restore the microbial balance and maintained the natural stability of indigenous bacterial microbiota in the gut (Lan *et al.*, 2003). Furthermore, a study by Khan *et al.* (2011) showed that probiotics increased antibody titre against Newcastle disease (ND). Therefore, probiotics can serve as suitable alternatives to antibiotics in non-ruminant nutrition and may be effectively used.

7.3. Use of probiotics in Kenya

There are feed additives which are marketed as probiotics by Agro-veterinary shops in Kenya. In experiment 1 of this study, 15 products were found in the Kenyan market which were sold as probiotics. The most common of these were, Product 1, Product 7, Product 2 and Product 4. The price of probiotic which is adequate for either one tonne of feed or for 1000 litres of water ranged from Ksh 750 to 3500. Poultry farmers in Kiambu, Kenya have accepted to use "probiotics" in poultry production and are reporting positive feedback on production. . Farmers reported that probiotics confer a positive effect on growth and chickens hardly get sick.

Poultry farmers reported that after using the probiotics, they observed an increase in feed intake among the chicken and hence faster growth rate. Samanya and Yamauchi (2002) indicated that the broilers fed on probiotics had a tendency to display pronounced intestinal

histological changes such as prominent villi height which increase the rate of absorption of available nutrients hence resulting in greater growth performance and increase in body weight gain. This enhanced performance could be due to the increased digestion and the observations by farmers. The farmers also indicated low mortality rates among the young chicks and good health among their flock after using probiotics. Similarly, Talebi *et al*. (2008) stated that probiotics feeding also have been reported to improve antibody titres against viral diseases like Newcastle Disease (ND) and Infectious Bursal Disease (IBD).

In layers, farmers noted reduction in stress factor especially during peak production, production of strong egg shells and maintainace of optimum egg production. Zarei *et al*. (2011) reported an increase in egg mass and weight and egg shell weight and thickness on feeding laying hens with diets supplemented with some commercial probiotics, prebiotics and synbiotics.

Poultry farmers reported the production of dry litter by the chicken on administering probiotics and reduction of bad odor in the pens. Probiotics have been found useful in reducing ammonia production in litter and faecal water contents by their antagonistic action towards ammonifying bacteria and reducing urease activity (Patterson and Burkholder, 2003). Chen *et al*. (2003) concluded that supplementing broiler with the lactobacilli type probiotics Ecozyme reduced the environmental ammonia and volatile organic compound levels. It also reduced the pH and moisture content of the excreta.

Probiotics have been reported to work through various modes like; suppression of viable count of bacteria populations by production of antibacterial compounds and competition for nutrients and adhesion sites which suppress growth of the bacteria colonies along the gut. Another mode of action is the alteration of microbial metabolism by increasing or decreasing the enzyme activity in the gut. Probiotics also stimulate the immunity of the host by increasing the antibody levels and macrophage activity.

7.4. Do the probiotics work?

In a controlled experiment, Product 1, Product 7, Product 2 and Product 4 were tested in broiler chicken to evaluate their effects on performance. The probiotics were found to be of no benefit at all in the starter phase (1-21 days). The performance of the broilers supplemented with probiotics was as good as that of the broilers in control group. Similar results were obtained by Fernandes *et al*. (2014) who showed that broilers fed on probiotic,

prebiotic, synbiotic and organic acids in the starter period were similar in weight gain to those in control group. Pelicano *et al*. (2004) also observed that there were no differences in weight gain for broilers receiving probiotics and control group in the starter phase.

During the finisher period (22-42 days), broilers fed diets containing Product 2 performed poorer those broilers on the other diets. Broilers offered diets contained Product 1 also tended to perform poorly. These two probiotics suppressed the feed intake and feed efficiency. Pelicano *et al*. (2004) reported similar results in their study in that a lower feed intake ($p<0.05$) was observed. This was associated with poor feed conversion resulting to lower weight gain in the broilers in the finisher phase. The reasons for the poorer performance are not apparent.

7.5. Antibiotic properties of the feed additives investigated

The disk diffusion test was used to test the inhibitory effect of the probiotics; Product 1, Product 4, Product 7 and Product 2 respectively, on the following pure bacteria cultures; *Escherichia coli***,** *Staphylococcus aureus, Bacillus cereus* and *Candida albicans.* The aim of this study was to determine whether the products sold in the market as probiotics are antibiotics or have any antimicrobial effect on some common bacterial pathogens.

The results of this study showed that two probiotic treatments; Product 4 and Product 7 showed antimicrobial effects on all the bacterial cultures used. Product 1 and Product 2 did not show any antimicrobial effect on the bacteria. Research has shown that different species produce different antimicrobial substances like: *Lactobacillus reuterii* produce a low molecular weight antimicrobial substance called reuterin and the subspecies of *Lactococcus lactis* produce a class I bacteriocin, known as nisin A. *Enterococcus feacalis* DS16 produces a class I bacteriocin cytolysin and *Lactobacillus plantarum* produces a class II bacteriocins plantaricin S. Lastly, *Lactobacillus acidophilus* produces a class III bacteriocin acidophilucin A (Quwehand and Vesterlund, 2004).

7.6. Antibody responses to infectious bursal disease

In the experiment to test the antibody response of the probiotic treatments to Infectious Bursal Disease, it was observed that, the addition of probiotics had no significant effect. Our results agree with Balevi *et al.* (2009) who reported that probiotic supplementation did not affect specific antibody synthesis to Newcastle Disease vaccine antigen administered via

drinking water. Similar results were observed in another study using the probiotic *B. longum* PCB133 in turkeys, where there was no significant immune response to NDV antibody production (Seifert *et al*., 2011).

7.7. Conclusions

a) The Kenyan farmer in Kiambu has accepted to use probiotics in poultry production with the most common being, Product 1, Product 7, Product 2 and Product 4.

b) However, the results demonstrated that the inclusion of feed additives marketed as "probiotics" were of no value on the performance of broilers. In fact some of them caused a negative effect in that it reduced feed intake and growth rate of the broilers especially in the finisher phase (22-42 days).

c) It was observed that the addition of these additives believed to be probiotics had no significant effect in the experiment to test the antibody response of the probiotic treatments to Infectious Bursal Disease.

d) Product 4 and Product 7 showed positive results in the experiments on the antimicrobial susceptibility test.

7.8. Recommendations

a) Analysis of the probiotics in the market is required to establish the micro-organisms involved.

b) The common feed additives in the Kenyan market should be classified scientifically as probiotics, prebiotics, and synbiotics based on their ingredients and mode of action in the gut.

c) Further research should be carried out to establish the circumstances under which probiotics in Kenya can positively impact on the performance of broilers.

d) Research to determine the effect of probiotics on immune response on various poultry diseases focusing more on experimental designs examining mucosal, cellular and humoral immunities.

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APPENDICES

Appendix 1. Questionnaire for evaluating the extent to which probiotics are available and used in Kiambu County.

Introduction

My name is Evelyn Nyathogora Ngunyangi. I am a graduate student from Egerton University majoring in MSc. Animal Nutrition. The purpose of this survey is to determine the 'feed additives' sold in the market as probiotics and their cost.

I appreciate you taking the time to complete the following survey. Your responses are voluntary and will be confidential. All responses will be compiled together and analyzed as a group. In case you have any questions regarding the survey, please contact me through my phone number…….. Thank you very much for your time and suggestions.

Agro vets' questionnaire

Name of Agro Vet; ----------

Location; ----------

DATE: --------/----------/2015

Product Identity

Which probiotic is available in the market?

Name of probiotic	Manufacturer/ Distributor	Purpose	Price
1			
2			
3			
4			

Appendix 2: Poultry farmer's questionnaire.

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OFFICE OF THE CHAIRMAN

DEPARTMENT OF ANIMAL SCIENCE

Date: _____

Introduction

My name is Evelyn Nyathogora Ngunyangi. I am a graduate student from Egerton University majoring in MSc. Animal Nutrition. The purpose of this survey is to determine the 'feed additives' used for poultry production by farmers and their effect on poultry performance.

I appreciate you taking the time to complete the following survey. Your responses are voluntary and will be confidential. All responses will be compiled together and analyzed as a group. In case you have any questions regarding the survey, please contact me through my phone number……. . Thank you very much for your time and suggestions.

Farmers' Questionnaire

Name of Sub County: _____

Location of the Farm: _____

Section A: Farmer's Details

Name of the Respondent:_____

Form of Training: _____

Type of the Farm: _____ **Household=1,**

Commercial =2

Section B: Poultry Production Practices and use of Feed Additives

1. **What is your flock size:** _____

2. **What type of poultry do you keep?** _____

   a) Broiler

   b) Layers

   c) Local

Do you use any booster on poultry?

Name of Booster	**Growth Stage**	**Purpose**	**Dosage**	**Frequency**

3. **Where did get information on use of the booster? [_____]**

   a) From veterinary

   b) From agro vet

   c) From extension officers

   d) From Lead Farmers

   e) Other, specify

4. **What quantity of the booster do you use per month?** _____

5. **Does it have any effect on the performance of the birds? Yes / No**

If yes, specify; _____

Section C: Marketing Plan of Production

6. **How often do you sell your birds?** _____

7. **What is the average weight of the birds at the time of sale?** _____

8. **What is the price of the birds per kg during sale?** _____

Farmer's contacts: _____

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Appendix 3. Coding of probiotics

Serial Number	Product	Coded Name
1	Vipro plus	Product 1
2	Diamond V (xpc)	Product 2
3	Molaplus	Product 3
4	Super Enzymes Poultry Microbes	Product 4
5	Magic Boost	Product 5
6	Magic set Broiler	Product 6
7	EM.1 (Effective Micro-organisms)	Product 7
8	Bio-active Microbes	Product 8
9	Super Booster	Product 9
10	Antox	Product 10
11	Rhonjas Super Poultry Microbes	Product 11
12	Brema- Bloom	Product 12
13	Elimin-8	Product 13
14	Bio spark V	Product 14
15	Caplix	Product 15
16	Zegg Booster	Product 16

Appendix 4: Effects of common probiotics on broiler performance in Kenya

The GLM Procedure

Dependent Variable: ADGg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	82.1759222	20.5439806	2.46	0.0594
Error	43	358.7674444	8.3434289		
Corrected Total	47	440.9433667			

Dependent Variable: ADGf

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2547.208872	636.802218	11.90	<.0001
Error	43	2300.406109	53.497816		
Corrected Total	47	4847.614981			

Dependent Variable: ADGo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	664.181980	166.045495	8.06	<.0001
Error	43	885.786020	20.599675		
Corrected Total	47	1549.968000			

Dependent Variable: ADFIg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	69.313054	17.328264	0.78	0.5439
Error	43	954.382212	22.194935		
Corrected Total	47	1023.695267			

Dependent Variable: ADFIf

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5620.01310	1405.00328	11.13	<.0001
Error	43	5427.58469	126.22290		
Corrected Total	47	11047.59779			

Dependent Variable: ADFIo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1385.153746	346.288437	5.83	0.0008
Error	43	2556.183646	59.446131		
Corrected Total	47	3941.337392			

Dependent Variable: FCEg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.28476278	0.07119069	1.96	0.1185
Error	43	1.56502889	0.03639602		
Corrected Total	47	1.84979167			

Dependent Variable: FCEf

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.90494347	0.72623587	3.17	0.0227
Error	43	9.84105444	0.22886173		
Corrected Total	47	12.74599792			

Dependent Variable: FCEo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.55805611	0.13951403	1.76	0.1544
Error	43	3.40746889	0.07924346		
Corrected Total	47	3.96552500			

t Tests (LSD) for ADGg

Means with the same letter are not significantly different.

t Grouping		Mean	N	TREATMENT
	A	22.592	10	1000
	A	22.156	9	1001
B	A	20.430	10	1003
B	A	20.316	9	1004
B		19.054	10	1002

t Tests (LSD) for ADGf

Means with the same letter are not significantly different.

t Grouping		Mean	N	TREATMENT
	A	48.749	10	1003
B	A	47.829	10	1002
B	A	47.384	10	1000
B		41.380	9	1001
	C	28.979	9	1004

t Tests (LSD) for ADGo

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	34.987	10	1000
A	34.590	10	1003
A	33.443	10	1002
A	31.767	9	1001
B	24.647	9	1004

t Tests (LSD) for ADFIg

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	37.669	10	1000
A	35.680	9	1001
A	35.537	10	1003
A	34.491	10	1002
A	34.346	9	1004

t Tests (LSD) for ADFIf

Means with the same letter are not significantly different.

t Grouping		Mean	N	TREATMENT
	A	118.159	10	1000
B	A	111.905	10	1002
B	A	110.666	10	1003
B		104.091	9	1001
	C	86.104	9	1004

t Tests (LSD) for ADFIo

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	72.776	10	1000
A	69.142	10	1003
A	68.181	10	1002
A	65.882	9	1001
B	56.543	9	1004

t Tests (LSD) for FCEg

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	1.84100	10	1002
B A	1.74700	10	1003
B A	1.70000	9	1004
B A	1.67400	10	1000
B	1.61111	9	1001

t Tests (LSD) for FCEf

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	3.0389	9	1004
B	2.5722	9	1001
B	2.5180	10	1000
B	2.4500	10	1002
B	2.2950	10	1003

t Tests (LSD) for FCEo

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREATMENT
A	2.3333	9	1004
B A	2.0930	10	1000
B A	2.0889	9	1001
B	2.0680	10	1002
B	2.0170	10	1003

Appendix 5. Effect of common probiotics fed to broilers on antibody production when vaccinated against Infectious Bursal Disease

The GLM Procedure

Dependent Variable: Logvalue

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.24312890	0.06078223	0.57	0.6868
Error	29	3.09495290	0.10672251		
Corrected Total	33	3.33808180			

t Tests (LSD) for Logvalue

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	2.8543	4	1004
A	2.7699	8	1003
A	2.6715	9	1002
A	2.6115	8	1000
A	2.6099	5	1001

Appendix 6. Photos of plates

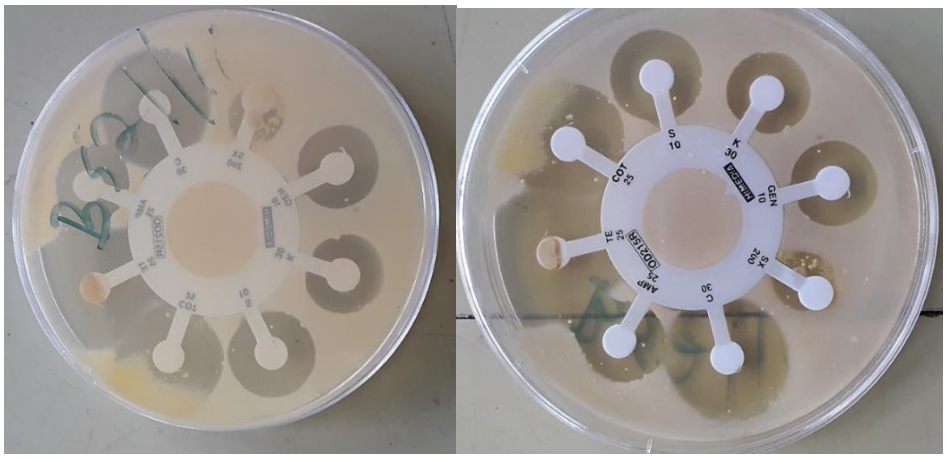

Plate 6.1 Disc plate showing the inhibitory effect of 8 different antibiotics on *Staphylococcus aureus.*

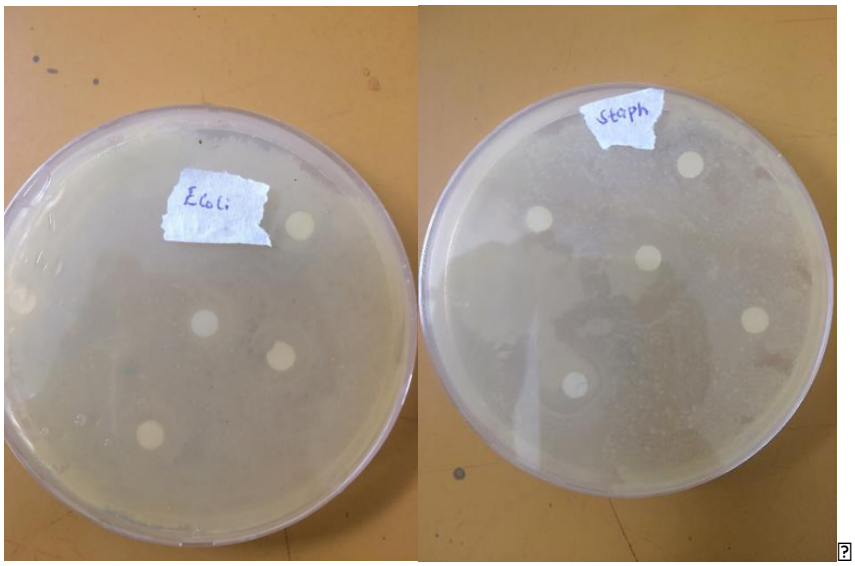

Plate 6.2 The control disc plates with *Staphylococcus aureus* and *Escherichia coli*

Plate 6.3 The inhibitory effect of sample A on *Staphylococcus aureus* and *Escherichia coli*

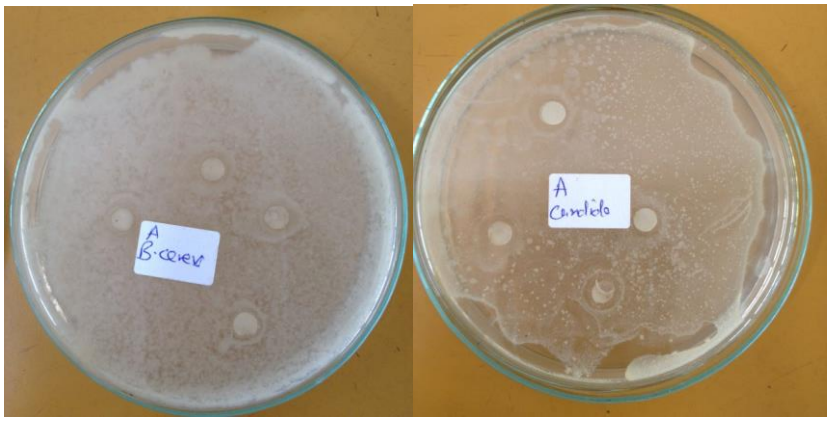

Plate 6.4 The inhibitory effect of sample A on *Bacillus cereus* and *Candida albicans*

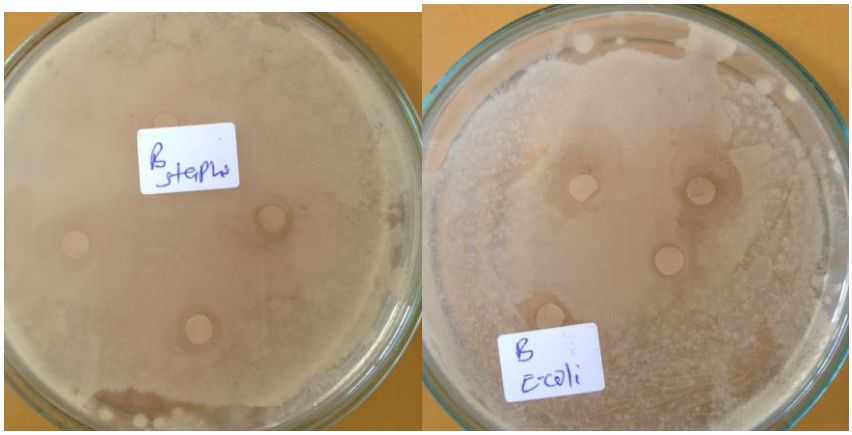

Plate 6.5 The inhibitory effect of sample B on *Staphylococcus aureus* and *Escherichia coli*

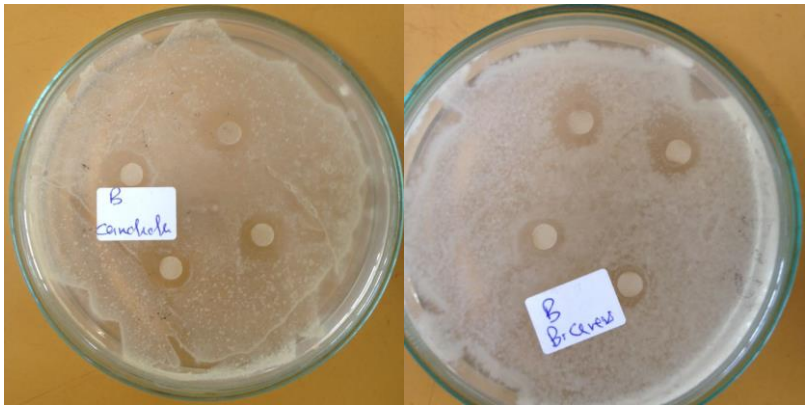

Plate 6.6 The inhibitory effect of sample B on *Candida albicans* and *Bacillus cereus*

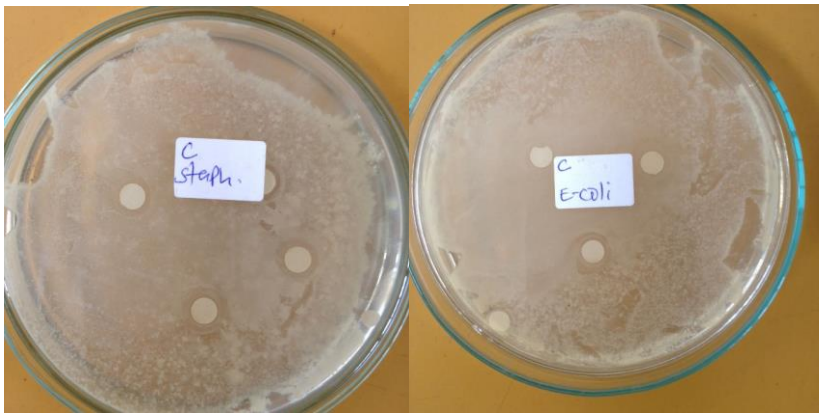

Plate 6.7 The inhibitory effect of sample C on *Staphylococcus aureus* and *Escherichia coli*

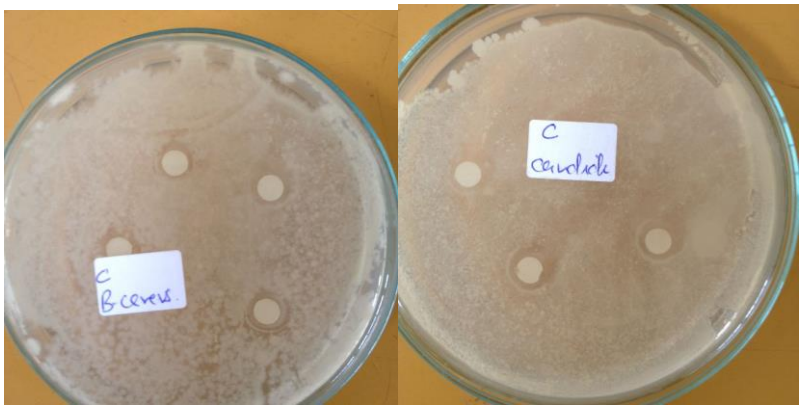

Plate 6.8 The inhibitory effect of sample C on *Bacillus cereus* and *Candida albicans*

Plate 6.9 The inhibitory effect of sample D on *Bacillus cereus* and *Candida albicans*

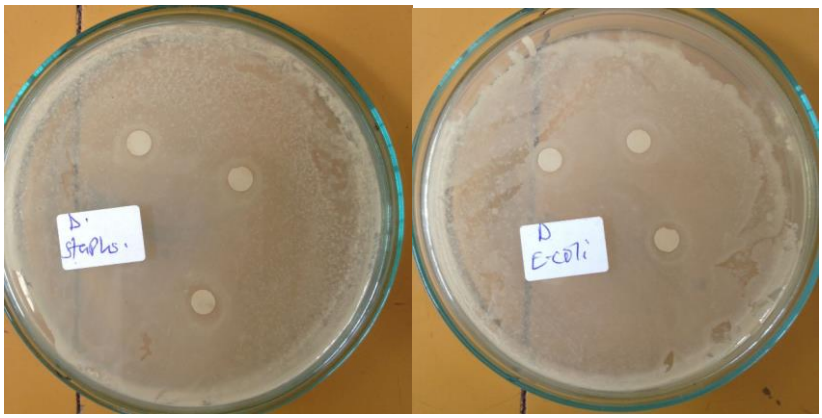

Plate 6.10 The inhibitory effect of sample D on *Staphylococcus aureus* and *Escherichia coli*

Appendix 7: Research Permit Authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/89373/29445**

Date: **30th April, 2019**

Evelyn Nyathogora Ngunyangi
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of efficacy of probiotics on performance and immune response in broiler chicken"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu and Nakuru Counties** for the period ending **30th April, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education, Kiambu and Nakuru Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

THIS IS TO CERTIFY THAT:
MS. EVELYN NYATHOGORA NGUNYANGI
of EGERTON UNIVERSITY, 536-20115
Nakuru,has been permitted to conduct
research in *Kiambu , Nakuru Counties*

on the topic: *EVALUATION OF EFFICACY OF PROBIOTICS ON PERFORMANCE AND IMMUNE RESPONSE IN BROILER CHICKEN*

for the period ending:
30th April,2020

.....
Applicant's Signature

Permit No : NACOSTI/P/19/89373/29445
Date Of Issue : 30th April,2019
Fee Recieved :Ksh 1000

.....
Director General
National Commission for Science, Technology & Innovation

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014.

CONDITIONS

1. The License is valid for the proposed research, location and specified period.
2. The License and any rights thereunder are non-transferable.
3. The Licensee shall inform the County Governor before commencement of the research.
4. Excavation, filming and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
5. The License does not give authority to transfer research materials.
6. NACOSTI may monitor and evaluate the licensed research project.
7. The Licensee shall submit one hard copy and upload a soft copy of their final report within one year of completion of the research.
8. NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice.

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Email: dg@nacosti.go.ke, registry@nacosti.go.ke
Website: www.nacosti.go.ke

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH LICENSE

Serial No.A 24420

CONDITIONS: see back page