USE OF NON-ANTIBIOTIC GROWTH PROMOTERS IN CHICKEN BROILER PRODUCTION IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my family: your love, sacrifice and support enabled me accomplish this enormous task. You were there materially and emotionally with me all this time. My wife Everlyne Nawiri, you made all the priceless sacrifices to support me on this project. To my brother Abel, your humbling generosity is beyond measure and the late Habakuk, for your eternal inspiration, I will cherish you forever.

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KEY TO ABBREVIATIONS

OA: Organic acids

Acidomix: Commercial brand of unprotected in-feed organic acids from Novus international

Activate: Commercial brand of unprotected organic acids from Novus international

AGP: Antibiotic growth promoters

Agrovets: Outlets that sell veterinary and agricultural imputs

AKEFEMA: Association of Kenyan feed manufacturers

ANOVA: Analysis of variance

Av: Available

Avimatrix: Commercial brand of protected infeed organic acids from Novus International

Cfu: Colony forming units

d.f.: Degrees of freedom

DM: Dry matter

DNA: Deoxyribonucleic acid

F pr: Probability of F value

F ratio: The statistic from ANOVA, revealing the significance of the hypothesis that Y depends on X

H_O1: Null hypothesis

H_O2: Alternative hypothesis

Kcal/Kg: Kilocalories per kilogram

KEBS: Kenya bureau of standards

m.s.: Mean of sum squared

m.v.: Mean variance

ME: Metabolizable energy

MRS agar: De Man Rogosa and Sharpe agar

N: Number of respondents

Nacl: Sodium chloride

P: Probability

PCA: Plate count agar

s.s.: Sum squared

SEM: Standard error of means

T1: Treatment one

T2: Treatment two

T3: Treatment three

T4: Treatment four

TMT: Treatment

USAID: United States Agency for International Development

v.r: Variance ratio

WD: Water dispersible

ABSTRACT

This study was done to investigate the viability of organic acids as alternative replacement to antibiotics as growth promoters and in managing broiler chickens in poultry farms around Nairobi in Kenya. The objectives of the study were to

(i) Assess the types and levels of antibiotics used in chicken broiler feeds and in the management of the birds; To compare the performance of chicken broilers fed on diets containing antibiotic growth promoters with those on a mixture of organic acids;

(ii) Evaluate the effects of antibiotics and organic acids on gut morphology and microflora in chicken broilers and

(iii) Compare performance of chicken broilers given drinking water containing organic acids with chicken broilers in which no organic acids are added in their drinking water.

The first part of this study was a survey and second part consisted of two feeding trials. The survey was conducted in the environs of Nairobi to determine the extent of antibiotic usage as a therapeutic agent and as a growth promoter. Structured questionnaires were administered to feed millers, agrovets and farmers. It was found that all the agrovets in the study area stocked antibiotics for use as therapeutic agent and as growth promoters. Both the feed millers and the agrovets were found to be in possession of antibiotics with components similar to those used in human treatment. It was found that only 28% of the 181 farmers interviewed observed antibiotic withdrawal period. This suggests that there is no proper management in the use of antibiotics in the study area, and the eminent risk of selecting resistant bacteria is not dealt with. Feeds for commercial poultry production are compounded with antibiotics such as salinomycin sodium and virginiamycin so as to promote growth and reduce mortality. Farmers routinely use antibiotics to

control or treat some of the common diseases encountered in their flocks. There is worldwide concern about use of these substances in intensive livestock production because of deleterious effects these substances can have on human health due to consumption of animal food products containing antibiotic residues. Thus the need to identify suitable substitutes to antibiotics for use in poultry industry in Kenya.

In the second part of the study, two feeding trials were done. The first feeding trial assessed the performance of chicken broilers fed on diets supplemented with AGP or OA. The second feeding trial assessed the performance of chicken broilers fed on AGP or OA supplemented diets with OA added to their drinking water. For the two feeding trials the diets were formulated to contain 2650 kcal/kg ME and 220g CP per kg in the starter diet and 2750 kcal ME and 180 g CP per kg in the finisher diet. Other nutrients were maintained at calcium 10g per kg, available phosphorous 4.5g per kg, lysine at 5% of CP and methionine at 2% of CP. The first trial had four treatments each replicated six times with 10 birds per replicate. The treatments were: T1 (control with no antibiotic, no organic acid), T2 (Salinomycin sodium), T3 (Acidomix) and T4 (Avimatrix). Salinomycin, Acidomix and Avimatrix were added at rates of 0.05, 0.3 and 0.05% respectively. The diets for trial two were similar to trial one except for addition of organic acid to their drinking water, having seven replicates with 10 birds per replicate. The treatments for trial two were: T1 (Activate only), T2 (Salinomycin + Activate), T3 (Acidomix + Activate) and T4 (Avimatrix + Activate). Activate was administered at the rate of 0.3 ml per litre of drinking water from Day 3 – 16 and Day 30 – 44. Feed intake were recorded daily and live weight weekly and Feed conversion ratio and weight gain calculated weekly. Jejunal samples were obtained on days 23 and 44 for histology and microbiology. Blood was collected for serology. There were no significant differences observed in feed intake, average daily gain, final body weights and feed conversion ratios between antibiotic and organic acid diets ($p<0.05$), Addition of organic acid to drinking water led to 9% improvement in feed conversion ratio from average of 2.66 to 2.42 and a 96% reduction total bacterial count from antibiotic, 25% increase from unprotected acid and 397% increase from protected acid dietary treatments. Protected organic acid treatments had significantly higher lactobacilli and yeast count, 92.86×10^5 cfu compared to 3.5×10^5 cfu for antibiotic treatment, no coliforms were isolated in unprotected organic acid compared to 0.287x 105 cfu for antibiotic treatment.

Antibiotic treatment had significantly taller villi, 1722µ compared to 1449 µ for protected organic acids ($p<0.05$). Inclusion of organic acid into the drinking water increased villi height for all the treatments except unprotected organic acid treatment. Organic acid treatments had broader villi, 117.1µ (protected) and 155µ (unprotected) compared to 138µ (antibiotic) and 108.4µ (control). Addition of organic acid to drinking water increased villi bread for both control and antibiotic. Tunic thickness and glandular area thickness were all significantly improved in treatments without inclusion of organic acid in the drinking water. Generally, inclusion of organic acid in broiler's drinking water, improved feed conversion ratio and gave a better gut health through increase in beneficial bacteria and reduction in coliform counts. Organic acids are viable alternatives for antibiotics in ensuring improved health and productivity of chicken broilers.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background on poultry sector in Kenya

Agricultural sector dominates the Kenyan economy accounting for approximately 24% of the gross domestic product. It is the largest foreign exchange earner and provides directly or indirectly employment and livelihood to about 75% of the population (Export Processing Zones Authority, 2005). Investment in this sector remains a priority for the Kenyan government. The sector has several connections with manufacturing industry, thereby providing most of the raw materials used therein and market for finished products.

The poultry sector contributes 7.8% of the Gross Domestic Product, 55% of livestock sector and 30% of the agricultural sector, employing two to three million people (USAID, 2011). Data from Export Processing Zone Authority (2005) shows that 19% of livestock products is white meat (poultry and pig meat).

Chicken is the most important type of poultry in Kenya with low scale production of ducks, turkeys and geese. There are two types of chicken production systems, the backyard and the commercial production (Mbugua, 2010). Poultry farming in the rural Kenya is exemplified by use of local chicken ecotypes, low inputs, low productivity and few birds per household. On the other hand, the commercial production system is characterized by use of hybrid chicken, high productivity, more inputs and larger flock sizes of 100 – 350,000 birds per household (Karuri, 2010). The high demand for meat and eggs in the urban areas has led to tremendous growth of the poultry industry. The hybrid chickens constitute a major part of this growth (Export Processing Zones Authority, 2005).

The hybrid chickens are fed on compounded feeds, which contain antibiotic growth promoters mainly salinomycin, virginiamycin and zinc bacitracin.

Farmers routinely treat or prophylactically dose their chickens with antibiotics and anti-coccidial drugs to control diseases (Montagne et al, 2003). Some of these antimicrobials are also used in treating disease conditions in humans thus the global concern on effects of antibiotic used in poultry and other livestock on antibiotic resistance in humans.

Antimicrobials help animals digest their food better, improve food conversion ratio and develop into stronger and healthier animals (Jensen, 2001). The antibiotics work by suppressing sensitive microbial population in the gastrointestinal tract. Antibiotics however, should only be used for treating diseases under qualified veterinarian's supervision. This approach will reduce antimicrobial resistance in important food animal reservoirs, which reduces the threat of resistance to humans. This precaution is not observed by both farmers and extension service providers. There is need to find viable alternative ways to reduce antibiotic usage (Hajati et al, 2010).

Antimicrobials are used in Kenya for managing livestock. According to Mitema and Associates (2001) about 14.6 metric tons of AGP were used in food animals in Kenya in 2001. A study on Kenyan animal feeds and fodder sub-sectors showed that Kenya has no regulatory framework for medicated feeds (ABS TCM LTD, 2013). Kenya Bureau of Standards (KEBS), which is charged with enforcing the quality standards, has no capacity to police the large and ill-structured feed industry. Sub-therapeutic feeding of antibiotics is well established practice in farming but there are concerns on the extent of antibiotic usage in feeds (ABS TCM LTD, 2013).

Organic acids have been used extensively in controlling fungal and bacterial contamination in feeds. The most common organic acids in animal nutrition are citric acid, propionic acid, fumaric acid, lactic acid, formic acid and benzoic acid. These acids have been shown to be effective in controlling salmonella among other bacterial species (Rusel et al, 1998). They have been extensively used in pigs than poultry production due to limited response in feed conversion ration and weight gain (Langout, 2000). thus the need to validate their efficacy in poultry production. Their activity increases with decrease in environmental pH (John & Ricke, 1998).

Organic acids have antibacterial, antifungal, and antiprotozoal activity particularly at low pH. Some organic acids have been found effective in controlling avian coccidiosis (Shobha and Ravindranath, 2012, Garcia. *et. al.,* 2007 and Abbas *et. al*, 2011). Organic acids provide alternative means of controlling coccidiosis in poultry (Mansoor et al, 2013). Most commercial preparations of organic acids contain blends of organic acids either protected or unprotected and can achieve this function. Organic acids can be used safely to improve performance and health of broiler chickens (Ghazalah, 2011).

1.2. Problem statement

Elimination of antibiotics in poultry production is a worldwide concern. Indiscriminate use of antibiotics increases microbial resistance which could leave the world without effective human antibiotics. (Scott, 2011). The European Union has banned the use of antibiotic growth promoters, in 2006, due to the hazards posed to both human and animal health (Niba et al,2009). There is need to ascertain the extent of antibiotic usage in poultry production in Kenya and identify viable alternatives to replace antibiotic use in poultry production in Kenya.

1.3. Hypotheses

The hypotheses for this study were;

H_O1: Kenyan feed millers do not add antimicrobials in feed compounding and Kenyan chicken broiler farmers do not use antibiotics in feeding and management of chicken broilers.

H_O2: Antimicrobial growth promoters and organic acids have no effects on chicken broiler performance.

H_O3: Chicken broilers would perform the same with or without supplementing their drinking water with organic acids.

1.4. General Objective

The purpose of this study was to collect data and information about antimicrobial use in feeds and at farm level in Kenya and evaluate the viability of organic acids as alternatives to antibiotics in chicken broiler production.

1.5 Specific objectives

(i) To assess the types and levels of antibiotics used in chicken broiler feeds and in the management of the birds.

(ii) To compare the performance of chicken broilers fed on diets containing antibiotic growth promoters with those on a mixture of organic acids.

(iii) To evaluate the effects of antibiotics and organic acids on gut morphology and microflora in chicken broilers.

(iv) To compare performance of chicken broilers given drinking water containing organic acids with chicken broilers in which no organic acids are added in their drinking water.

CHAPTER TWO

LITERATURE REVIEW

2.0. Use of antibiotics in poultry production

An antibiotic is a chemical produced naturally by a bacteria or fungus to inhibit the growth of bacteria. Antimicrobials include antibiotics and synthetic compounds like sulphur drugs that inhibit growth of bacteria. Antimicrobial growth promoters are compounds that destroy or inhibit bacteria when administered at sub-therapeutic doses (Baurhoo et al., 2007).

The hybrid chickens are fed on compounded feeds. In Kenya, coccidiostat like Salinomycin, virginiamycin or zinc bacitracin, are added into these feeds to promote growth. Kenya feeds standards allow inclusion of medicaments in poultry feed premixes (Kenya Bureau of standards, 1990) Antimicrobials help animals digest their food better, improve food conversion ratio and develop into stronger and healthier animals (Jensen, 2001). The antibiotics work by suppressing sensitive microbial population in the gastrointestinal track. Microbial fermentation causes 6% energy loss in pigs. This energy loss can be prevented and converted to growth if antimicrobials are used (Dibner & Richards, 2005).

Salinomycin is a polyether (ionophoric) antibiotic, produced by *Streptomyces albus*, exhibits activity against gram positive bacteria including mycobacteria and some filamentous fungi and is effective in treatment of coccidial infection of poultry (Yukio et al, 1974). Due to the antibacterial and anticoccidial properties of salinomycin sodium it promotes growth in chickens and improve feed efficiency in growing cattle. It is widely used in chicken diets as it improves the composition of chicken gut microflora by reducing growth of *Clostridium perfringens*, which causes necrotic enteritis in chicken, thus leading to increased growth performance. Salinomycin has been shown to control coccidiosis infection and reduce prevalence of salmonella infection. (Charlotte et al, 2007).

2.1. Use of antimicrobials in the Kenyan feed industry.

According to Mitema and associates (2001), 14.6 metric tonne of antimicrobials were used in food animals in the year 2001, with tetracyclines and cotrimoxazole constituting 78% of these antimicrobials (Mitema et al 2001). The researchers recommended a robust surveillance system to monitor feed additives, a review of the contents of broiler diets to ascertain antimicrobial contents and education of farmers on how to maximize profits without antibiotics or antibiotic growth promoters, proper use of antibiotics and the role of hygiene in reducing antibiotic use in animal production (Mitema et al, 2001). The report from a study on Kenyan animal feeds and fodder sub sectors showed that Kenya had no regulatory framework for medicated feeds, KEBS (Kenya Bureau of Standards), the body in charge of enforcing the quality standards, has no capacity to police the large and ill structured feed industry, the feed sector lacks integral chain management and control, there is a conflict between the roles of Livestock and Veterinary departments leaving a vacuum for regulation, sub therapeutic feeding of antibiotics is well established practice in farming and there was concern on the extent of antibiotic usage in feeds. They identified the need for the Kenyan policy and regulatory authority, KEBS, to document the availability, usage and to ascertain empherical data on efficacy of these substances. (ABS TCM LTD, 2013). There is need to ascertain the extent and type of antibiotic usage in poultry rearing, the reasons for the usage and find viable alternatives to antibiotics in addressing the need for antibiotics while improving chicken performance.

2.2. Global trend

Farmers routinely treat or prophylactically dose their chickens with antibiotics and anticoccidials to control diseases (Montagne et al, 2003). Some of these antimicrobials are used in treating disease conditions in humans. (Mitema et al, 2001, Naliaka, 2011)). This aspect has caused global concern on effects of antibiotic used in poultry and other livestock on antibiotic resistance in humans. (Naliaka, 2011). Several European countries have withdrawn the use of antibiotics in food animal production. The withdrawal, was however with consequences. (Mitema et al, 2001) reported considerable speculation about the effects of antimicrobial growth promoter termination on efficiency of food animal production, animal health, and food safety and consumer prices, however, the study showed that withdrawal of antimicrobials in livestock production had no serious negative effects. They demonstrated that the use of antimicrobials for the sole purpose of growth promotion can be discontinued. The use of antimicrobial growth promoters can however be very effective in reducing the overall quantities of antibiotics administered to food animals (Montagne et al, 2003). They suggested that antibiotics should only be used for treating diseases under qualified veterinarian's supervision. This approach will reduce antimicrobial resistance in important food animal reservoirs, which reduces the threat of resistance to public health. Based on the above arguments, there is need to eliminate or reduce antibiotic use in broilers in Kenya to achieve these outcomes.

2.3. Alternatives to antibiotic growth promoters

The three main ways in which to reduce our dependence on antibiotic use in animals are-

i. The development of alternatives to antibiotics that promote growth by enhancing the efficiency of feed conversion.

ii. To improve animal health through good husbandry practices, which may be difficult to achieve under most practical conditions (Adams, 1999).

Growth promoters have been shown to perform best under worst conditions; i.e. animals in poor health and under unhygienic living conditions. With improved environment through reduced overcrowding and improved infection control techniques, the need for growth promoters may be removed (Prescott et al, 2000). The use of antibiotic growth promoters and development of antibiotic resistance are closely related. There is increasing concern about the potential for antibiotic resistant strains of bacteria developing from exposure to these sub therapeutic antibiotic doses. This has compelled the researchers to explore alternatives such as enzymes, probiotics, prebiotics, herbs, immunostimulants, organic acids, bacteriocins and phytotherapeutic plants (Langout, 2000).

2.3.1 Organic acids

Organic acids are organic compounds with acidic properties. The most common examples are carboxylic acids like lactic acid, propionic acid, acetic acid, formic acid, sorbic acid, citric acid, oxalic acid, uric acid, butyric acid (Dibner & Buttin, 2002). Organic acids are not antibiotics but when combined with good nutritional, managerial and bio-security measures, they can prove powerful in maintaining the gut health of poultry, thus improving their livability, feed conversion ratios, weight gain, live weight and immune responses (Sheikh et al, 2011).

2.3.2. Effects of organic acids on performance of broilers, gut health and morphology

The antibacterial activity of organic acids is related to the reduction of pH and their ability to dissociate. Organic acids have a relatively high dissociation constant and are thus relatively reluctant proton donors in aqueous solution and thus weak acids. Dissociation of a weak acid is pH dependent. Thus, the antibacterial activity increases with decreasing pH-value. Organic acids are lipid soluble in the un-dissociated form thus they easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. The organic acid releases the proton H^+ in the more alkaline environment, resulting in a decrease of intracellular pH. This alters microbial metabolism by inhibiting the action of important microbial enzymes which forces the bacterial cell to use energy to export the excess of protons H^+ leading to death by starvation. The protons H^+ can denature bacterial acid sensitive proteins and DNA. Lactic acid bacteria are able to grow at relatively low pH thus more resistant to organic acids than other bacterial species, such as *E. coli* and *Salmonella*. Gram-positive bacteria (like Lactobacilli) have a high intracellular potassium concentration, which counteracts acid anions (Russel & Diaz-Gonzalles, 1998). Due to antimicrobial effect, organic acids inhibits pathogenic intestinal bacteria leading to reduced bacterial competition with the host for available nutrients, reduction in the level of toxic bacterial metabolites, improvement of protein and energy digestibility and improvement in performance of birds (Baurhoo et al, 2007).

Administration of organic acids affects the morphology of the gut. Organic acids lead to increased villus height in the small intestines thus increasing the absorptive intestinal surface area for better nutrient absorption and growth performance. Reduced pH value in different segments of gastro-intestinal tract promotes growth of favorable bacteria while inhibiting the growth of pathogenic bacteria, which grow at relatively higher pH. These acid anions complex with calcium, phosphorus, magnesium and zinc thus improve the digestibility of these minerals. Resultant reduction in gastric pH increases pepsin activity and the peptides arising from pepsin proteolysis trigger the release of hormones, including gastrin and cholecystokinin, which regulate the digestion and absorption of proteins (Lan et al, 2005).

Examples of commercially available organic acids are Acidomix® FG, a micro-granulated feed acidifier based on formic acid, lactic acid, fumaric acid and ammonium formate. It is an unprotected organic acid mixture which is active in the foregut as it is neutralized by bile. Avimatrix, which contains 3.0% calcium formate and 49.0% benzoic acid, is a protected organic acid mixture thus active in the midgut as it dissociates in the alkaline environment within the small intestines (jejunum). Activate WD is an unprotected organic acid which contains methionine hydroxyl analogue (HMTBa), formic acid and propionic acid acts as a water sanitizer and influences the upper gut by lowering gastric pH, increasing pepsin activity hence better protein digestibility. Low pH environment prevents growth of acid-labile pathogenic microbes while promoting acid tolerant beneficial microbes. Organic acids aid in absorption of minerals like calcium and phosphorous thus improving mineral digestibility (Ramana et al, 2015).

2.3.3. Use of organic acids in poultry production

Prophylactic and therapeutic antibiotics have been in use in poultry production for management gastrointestinal infections. The realization of the challenges involved with the use of antibiotics in food animal production has led to efforts to utilize the existing commensal intestinal microflora in managing pathogenic microflora. This involves stabilizing the microflora at required levels or modifying them by use of natural alternatives (Lan et al, 2005). These natural alternatives include prebiotics, probiotics, enzymes, acidifiers, herbs, essential oils, and immunomodulators.

Organic acids have been used extensively in controlling fungal and bacterial contamination of feeds. The most common organic acids in animal nutrition include citric, propionic, fumaric, lactic, formic and benzoic acids and are effective in controlling bacterial conditions caused by *salmonella*, *Campylobacter Clostridium perfrigens* among others (Rusel et al, 1998; Marco et al, 2013). Organic acids can thus replace antibiotic growth promoters in chicken as in swine. They were aslo found to be potential inhibitors of *Campylobacter* spp. in water (Chaveerach et al., 2001). Addition of organic acid in drinking water has been shown to reduce presence of Salmonella spp if administered in the first and last week of chicken broilers life (Montonya et al, 2011).

2.3.4. Controlling coccidiosis without antibiotic growth promoters

Avian coccidiosis is a fatal disease caused by intracellular parasites of genus *Eimeria* which cause damage to the gut epithelium leading to severe hemorrhages. This causes weight loss, poor feed efficiency and even death in poultry. Coccidiosis causes significant economic losses globally (Masood et al, 2013). Coccidiosis is controlled by anticoccidials or antibiotics such salinomycin, monensin, sulfonamides, amprolium among others, vaccination using either live virulent, live attenuated or live ionophore resistant strains, nutritional supplements like herbs, fats and oils, vitamin A or oligodeoxynucleotides, probiotics and organic acids (Masood et al, 2013, *Kitandu* et al, 2006). Organic acids have antibacterial, antifungal, and antiprotozoal activity particularly at low pH. Formic, butyric, anacardic, acetic and hydrochloric acids are effective in controlling avian coccidiosis (Shobha and Ravindranath, 2012*;* Garcia et al, 2007 and Rao Z. Abbas et al, 2011). Acetic acid has been shown to have anticoccidial activity (Abbas et al, 2011). Low doses of hydrochloric and formic acids have anticoccidial activities against Eimeria tenella in broiler chickens. Organic acids provide alternative means of controlling coccidiosis in poultry (Abbas et al, 2011). Most commercial preparations of organic acids contain mixture of organic acids either protected or unprotected and achieves this function.

2.4. In-feed enzymes

Feed enzymes are routinely added to pig and poultry feeds to help in the breakdown of less digestible components of the feed like glucans, pectin and phytates. They are produced as fermentation products from fungi and bacteria and seem to only have a positive effect on the animal. Feed enzymes are very effective at maximizing feed conversion efficiency and have few drawbacks. The enzymes have been found to be safer than antibiotics (Jensen, 2001). The most commonly used enzymes in poultry and pig nutrition include phytases, β-glucanases, xylanases, α-galactosidases, proteases, amylase, lipases, mannanases, cellulases, hemicellulases and pectinases (Ravindran, 2013).

2.5. Competitive exclusion products

Competitive exclusion describes the protective effect of the natural or native bacterial flora of the intestine in limiting the colonization of some bacterial pathogens. The gut of new born chicks is devoid of any bacteria but gets colonized briefly after hatch. When these birds are fed on probiotics it influences the colonization of the chicks guts with the microbes contained in the probiotic. Probiotic bacteria are non-pathogenic bacteria thus by binding to all available sites in the chicks' guts, pathogenic bacteria like *E. coli* and *Salmonella* are inhibited by exclusion from the binding sites. These bacteria produce organic acids and other immune modulating substances that kill or prevent growth of harmful bacteria (Jeffrey, 1999) Competitive exclusion products include prebiotics, probiotics, organic acids, phytogenic compounds among others that selectively promotes the growth beneficial species of gut bacteria while inhibiting the pathogenic gut microflora (Hajati et al, 2010). They are often administered in day old chicks to colonize their gut preventing *Salmonella* and *Campylobacter* infections. This reduces diarrhea and reduce levels of mortality. These competitive exclusion products are preferably administered after treatment with antibiotics, to re-colonize a gut depopulated by the antimicrobial action of the drugs (Ferkett, 2004).

2.6. Probiotics

Probiotics are living microorganisms, such as *Lactobacilli*, that provides a health benefit to their host when administered in adequate amounts. (Niewold, 2007; Alloui et al, 2013). Gut flora are the first line of defence in the gut. Probiotic supplementation has been shown to enhance gut defence mechanisms in poultry due to their influence on the gut microflora. Gut flora influence resistance to enteric infections by various pathogens (Chateau et al, 1993; Kitandu et al, 2006). (Dalloul et al, 2003) demonstrated that feeding broiler chickens on feeds containing *Lactobacillus* based probiotic resulted in an immunomodulatory effect on local gut system in the broilers. This led to improved resistance to *Eimeria tenella* as demonstrated by reduced shedding of oocysts. Thus probiotics can be used to control coccidiosis.

They influence the digestive microflora positively to promote performance and to protect against colonization by harmful bacteria and enteropathogens of human importance (Niewold, 2007) thus improve the overall health of an animal by improving the microbial balance in its gut.

2.7 Effects of probiotics on performance of broilers, on gut health and morphology

Probiotics exert their influence by colonizing the gut in large numbers, the probiotic bacteria exclude pathogens and thus prevent them from causing infection (Jeffrey, 1999). Probiotics stimulate the immune system leading to increased surveillance of the gut by leukocytes to the probiotic bacteria and other potential pathogens ensuring elimination of pathogenic bacteria by the gut immune cells (Revolledo et al, 2006). Probiotics stimulate production of vitamin B_{12}, bacteriocins, and propionic acid vital for the host metabolism. Prophylactic administration of probiotics from day one to chicks has been shown to prevent lameness in broilers due to bacterial chondronecrosis with osteomyelitis (Richards et al, 2005). Probiotics may lessen or even eliminate the need for antibiotic treatment of related disease conditions in growing broilers, according to the results.

(Yegani & Korver 2008) demonstrated their anti- tumors activity in mice fed with fermented colostrum before onset of tumor growths. (Prescott et al,2000) demonstrated that intraperitoneal administration of *Lactobacillus casei* inhibited tumour growth. It has been shown that *L. Casei* has immunopotentiator properties similar to those of BCG (Bacille Calmette–Guérin): thus a possible vaccine to tumours and a stimulus to the immune system. Probiotics acts best by in-feed delivery system (John and Ricke, 1998). Probiotics improve weight gain, feed conversion rates and have proved very effective for newborn animals or following antibiotic treatment. These beneficial effects of probiotics have been demonstrated to support the health promoting claims of probiotic therapy (Richards et al, 2005).

2.8. Prebiotics

Prebiotics are indigestible feed ingredients like oligosaccharides. They have selective effects on micro biota that helps improve the health of the host. Examples include mannan oligosaccharide (MOS) products, which are derivatives of yeast cell walls, and fructose oligosaccharide (FOS) products, which can be extracted from fruits (Niewold, 2007).

2.9. Effects of withdrawal of antibiotics from food animal production in the European Union

Antibiotics have undoubtedly improved animal performance by improving their feed conversion efficiency and general health of animals. Examples of countries that withdrew from the use of antibiotics from their agricultural sector are Sweden and Denmark among others. Despite these two countries' restrictions on antibiotic usage in food animal production, their production continues to thrive. However, this success has been coupled with tremendous extension services on therapeutic usage of antibiotics (Scott, 2011).

2.9.1. Effects of organic acids and antibiotics on broiler chicken performance

Organic acids administered to chicken broilers help in establishing a stable microbiota that help in reducing colonization of the food borne pathogens such as salmonella (Richards et al, 2005). Lessening subclinical enteritis aid the mitigation of the negative enteric effects of *Clostridium* species. The causes of these enteric disorders are multifactorial thus cannot be eradicated by a single treatment approach (Richards et al, 2005). In precision, care must be taken to frontier production of mucous in the small intestine and prevent overgrowth of *Clostridium* species. The availability of nutrients in the small intestine due to poor protein digestibility promotes proliferation of *Clostridia*.

2.9.2. Use of antimicrobials by poultry farmers

The use of antimicrobials is a prevalent practice in food animal production like the chicken broilers keeping (Baurhoo et al, 2007). In chicken broilers farming antimicrobials are administered for therapeutic means for treatment of infection, prophylactic purposes in advance of symptomatic and asymptomatic conditions and for non-therapeutic purposes for growth promotion and improved feed efficiency (Mitema et al, 2001).

There is a close relationship between antibiotic growth promoters and antibiotic resistance (Sheikh et al, 2011). Sub therapeutic and uncontrolled therapeutic use of antibiotics pose a great danger of development of antibiotic resistance by important human pathogens (Montagne et al, 2003; Naliaka, 2011). There is thus a global need to find safer alternatives to antibiotics in the management of food animals. This has driven a lot of research non-therapeutic alternatives like enzymes, probiotics, prebiotics, herbs, essential oils, immunostimulants and organic acids as feed additives in animal production. Organic acids have shown their viability as alternatives to antibiotics in poultry production (Sheikh et al, 2011). This project aims at exploring the use of organic acids to replace therapeutic and subtherapeutic use in chicken broiler production usage (Hajati et al, 2010).

CHAPTER THREE

Assessing the use of antimicrobial growth promoters in chicken broiler production

3.0. Introduction

Field surveys were conducted among farmers, dealers in veterinary products and feed manufacturers to determine the types and levels of antibiotics used in management of chicken broilers in Kenya. The objective in this part of the study was to assess the types and levels of antibiotics used in chicken broiler feeds and in the management of the birds.

3.1. Materials and methods

3.1.1. The Study area

The survey was conducted in Kiambu, Nairobi, Kajiado and Machakos counties as shown in figure 1. The sites were selected due to their proximity to Nairobi city and the high concentration of poultry (USAID, 2011).

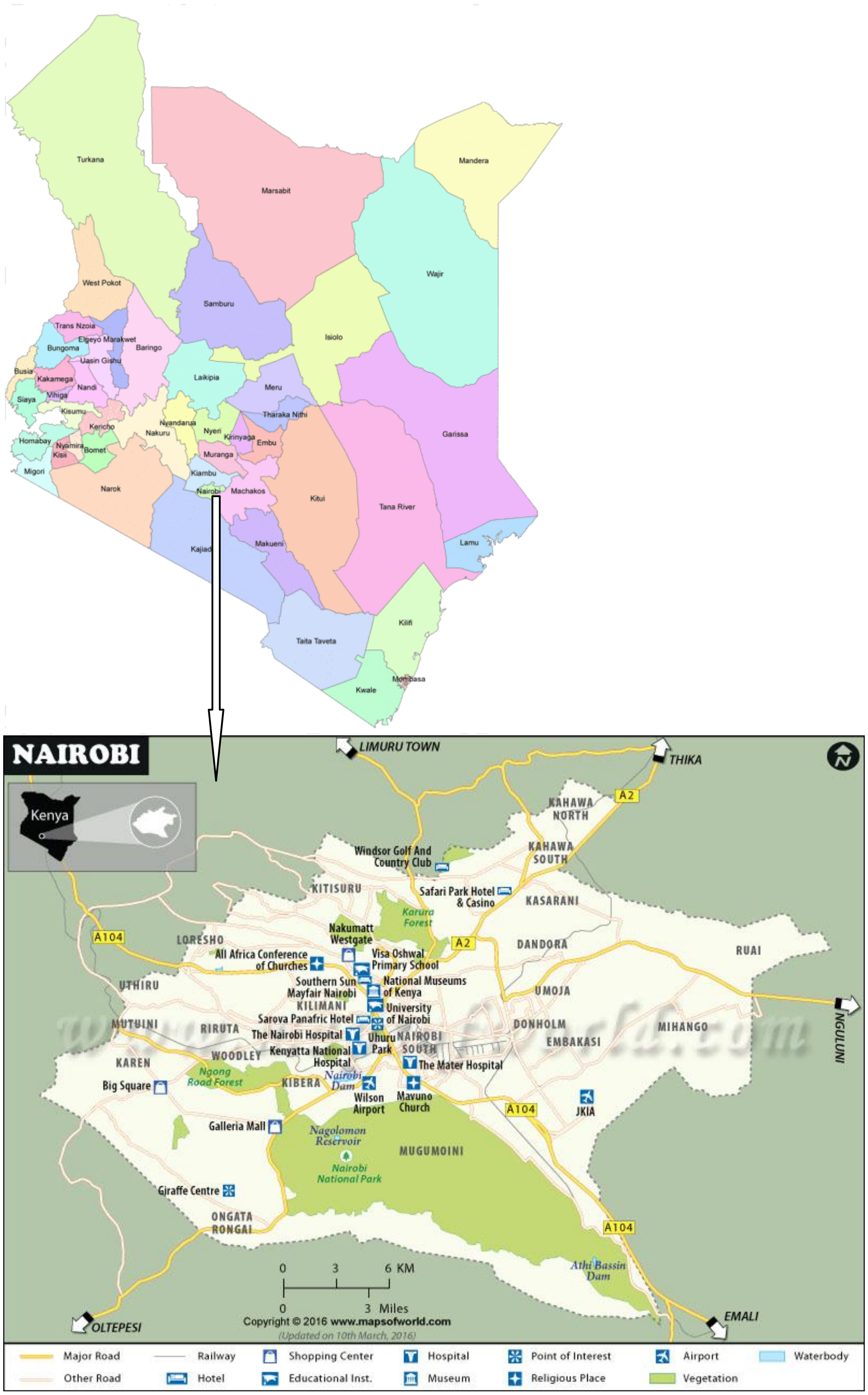


Figure 1: Map of the study area

(http://www.mapsofworld.com/kenya/cities/nairobi.html, 2016)

3.1.2. Target population

Information was collected from chicken broiler farmers, Agrovets (outlets that sell veterinary and agricultural inputs) and feed manufacturers.

A list of the farmers interviewed was obtained from hatcheries and feed millers. In relation to broiler production, the other stake-holders targeted were veterinarians, Para veterinarians, over-the- counter attendants in retailing and wholesale agrovet shops, production managers and nutritionist in animal feed mills.

To ensure accuracy of the data provided by the respondents, all the people interviewed were persons owning the poultry enterprises or directly managing the birds.

The agrovet targeted were those who supplied the farmers with medicines and supplements so as to get information on the types of antibiotics stocked and sold, the prevalent diseases, how farmers chose drugs for their broilers and the most commonly prescribed antibiotics.

3.1.3. Sampling procedure

Commercial poultry farmers were identified to the researcher by traders selling day old chicks and personnel offering extension services to them during meetings and field days. During the meetings, individual questionnaires were administered to the farmers. Those farmers identified through the hatcheries and agrovets were visited in their farms and questionnaires administered.

All the agrovet outlets mentioned by the farmers as the source of drugs were interviewed.

The survey targeted the feed millers who were mentioned by the farmers as their source of feeds were interviewed.

3.1.4. Data collection procedure

Semi structured questionnaires were administered to farmers, Agrovets and feed millers. Information collected from the farmers through the questionnaires included use of antibiotics (for therapeutic, prophylaxis or growth promotion), types and sources of antibiotics.

For the agrovets information on types of antibiotics sold, choice of antibiotics sold to the farmers, common diseases reported by the farmers and instruction on use of antibiotics was captured. Finally, for the feed millers' information was collected on the type and level of antibiotics inclusion in the feed.

3.1.5. Data analysis

The raw data was entered into Microsoft Excel Spread sheets. The data was subjected to descriptive statistics to determine the frequencies and means of the occurrences of the different variables being assessed.

3.2. Results and discussion

DISTRIBUTION OF RESPONDENTS IN STUDY AREA.

The farmers, Agrovets and Feed millers interviewed are shown in Table 1.

Table 1: Distribution of respondents in the study area

County	Area	No. of Farmers	No. of agrovets	No. of millers
Nairobi	Embakasi	14	6	
	Kasarani	11	3	
	Makadara			2
	Starehe		3	
	Westlands	1		
Kiambu	Ruiru	36	3	
	Thika	18	2	4
	Gatanga	5		
	Gatundu	5		
	Juja	3		
	Kiambu	18	1	
	Limuru	4		
	Githunguri	39		
Kajiado	Loitoktok	22		
	Isinya			1
Machakos	Yatta	2		
	Kagundo	1		
	Athi river	2		
TOTAL		**181**	**18**	**7**

3.2.1. Results from the survey of chicken broiler farmers

SOCIO ECONOMIC CHARACTERISTICS OF THE RESPONDENTS

Table 2 below shows the social economic characteristics of the farmers this study, which includes gender, education level and age, of respondents as well as types of poultry kept.

Gender of respondents

Sixty-seven percent of the respondents were females and the rest were males.

Level of education of the respondents

The respondents were well educated with 78% of them having either secondary or tertiary education. University graduates formed only nine percent of the sampled population, followed by primary school graduates at 14%. Of the female respondents, 69% of them had secondary or tertiary level of education.

Age of respondents

The Majority of farmers (39 %), were between the ages of 30 and 40 years, while63 % were below 40 years (Table 2). Only 4 % of respondents were above 60 years of age. The majority were therefore young and energetic. Efforts to improve productivity and safety in the poultry industry must thus target farmers below 40 years of age.

Table 2: The socio economic characteristics of farmers in the study area

Gender of respondents	**Frequency n =180**	**%**
Male	72	40
Female	108	60
Total	180	100
Education level of respondents	**Frequency n = 57**	**%**
Primary	8	14
Secondary	22	39
Tertiary	22	39
University	5	9
Total	57	101
Age group of respondents (years)	**Frequency n = 57**	**Percentage**
18 – 30	14	24
30 – 40	22	39
40 – 50	9	16
50 – 60	8	14
Over 60	4	7
Total	57	100
Type of birds kept	**Frequency n = 57**	**%**
Broiler	37	65
Layers	17	30
Indigenous	1	1
Mixture	2	4
Total	57	100

Types of chicken kept in the study area

The types of chicken kept by farmers are shown in Table 2. Majority of farmers, (95%), kept commercial hybrid birds with 65% keeping broilers, 30% layers and 1% indigenous chicken. This reflects the trend in urban areas and may not reflect the trends in rural areas.

HUSBANDRY PRACTICES BY THE FARMERS IN THE STUDY AREA

The preferred source of extension services by the farmers interviewed

The extension service providers for respondents are shown in Table 3. Majority (72%) of farmers got extension services over the counter at the Agrovet outlets, while 17% got extension services from feed millers and 3% relied on Government extension services. The quality of extension services received depended on the competence of extension staff at Agrovet outlets. The quality of technical staff at these outlets thus determined the quality of extension services offered to the farmers in the study area.

Common diseases reported by farmers

The most frequently occurring poultry diseases in the study area are shown in Table 3.

The diseases reported by the farmers by farmers were coccidiosis (20%), newcastle (18%), chronic respiratory disease (14%), gumboro (13 %) and ascites (10%). These five disease conditions constituted 75% of the diseases occurring in the farms sampled. Bacterial infections contributed 24% of reported cases (typhoid, omphalitis and chronic respiratory disease) While viral diseases such as gumboro, newcastle and fowl pox constituted 37% of the conditions (Table 3).

Table 3: The husbandry practices by farmers in the study area

Preferred source of extension by farmers	**Frequency n = 72**	**%**
Agrovets	52	72
Feed millers	12	17
Breeders	6	8
Public extension staff	2	3
Total	72	100
Why farmers gave medications to birds	**Frequency n = 142**	**%**
To treat sick birds	48	34
To prevent known diseases from occurring	44	31
As a routine practice	35	25
To make birds grow faster	12	8
To increase egg production by the birds	3	2
Total	142	100
Diseases reported by the farmers	**Frequency n = 69**	**%**
Coccidiosis	14	20
Newcastle	13	19
Chronic respiratory disease	10	14
Gumboro	9	13
Ascites	7	10
Diarrhoea	6	8
Fowl pox	4	6
Omphalitis	4	6
Typhoid	2	4
Total	69	100
Source of instructions on antibiotic use	**Frequency n = 77**	**%**
Agrovets	51	66
Private veterinarians	14	18
Feed millers	7	9
Public extension service	5	6
Total	77	100
Observance of withdrawal period	**Frequency n = 172**	**%**
Yes	49	28
No	123	72
Total	172	100

The number of respondents differed for the different questions. Not all questions were answered by all respondents thus the variation in number of respondents for different questions.

Reasons why farmers gave medications to their birds

Majority of farmers (90%) used antibiotics for treatment, prevention or as routine management practices in their poultry units (Table 4). Thirty-four percent (34%)of respondents used antibiotics for treating sick birds, 31% for preventing diseases and 25% used them as part of routine management. 10% gave antibiotics to either improve growth or egg production from their birds.

The source of instructions on antibiotic usage to the farmers in the study area

Agrovets were the main source of advice to farmers on the use of antibiotics (66%).

Observance of antimicrobial withdrawal periods by the farmers

Majority, (72%), of the interviewed farmers did not observe a withdrawal period before harvesting their broilers for slaughter despite using antibiotics. This may be attributed to prevalence of diseases in the farm as shown in table 4.

COMMON ANTIBIOTIC BRANDS USED BY FARMERS IN THE STUDY AREA

Table 4 represents 50% of the preferred medications used by farmers in the study area. The products found in the market had different active ingredients with sulfonamides and oxytetracyclines being the most preferred ingredient in most of the products (see appendix) and erythromycin and streptomycin being the least preferred. Fifty percent (50%) of the products (n = 28) that farmers used in managing their poultry were combinations of different antibiotics.

Table: 4. Common brands of antimicrobials used by chicken farmers in the study area

Brand	Frequency	%	Type of antimicrobial
Fosbac	42	19	Antibiotic
Aliseryl	24	11	Antibiotic
Broiler booster	20	9	Probiotic
Miramed	20	9	Antibiotic
Biotrim	19	9	Anticoccidial
Vetacox	11	5	Anticoccidial
Oxytetracyline+vitamins	11	5	Antibiotic
Anticox	9	4	Anticoccidial
Livergen	9	4	Herbal
Herbs	9	4	Herbal
Hipradoxy	8	4	Antibiotic
Supermed	7	3	Anticoccidial
Tylodoxy	5	2	Antibiotic
Doxycycline	5	2	Antibiotic
Agracox	5	2	Anticoccidial
Tylosin	4	2	Antibiotic
Quinocol	4	2	Antibiotic
Agraryl	4	2	Antibiotic
Total	216	100	

Source: Products or empty packets at the interviewed farms

THE VACCINES ADMINISTERED TO BIRDS IN THE STUDY AREA

Vaccines administered to birds in the study area are shown in table 6. Both gumboro and new-castle vaccinations are done in equal proportions by 38 percent of the respondents respectively. Vaccinations against fowl pox and typhoid were also done in equal proportions (17) of each of them respectively (Table 5).

Table: 5. Vaccines administered to birds in the study area.

Vaccines given to birds in the study area	Frequency n = 141	%
Gumboro	54	38
Newcastle	53	38
Fowl typhoid	17	12
Fowl pox	17	12
Total	141	100

MAJOR CHALLENGES ENCOUNTERED BY BROILER CHICKEN FARMERS

Challenges encountered by broiler chicken farmers are shown in Table 6. The most important challenge was the cost of inputs (26%), marketing of poultry products (16%), access to capital to finance their enterprises (14%), poor quality of available inputs (12%), disease outbreaks (12%) and high mortality of their birds (8%). These six challenges accounted for 87% of the challenges stated by respondents.

Table 6: The most important challenges reported by farmers in and around Nairobi

Important challenges n=182	Frequency n=182	%
High input costs	48	26
Market availability	29	16
Capital availability	25	14
Input quality	21	12
Diseases	21	12
Deaths	15	8
Growth rate of birds	8	4
Technical support by extension staff	7	4
Knowledge about good management	5	3
Conmen getting lost with their money	2	1
Ineffective treatment – treatment not working	1	1
Total	182	100

DISCUSSION

Majority of the farmers (82%) used antibiotics on their birds. Eighteen percent of respondents used any product recommended to them by extension service providers (table 3) without the recommendation of qualified veterinary practitioners, thereby risking the challenge of drug abuse.

Despite the widespread use of antibiotics as indicated by this study, only 28% of the farmers were found to observe antibiotic withdrawal period (table 4). This implies that there are extremely high chances of antimicrobials getting into humans after consumption of broiler chicken from the 72% of the farmers (Sirdar, 2012).

3.2.2. Results and discussions from the agrovets survey

FACTORS DETERMING THE CHOICE OF DRUGS

The types of antibiotics stocked by agrovets in the study are was investigated. The products are grouped according to the diseases they are used to treat or control. All the four categories of products were sold by the agrovets interviewed. All the 18 agrovet outlets interviewed in this survey sold antibiotics, anticoccidials, vaccines and dewormers to poultry farmers.

PREVAILING POULTRY DISEASES IN THE STUDY AREA

Table 7 below shows the prevailing diseases in the study area and drugs used to treat them. The most common diseases in broiler chickens reported by agrovets in the study area were coccidiosis, chronic respiratory disease complex and gumboro. Coccidiosis was the most prevelant at 61percent followed by chronic respiratory disease at 33 percent. The least reported disease was Gumboro disease at 6 percent as shown in Table 8 below.

The data collected from the 18 agrovets in the study area, on the types of products sold to broiler chicken farmers showed that potentiated sulfurs are the most commonly used at 18% (10% and 8%) followed by, amprolium at 8%, oxytetracyclines at 8% and tylosin and tylosin combinations at 14% (7% and 7%). Potentiated sulfurs (sulphonamide containing compounds) and amprolium are mainly used for treatment and control of coccidiosis, thus anticoccidials constitute 26% of all the medicines bought by broiler chicken farmers from the agrovets in this region.

Table 7: Prevailing diseases and commonly sold medicine brands reported in the study

Disease type	Frequency n = 18	%
Coccidiosis	11	61
Chronic respiratory disease (CRD)	6	33
Gumboro	1	6
Total	18	100

DRUGS SOLD MOST FREQUENTLY

Table 8:Frequently sold medicine brands reported in the study

Antimicrobial/ compound n = 235	Frequency	%	Category
Trimethoprim/Sulphadimethoxine	24	10	Anticoccidial
Amprolium	18	8	Anticoccidial
Oxytetracyclines	18	8	Antibiotic
Diveridine+sulphadimidine	18	8	Anticoccidial

DISCUSSION

The study found out that all the agrovets sampled sold antibiotics and anticoccidials to famers. The antimicrobial products sold by agrovet outlets sampled were shown in table 8. Seventy-two percent (72%) of drugs were antibiotics or anticoccidials. Since sale of products from agrovets is mostly demand driven this observation shows that most farmers used antibiotics in their farms for various purposes. The antibiotic profile corresponds to the reported disease cases from the farmers. Some of the molecules and active ingredients are also used in human medicine like Oxytetracyclines, Doxycycline, Piperazine, Levamisole, erythromycin, streptomycin, potentiated sulfurs among others. This calls for care in the use of these molecules to avoid cross resistance.

This scenario has been reported in developed countries as evidenced by European Union ban on the use of antibiotic growth promoters due to challenges posed to human and animal health (Niba et al., 2009), and there are attempts to reduce their usage globally (Yegani & Korver, 2008) The realization of extensive disposal of antibiotics for use in poutlry production to levels of (72%) is a clear signal of the challeges already documented in the European Union soon coming to be a reality in the studied area. The hazard being selection and survival of resistant bacterial strains.

3.2.3 Results and discussion from the feed millers survey

TYPES OF FEEDS COMPOUNDED FEEDS PRODUCED BY THE MILLERS

In the panning of the study it was hoped to interview seven millers but only six responded. These millers were producing feeds for chickens, pigs and dairy cattle. All the six millers produced both broiler and layer feeds. None specialized in a particular feed.

THE TYPES OF ANTIBIOTICS USED IN COMPOUNDING FEEDS

These millers included antibiotics in compounding the different types chicken broiler feeds. Salinomycin sodium was the most commonly used by 46 % followed by Zinc bacitracin 31%. The two antibiotics constituted 77 % of all the antibiotics used in feed formulation. Some millers were using more than one antibiotic for same feeds or different stages of feeds (Table 9).

Table 9: Types of antibiotics used by the feed millers

Types of antibiotics used by the millers	**Frequency n =6**	**%**
Salinomycin sodium	6	46
Zinc bacitracin	4	31
Virginiamycin	2	15
Robedine	1	8
Total	13	100

THE INCLUSION RATES OF THE ANTIBIOTICS IN DIFFERENT FEED TYPES

The antibiotics commonly used by the feed millers were salinomycin, zinc bacitracin; Virginiamycin and Robedine (Table 10). They were included at 0.025% to 0.1%. (250gm to 1kg per metric tonne). None included antibiotics in layers' diet. The antimicrobials are used for prevention of coccidiosis and bacterial infections.

Table 10: The inclusion rates of the antimicrobials in different feed types

Type of feed	Antibiotic used	Inclusion rate g/1000kg
Broiler starter	Salinomycin	500-1000
	Zinc bacitracin	250-500
	Robedine	250
Broiler finisher	Salinomycin	500
	Zinc bacitracin	500
	Virginiamycin	500
Chick mash/starter	Salinomycin	500-1000
	Zinc bacitracin	0
Growers diet	Salinomycin	500
	Zinc bacitracin	0
Layers diet		0

The nutritionists reported the variation of inclusion rate to respond to specific disease challenges in the field.

DISCUSSION

All the millers interviewed included antibiotic growth promoters in feeds for poultry. The most frequently used antibiotics were as follows: Salinomycin (46%), Zinc bacitracin (31%), Virginiamycin (15%) and Robedine (8%). Most of the millers surveyed used Salinomycin or Zinc Bacitracin in both broiler starter and finisher diets (Table 10). The use in finisher poses a risk of contaminating broiler meat which ends up in human food chain. None of the surveyed millers included antibiotic growth promoters in layer diets. All the millers surveyed included antibiotics in broiler feeds with no provision for withdrawal periods. Salinomycin was the most commonly used antimicrobial growth promoter due to its spectrum of activity. Salinomycin is both antibacterial and anticoccidial thus the preference by many millers. Zinc bacitracin is poorly absorbed from the gut so the risk of contaminating the meat was low except possibly at slaughter. Salinomycin is the latest anticancer drug recently discovered for humans (Scott, 2011). Continued use in feeds may pose a risk of failure in treating cancer, cases of which are in the rise. It was noted that antibiotics were intensively used in Kenya, including a majority that were long banned in the European Union (Mitema et al, 2001) like virginiamycin and olaquindox. If we are to increase our capacity in poultry production and possibly export our products, then a withdrawal program from use of such antibiotics should be considered.

CHAPTER FOUR

4.0 EFFECTS OF ANTIBIOTICS AND ORGANIC ACIDS ON PERFORMANCE, GUT MICRO-FLORA AND MORPHOLOGY OF CHICKEN BROILER OF CHICKEN BROILER

4.1. Introduction

This part of the study involved two feeding experiments to evaluate the effects of antibiotics and organic acids on the performance of broiler chickens. In the first feeding trial, the effects of dietary antibiotics and organic acids were compared. In second feeding trial, the effect of administering organic acid through drinking water was assessed.

The objectives of the study were

(i) To compare the performance of chicken broilers fed on diets containing antibiotic growth promoters with those on a mixture of organic acids

(ii) To evaluate the effects of antibiotics and organic acids on gut morphology and microflora in chicken broilers

(iii) To compare performance of chicken broilers given drinking water containing organic acids with chicken broilers in which no organic acids are added in their drinking water.

4.2 Material and methods

4.2.1 Experiment 1

EXPERIMENTAL DIETS

Broiler starter and finisher diets were formulated according to Kenya Bureau of Standards specifications and used for this study. The starter diet contained 2650 kcal of ME and 220 g of crude protein (CP), while the finisher diet had 2750 kcal of ME and 180 g of CP per Kg as shown in Table 11.

Table 11: Composition of experimental diets used in the study (Air-dry basis)

Raw material content	% in Starter diet	% in finisher diet
Maize germ cake	5.0	30.0
Maize germ bran	20.0	5.0
Maize	34.7	5.0
Pollard	0.0	34.0
Dicalcium phosphate	0.5	0.0
Bone meal	0.0	3.2
Stock feed lime	1.6	0.0
Cotton seed cake	2.2	0.0
Soya meal	22.3	4.0
Sunflower seed cake	6.0	12.0
Broiler premix	1.0	2.0
Dagga fishmeal	6.0	2.0
Lysine premix	0.2	1.8
Methionine premix	0.1	0.5
Salinomycin[2]	0.05	0.05
Acidomix[3]	0.3	0.3
Avimatrix[4]	0.05	0.05
Total	100	100
Calculated composition		
ME Kcal/kg[1]	2650	2750
Crude Protein (%)	22	18
Calcium (%)	1	1
Available Phosphorous (%)	0.4	0.5
Lysine (%)	1.1	0.96
Methionine (%)	0.44	0.4
Salt (Nacl (%)	0.3	0.4

[1]*ME of diet was determined by calculation*

[2] *= Salinomycin, an antibiotic with anticoccidial properties used in treatment 2 only*

[3] *= Acidomix, an unprotected organic acid blend used in treatment 3 only*
[4] *= Avimatrix, a protected organic acid blend used in treatment 4 only*

Unga Farm Care E.A. Ltd provided the feed for the feeding trials, while Novus international provided the organic acids. The organic acid mixtures produced by NOVUS and marketed by the trade names Acidomix, Avimatrix and Activate WD were used. The antibiotic, Salinomycin Sodium, was used for being the most commonly used in poultry feeds in Kenya according to our survey.

EXPERIMENTAL DESIGN

A completely randomized block design was used for this investigation. The replicates for each treatment were randomly assigned within the poultry unit. Trial one had four treatments with six replicates of ten birds each per each with no organic acid added to the drinking water.

EXPERIMENTAL BIRDS

Day old chicken broilers were obtained from a commercial hatchery and placed in the experimental floor cages and reared up to 44 days of age. Each cage was 1x1 meter and had the capacity to hold 10 birds. The floor of the cage was covered with wood shaving and had feeding and watering facilities. In the first three weeks, infrared bulbs suspended above the floor were used for brooding.

TREATMENTS

Two hundred and forty broiler chickens were used. There were four dietary treatments, i.e. control, antibiotic, unprotected organic acid (Acidomix) and protected organic acid (Avimatrix). Each treatment was fed to a group of 10 chicks replicated six times, giving 60 birds per treatment.

EXPERIMENTAL PERIOD

The trials took 44 days.

MANAGEMENT OF EXPERIMENTAL BIRDS AND EXPERIMENTAL PROCEDURES

Two hundred and forty-day old broiler chicks were obtained from a commercial hatchery. They were fed on the control diet, which did not contain organic acids or antibiotics, for the first 2 days as they were acclimatized to experimental conditions. The chicks were then feather sexed, and weighed in groups of ten birds selected at random and allocated to the experimental pens each measuring 1.0 m by 1.0 m by 1.0 m. The purpose of sexing was to ensure that each treatment had equal number of male and female birds to remove any variation due to gender. They were introduced to experimental diets from the 3rd day and fed on them to the end of the experiment at forty-four days.

Wood shavings, 10 cm deep, were used as bedding in each pen. For the first three weeks, the pens were heated using infrared bulbs. Temperatures were maintained at 32 - 35°C which was reduced at 2°C every week by adjusting the height of the bulb and the curtains up to the fourth week. Feed and water was provided *ad-libitum*. The feeding trial lasted for a period of six weeks. The birds were fed on a broiler starter diet for the first three weeks and a broiler finisher for the final three weeks.

DATA COLLECTION

i. GROSS PARAMETERS

Data on feed intake, body weight of birds and the feed consumed were taken at day three and thereafter on weekly intervals. Body weight gain was calculated as the difference in weight between two consecutive weighing. Feed intake was computed as the difference in feed offered and feed left over at the end of every week. Feed conversion ratio was calculated as the ratio between feed intake and body weight gain. Mortality was assessed daily by counting the number of birds that died throughout the experimental period.

ii. GUT HEALTH

This was assessed by examining jejunal morphometry and bacteriology of the birds from the different treatments. Bacteriology was assessed by the total coliforms count, total bacterial count and lactobacilli counts in the jejunum contents. Histology of the jejunal section was done to assess villi basal breadth, villi height, glandular base thickness and tunics or mucosal thickness.

Gut samples were aseptically taken at days 23 and 44 using one bird from each replicate thus six birds from each treatment. Bacterial count was done using a 10 cm section of the jejunum from Markel's diverticulum which was sectioned and kept in peptone water. The histology section was the next 10 cm section after the bacteriology section. Thus 20cm section of jejunum, from the Merkel's diverticulum, was taken for the two procedures.

Bacterial count

A 10cm section of the jejunum from Markel's diverticulum was aseptically removed from one bird from each replicate and kept in peptone water. Coliforms were isolated using MaConkey Agar while MRS (Merck, Sharpe & Rogosa) Agar was used for Lactobacilli and PCA (Plate Count Agar) for total bacterial count. O.5ml of each sample was taken through four 90% serial dilutions. From the original sample, 0.5ml was taken and diluted with 4.5ml of peptone water, forming Dilution 1. 0.5ml of dilution one was then mixed with 4.5ml of peptone water to form Dilution 2, then 0.5ml of Dilution 2 used to form Dilution 3 and 0.5ml of Dilution 3 mixed with 0.5ml peptone water to form dilution 4. Dilutions 3 and 4 were used for bacterial cultures for this experiment. Pour plate method was used for PCA – the sample was mixed with warm liquid PCA and then poured into petri dishes then allowed to dry. The plates were incubated at 37ºC for 48 hours then checked for colony growths every 24 hours.

Spread plate method was used for MaConkey and MRS Agar. The 0.5ml samples were poured onto dried MRS agar and MaConkey Agar in a petridish then evenly spread out using sterile grass rods. The MaConkey plates were incubated at 37ºC for 48 hours and checked for colony growths every 24 hours. MRS plates were incubated under similar conditions but anaerobically in anaerobic jars, some modified from plastic containers with burning candles enclosed to eliminate oxygen.

Gut histology

Gut histology was assessed using a 10cm section taken from the jejunum, anterior to bacteriology sample, and preserved in 10% formalin and routinely processed, sectioned, fixed and stained for histology (M. Nasrin et al, 2012). Light microscopy of gut sections was used to evaluate villi basal breadth, villi height, glandular base thickness and tunics or mucosal thickness. Longitudinal and transverse samples of mid-portions of jejunum were fixed in 10% formalin and then processed routinely for histology. Gut sections were embedded in paraffin wax and sectioned on microtome at a thickness of 5 µm. The sections were then stained with haematoxyline and eosin (Mohammadpour, 2006). The heights of the villi, villi breadth, glandular area thickness and tunics/ mucosa thickness, in µm, were measured using a Leica DM 500 light microscope (Leica Microsystem Cambridge Limited) with Leica Application Suit system and a magnification of X4 and converted into millimeters (mm) for further analyses.

4.2.2. EXPERIMENT 2

EXPERIMENTAL DIET

The composition of experimental diets was same as in experiment one (Table 11). The only difference was the administration of organic acids through drinking water.

Table 12: Experimental diets and water treatment

Treatment	Dietary treatment	Water treatment
1	Control	Organic acid
2	Antibiotic	Organic acid
3	Unprotected organic acid	Organic acid

4	Protected organic acid	Organic acid

Activate WD is a commercial blend organic acid used for treating poultry drinking water

The dietary treatments were similar to experiment one except that the drinking water was treated with a mixture of organic acids, (Activate WD). The water was treated with the organic acid mixture, at the rate of 0.3 ml per litre of drinking water, from day 3 to day 16, stopped between days 17 to 31 then resumed from day 31 to 44. The rest of the procedure was as in experiment one. The birds were feed on starter diet from day 3 – 23 and finisher diet from day 24 – 44. The broiler starter and finisher diets were formulated as shown in table 16. Appropriate additives were added to the diets as shown below to make four dietary treatments. Diet 1, the control diet, contained neither organic acid nor antibiotic, while diet 2, 3, and 4 contained an antibiotic, un-protected organic acids and protected organic acids, respectively. Each diet was replicated seven times with ten chicks per replicate making a total of 280 birds for this experiment.

EXPERIMENTAL DESIGN

A completely randomized block design was used for this investigation. The replicates for each treatment were randomly assigned within the poultry unit. Trial two had four treatments with seven replicates of ten birds each per each but with organic acid added to the drinking water. The management was similar to experiment one.

EXPERIMENTAL BIRDS

Day old chicken broilers were obtained from a commercial hatchery, placed in the experimental units and reared up to 44 days of age.

TREATMENTS

Similar to experiment one except for the addition of organic acids in drinking water.

EXPERIMENTAL PERIOD

The trials took 44 days.

MANAGEMENT OF EXPERIMENTAL BIRDS AND PROCEDURES

Two hundred and eighty-day old broiler chicks were obtained from a commercial hatchery compared to two hundred and forty in experiment one. The management of the experimental birds was similar to trial one except for the addition of organic acids to the drinking water given to the birds.

DATA COLLECTION

The data collected and procedure for this trial was same as in experiment one.

4.3. Results and discussion

4.3.1. EXPERIMENT ONE.

EFFECTS OF DIETARY ORGANIC ACIDS AND ANTIBIOTICS ON BROILER CHICKEN PERFORMANCE

Birds fed on diet 1 (control) had the best feed conversion ratio of 2.59 followed by those fed on diets containing the antibiotic (2.69), Those fed on diets with protected organic acid had a feed conversion ratio of 2.71 and 2.73 for those fed on diets with unprotected acid. The group fed on the diet containing antibiotics had the highest average Feed intake (5876g) followed by those fed on the diet with protected organic acid (5761g). The highest average live weight was from birds on diets containing antibiotics 2243g, followed by those fed on the control diets, 2154g, protected acid, 2130g, and then unprotected acid, 2044g. Average daily gain followed a similar trend but these observed differences were not statistically significant as shown in table 13.

Table 13: Effects of organic acids and antibiotics fed through the feed on broiler chicken performance at 44 days

Treatment	Feed intake g/bird	Average daily Gain g/bird	Bodyweight /g/bird	Feed/ Gain
Control	5590	48.97	2154	2.59
Antibiotic	5846	50.98	2243	2.61
Unprotected organic acid	5578	46.45	2044	2.73
Protected organic acid	5761	48.41	2130	2.71
SEM	505.94	2.08	31.85	0.18

Means with no superscripts in a column are not significantly different (P > 0.05)

From Table 13, dietary treatments had no effect on (P > 0.05) feed intake, daily gain, final body weight and feed conversion. Thus there were no adverse effects on feed intake due to addition of organic acid mixtures in the broiler diets. The performance compared well with birds fed on antibiotic growth promoters.

FEED CONVERSION RATIOS OF BROILER CHICKENS

As shown in Table 13, the observed feed conversion ratios between treatments were not statistically significant (P>0.05). The addition of organic acid blends in the broiler diets did not have adverse effects on feed conversion ratio. Birds fed on diets containing organic acids performed just as well as those fed on antibiotic growth promoters. Differences observed between the final body weights from the different treatments were not statistically significant (P > 0.05) (Table 14). Birds fed diets containing organic acids performed just as well as those fed diets containing antibiotic growth promoters.

Feed intake, feed conversion ratio and weight gain of the broiler chicken were not significantly affected by dietary treatments (P>0.05). This is in contrary to studies by other researchers (Chaveerach et al., 2001; Dibner & Richards, 2005; Yegani & Korver, 2008*)* who reported a decrease in feed conversion ratio with inclusion of antibiotics and organic acids in chicken diets The effect on body weight was consistent with findings of Jan Kopecky (Ján Kopecký et al, 2012, sheikh Adil et al, 2010). (sheikh Adil et al, 2010, Azza M. Kamal & Naela M. Ragaa, 2014) demonstrated significant improvement in feed conversion ratio. The differences could be due to dosage of organic acids or and differences in feeding and water management. Jan Kopecky et al (2012) demonstrated that organic acids have optimum concentrations at which best performance is realized. There is thus need to evaluate the based dosage and or regime for the organic acid blends that we used in these trials.

Table: 14: Effects of organic acids and antibiotics fed through the feed on gut morphology at the jejunum area of broiler chickens at 44 days in microns (µ)

Treatment	Glandular area thickness µ	Tunic thickness µ	Villi breadth µ	Villi height µ
Control	225.4[a]	198.1[b]	108.4 [a]	1286[b]
Antibiotic	317[c]	246.9[c]	138[b]	1500[c]
Unprotected organic acid	270.4[b]	231.6[c]	151[b]	1125 [a]
Protected organic acid	215.4[a]	161 [a]	177.7[c]	1337[b]
SEM	53.209	42.408	36.453	212.414

Means with different superscripts in a column are significantly different (P > 0.05)

i. **Effects dietary treatments on the jejunal villi height**

Antibiotic treatment (T2) yielded villi height of 1500 µ, which was significantly taller than from protected organic acid 1337 µ and unprotected organic acid 1125 µ. Unprotected organic acid (T3) had the shortest observable villi. The observed difference between these means were statistically significant, p = <0.001(Table 14). This is inconsistent with findings by Sheik and associates, in 2010, that villi heights were only significantly higher in the duodenum but not jejunum and ileum. The villi heights from control and protected organic acids treatments were not statistically different from each other but differed from antibiotic and unprotected organic acid treatment. Antibiotic treatment had yielded the best villi heights. Villi increase surface area for digestion and absorption thus improvement in villi height should increase digestive efficiency (De Verdal et al, 2010).

ii. Effects on Tunic thickness at the jejunum

Diets containing unprotected organic acids 246 µ and antibiotics 231 µ, produced tunics thickness which were non-significantly different from each other but significantly thicker than control and protected organic acid diets. The observed differences in the thickness of the tunics of the jejunum region between the means of the four treatments were statistically significant, p = <0.001(Table 14). The tunic thicknesses from antibiotic and organic acid dietary treatments had no statistically significant differences. Treatment with protected organic acid yielded the thicker jejunal tunics than antibiotics and unprotected organic acid but lower than control. The dietary treatments thus affect the development of the layers forming the walls of the jejunum in broiler chicken. Thinner muscular is layer improves digestion and absorption of nutrients (Sheikh et al, 2010). Protected organic acids produced the best tunica thickness.

iii. Effects on thickness of glandular area of the chicken jejunum.

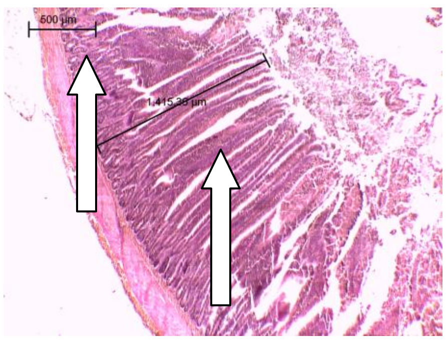
Antibiotic diet produced significant improvement in size of glandular area, 317 µ compared to unprotected organic acid 270 µ and protected acid diets 215 µ. Unprotected acid diets had significantly bigger glandular area than control diet. The observed difference in the thickness of glandular area at the jejunum region between the means of the four treatments were statistically significant, p = <0.001 (Table 14). The dietary treatments affect the development of glands within the jejunum area of broiler chicken gut. Glandular area thickness was found to be significantly greater from antibiotic diet than in the organic acid diets.

iv. Effects of the treatments on the breadth of the jejunum villi of broiler chickens

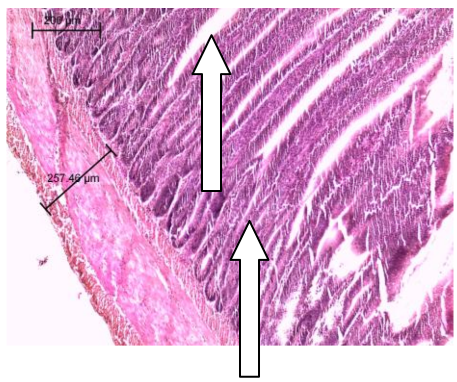
Diets containing protected organic acids yielded significantly broadest villi, 177 µ, compared to unprotected organic acids and antibiotic diets. The unprotected organic acids, 151 µ and antibiotic diets, 138 µ, yielded villi breadth which were non-significantly different. Both antibiotic treatment and the control had measurements below the average. The observed differences between the mean villi breadth at the jejunum from the different treatments were statistically significant, p = <0.001. The dietary treatments had an influence on the development of the jejunal villi of broiler chickens (Table 14). Organic acids promote development of broader villi leading to increased surface area for digestion and absorption thus improving feed conversion efficiency

The gut morphometry was measured as follows

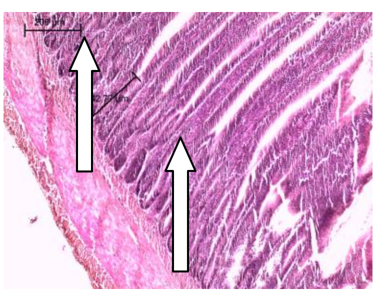
Villi height measurement



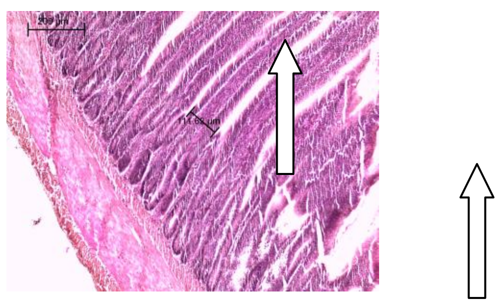
Tunics thickness of the jejunal wall



Glandular are thickness in the jejunal region



Villi breadth in jejunal area



EFFECTS OF ORGANIC ACIDS AND ANTIBIOTICS FED THROUGH THE FEED ON THE GUT MICROFLORA OF BROILER CHICKENS AT DAY 44 IN MICRONS (µ)

No observable significant difference in total bacterial counts p = 0.347 between the diets but control despite the observed higher counts from unprotected organic acids. Unprotected organic acid diets yielded significantly higher lactobacilli and yeast counts p = 0.009. Yeast and lactobacilli are beneficial in improving gut health and feed efficiency through competitive exclusion and production of metabolites which aid in digestion and absorption. All the treatments had a non-significant differences in reduction in coliform counts p = 0.542. Reduction in coliforms is important in improving gut health and performance. The control and unprotected organic acid were not significantly different but both were different from antibiotic and protected organic acid. Both antibiotic and protected organic acids had no significant differences on lactobacilli and yeast counts in the jejunum. (Table 15).

Table: 15: Effects of organic acids and antibiotics fed through the feed on the gut microflora of broiler chickens at day 44

Treatment	Total bacteria count$\times 10^5$	Lactobacilli and yeast$\times 10^5$	Coliforms $\times 10^5$
Control	223	381.3[b]	3.25
Antibiotic	92.4	92 [a]	0.3
Unprotected organic acid	118.2	136.5[ab]	0.917
Protected organic acid	58.6	40.4 [a]	0.75
SEM	21.12	207.48	3.65

Means with similar or no superscripts in a column are not significantly different (P > 0.05)

The presence of intestinal microbes reduces poultry digestive efficiency either by competition with the host for intestinal nutrients, appetite reduction due to immune response and catabolism of muscle protein to meet this demand, degradation of the intestinal tract and disease, mainly necrotic enteritis (Dibner & Buttin, 2002). The reduction of total bacterial count was expected to stimulate an increase in feed conversion efficiency as exhibited in previous studies (Ferkett, 2004; Lan et al., 2005; Dibner & Buttin, 2002; Yegani & Korver, 2008).

EFFECTS OF DIETARY ANTIBIOTIC OR ORGANIC ACID SUPPLEMENTATION ON THE IMMUNE RESPONSE TO NEWCASTLE VACCINATION IN BROILER CHICKENS

There were no significant differences observed in the immune response to Newcastle disease vaccine between treatments. The addition of organic acids in diets has no adverse effect on immune response of the broiler chickens as shown in table 16.

Table: 16: Immune response to Newcastle vaccine

Treatment	Newcastle antibody titers
Control	170.67
Antibiotic	100
Unprotected organic acid	24.67
Protected organic acid	78.67
SEM	24.66

Means with no superscripts are not significantly different (P > 0.05)

4.3.2. EXPERIMENT 2

EFFECT OF SUPPLEMENTING DRINKING WATER WITH ORGANIC ACID ON BROILER PERFORMANCE

The highest recorded feed intake, final body weight and average daily gain was recorded from birds fed on diets containing protected organic acid. Broilers fed on diets with unprotected organic acid had the lowest feed conversion ratio followed by Treatment 4, (protected organic acid). This finding is in contrast to table 13, where antibiotic had the best observable performance. Drinking water organic acids seem to improve the performance of broilers fed on diets containing organic acids.

These observed feed intake, feed conversion ratio and weight gain of the broiler chicken seemed not to be significantly affected with the dietary treatments ($p>0.05$) both with and without inclusion of organic acid in the chickens' drinking water. This is despite the inherent expectation of feed conversion ratio to reduce with introduction of antibiotic and organic acids into the meals. This compares with studies by other researchers in the poultry industry (Chaveerach et al, 2001; Dibner & Richards, 2005; Yegani & Korver, 2008; (Sheikh Adil et al, 2010)),who reported a decrease in feed conversion ratio with inclussion of antibiotics and organic acids in chicken feed. The ratio averaged at 2.41 as illustrated in Table 17. Application of organic acids in drinking water has been shown to influence contamination of drinking water leading to a lower enterobactericae counts compared to normal drinking water, with no significant effect of body weights of birds (Chaveerah et al, 2004). Short chain fatty acids like propionic and butyric acids have been shown to be essential for normal structure and function of the gut epithelium. Maximum nutrient utilization from feed is achievable from healthy gut (Yegani & Korver, 2008).

Table 17: Effects of treating drinking water with organic acids on their performance at day 44.

Treatment	Feed intake g/bird	Average daily gain g/bird	Body weight g	Feed/gain
1.Control	4190	38.52	1695	2.466
2.Antibiotic	4154	38.6	1699	2.449
3.Unprotected organic acid	4155	39.87	1754	2.366
4.Protected organic acid	4385	41.96	1846	2.375
SEM	**392.088**	**1.606**	**21.62**	**0.165**

Means with no superscripts in a column are not significantly different (P > 0.05).

No observable significant differences in feed intake, average daily gain, final weight and feed conversion ratio between antibiotic and organic acid diets (P > 0.0%). This was similar to experiment one in which there was no addition of organic acids to drinking water for the chickens (Table 17). Organic acids thus have no observable adverse effects on broiler performance.

Table 18: Effects of treating drinking water with organic acids on their gut morphology at the jejunum area at 44 days of age.

	Glandular area thickness µ	Tunic thickness µ	Villi breadth µ	Villi height µ
1.Control	242 [a]	251.7 [d]	120 [a]	1431 [b]
2.Antibiotic	323.8 [b]	219.4 [c]	184.4 [c]	1722 [c]
3.Unprotected organic acids	245 [a]	156.5 [a]	146.5 [b]	1065 [a]
4.Protected organic acids	306.8 [b]	195.6 [b]	132.4 [ab]	1449 [b]
SEM	39.572	37.801	27.902	238.285

Means with different superscripts in a column are significantly different (P > 0.05)

Antibiotic, 323 µ, and protected organic acid, 306 µ, diets had significantly larger glandular area than unprotected acid diets, 245 µ and control, 242 µ. There was a significant increase in the size of glandular area with addition of organic acids to drinking water on birds fed on protected acids. Glandular area secretions aid digestion, absorption and immunity of the gut.

Antibiotic, 219.4 µ and control, 251.0 µ diets yielded significantly thicker tunics than unprotected organic acids, 156.5 µ and protected organic acids, 195.6 µ. Thinner tunics is important for improved absorption of nutrients (Taylor-Packard et al, 2008).

All the treatments had significant improvement on villi breadth. Antibiotic diets, 184 µ had significantly wider villi than protected acid, 132.4 µ and unprotected acid, 146.5 µ, diets.

Antibiotic diets, 1722 µ yielded significantly taller villi than organic acid diets.

Villi breadth and length are important for increasing absorptive surface area (Sheikh et al, 2010). Addition of organic acid to drinking water had a positive effect on gut morphometry thus may improve gut health and performance.

Table: 19: Effects of treating drinking water with organic acids on their gut microbiology.

	Total bacterial count x 10^5	Lactobacilli and yeast x 10^5	Coliforms x 10^5
1.Control	159.1	21.07^{ab}	8.286
2.Antibiotic	3.5	4.43^{a}	0.286
3.Unprotected organic acids	147.9	14.29^{ab}	2.286
4.Protected organic acids	291.3	92.86^{b}	0
SEM	353.660	61.907	11.039

Means with no or similar superscripts in a column are not significantly different (P > 0.05).

Addition of organic acid lead to 28% and 96% reduction in gut microbial counts for control and antibiotic treatments respectively but an increase of 25% and 397% for unprotected and protected organic acid diets respectively. Despite no statistically significant difference in coliform counts, protected organic acid diets had undetectable coliform counts. There were significant differences in lactobacilli and yeast counts between antibiotic, 4.43 $x10^5$ cfu per ml, and unprotected organic acid, 14.29 $x10^5$ cfu per ml, and protected organic acid, 92.86 $x10^5$ cfu per ml, diets. Protected organic acid diet yielded significantly higher lactobacilli and yeast counts.

Addition of organic acids to drinking water thus improves the population of good gut microflora while reducing the population of pathogenic gut microflora. This is consistent with earlier findings that organic acids cause a reduction in pH suppressing growth of pathogenic microbes like campylobacter while promoting acid tolerant lactobacilli and yeast. According to Callaway & Ricke (2012), Lactobacilli and yeast are key components of probiotics that promote gut health.

Table: 20: Effects of the treatments on immune response to Newcastle vaccine

Treatment	Newcastle virus antibody titers
1.Control	74.67
2.Antibiotic	121.33
3.Unprotected organic acids	121.33
4.Protected organic acids	40
SEM	66.84

Means with no superscripts are not significantly different (P > 0.05)

There were no significant differences in antibody titers between the treatments p = 0.565. Organic acid treatments have no negative effects on immune response.

DISCUSSION

Digestible and non-digestible materials enter the gut of chicken after ingestion exposing the gut to pathogens and mechanical injuries. The gastrointestinal tract in optimum working condition must handle feed and its constituents, indigenous and foreign microflora and digestive secretions, while allowing nutrients to diffuse into the body, while keeping off harmful fractions of the feed from passing through the intestinal wall (Korver, 2006). The major components of the gastrointestinal tract that allow this efficient mechanism include; villi height, tunic thickness, glandular area thickness and villi breadth.

There was observed a general improvement in feed conversion ratio in treatments with organic acid included in drinking water. This was despite the reduction in microflora in the gut on treatment of drinking water with organic acid. Microbial load was reduced in the gut of the birds from all the treatments. Improved gut integrity was however observed without inclusion of organic acid in the chicken’s drinking water. The study recommended further studies to determine which combinations of gut morphometry parameters gives the best gut integrity and best performance in broiler chickens. Improvement in non-pathogenic flora has been shown to be associated with improvement in broiler chicken performance (Niewold, 2007) though not found to be statistically significant. Organic acid treatments resulted in increase in lactobacilli and yeast. Lactobacilli and yeast have a positive influence on gut health by creating conditions unfavourable to pathogenic bacteria. Addition of organic acids to drinking water resulted in improvement in most parameters thus should be adopted. Organoleptic taste improvement of the broiler meat was reported by all those who ate the birds.Organic acids can thus be used as alternatives to antibiotics in broiler diets and in broiler chicken management without any negative effect on production.

In this study, villi height from the antibiotic treatment was significantly higher than that from organic acids. The villi height provides an effective surface area for absorption nutrients, increasing feed conversion efficiency.

Villi breadth, contributes to increment of the villi surface area for nutrient absorption (Taylor-Packard et al, 2008), was significantly higher from diets containing protected organic acids than antibiotic but the antibiotic diets yielded similar results to unprotected organic acid diet.

Tunic thickness is the major component of the gastrointestinal wall. It selectively allows passage of nutrients to the blood stream (Taylor-Pickard et al, 2008). The diets with unprotected organic acid and antibiotic yielded tunics with significant difference and were significantly higher than control diet and diet containing protected organic acid.

These observed differences in gut morphology among the treatments did however not contribute to any significant differences in growth performance of the broiler chickens. There is need to investigate on which of these four parameters determine the growth performance of broiler chickens.

Lactobacillus and yeast were found to be significantly higher in chicken fed control diets and diet containing unprotected organic acid than in chicken fed the diet containing antibiotic and protected organic acid. There were no significant variations between diets containing the antibiotic and the organic acids on total bacteria counts and coliforms counts in the jejunum.

These significant differences in gut parameters did not result in in significant differences in gross performance parameters. There is need to correlate the significance of each or combination of gut parameters on performance of chicken broilers.

Organic acids have no observable negative effect on chicken broiler performance.

Intestinal microflora

The presence of intestinal microflora is thought to reduce poultry digestive efficiency by several mechanisms; competition for intestinal nutrients, appetite reduction due to immune response and catabolism of muscle protein to meet this demand, degradation of the intestinal tract and disease mainly by necrotic enteritis (Dibner and Buttin, 2002). This was evident in trial one where the total bacterial count was significantly higher in the control diet. The addition of organic acid in drinking water led to significant reduction of total gut microflora while shifting the balance in favour of friendly microbes. The reduction of total bacterial count is expected to stimulate an increase in feed conversion efficiency as exhibited in previous studies (Ferkett, 2004; Lan et al., 2005; Dibner and Buttin, 2002; Yegani & Korver, 2008).

Lactobacillus and yeast were found to be significantly higher in chicken fed diets containing antibiotic and organic acid without organic acid in drinking water than the control diet. There were significant variations between diets containing the antibiotic and the organic acids on addition of organic acids to their drinking water. Introduction of organic acid in drinking water, significantly reduced coliform counts ($p<0.05$) protected organic acid diet. This illustrates the synergistic effects of both the organic acid and the antibiotic in reduction of the coliforms. This reduction in coliforms was associated with a reduction in feed conversion ratio in all the dietary treatments. The addition of organic acids to drinking water for both protected and unprotected organic acid treatments lead to more reduction in coliform counts.

The coliforms count was found to be significantly lower in all the treatments when compared with the control diet. The lactobacillus and yeast counts were increased for protected organic acid diet by addition of organic acid in drinking water but was reduced in control, antibiotic and unprotected organic acid treatments. The antibiotics reduced gut microbial counts while organic acids caused an increase in total microbial counts, this could have contributed to observed effects.

Gut morphology

Digestible and non-digestible materials enter the gut of chicken after ingestion exposing the gut to pathogens and mechanical injuries. The gastrointestinal tract should have the capacity to contain feed and its constituents, indigenous and foreign microflora and digestive secretions. It should allow nutrients to diffuse into the body but keep off harmful fractions of the feed from passing through the intestinal wall (Korver, 2006). This function of the gastrointestinal tract is aided by villi height, tunic thickness, glandular area thickness and villi breadth.

In this study, villi height among the treatments was significantly higher that control. This is in exception of the diets treated with Acidomix (unprotected organic acid), which was lower than the control diet. The villi height provides an effective surface area for absorption of nutrients thus increasing feed conversion efficiency. Treatment of drinking water with organic acid was found to give the best villi height with an average of 1416 microns.

Villi breadth contributes to increment of the villi surface area for nutrient absorption, was found to improve in all the treatments in comparison to the control diet. Treatment without inclusion of organic acid in drinking water gave the largest villi breadth at day 44. This indicates that villi breadth improves with age unlike villi height which showed no significant change with time ($p<0.05$).

Tunic thickness is a major component of the gastrointestinal wall that selectively allows passage of nutrients to the blood stream. Tunic thickness was more emphasized in the treatments when organic acid was not included in drinking water. Addition of organic acid in drinking water resulted in thinner tunics.

Glandular area thickness, which enhances the efficiency of the gastrointestinal tract, was found to be significantly greater than in the control diet. Just like in the case of tunic thickness, without addition of organic acid into the drinking water reduced the thickness of this vital area.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Antibiotic use

(i) The Agrovets sold poultry antimicrobials to farmers for their own use.

(ii) The choice of antimicrobials was based on the veterinarians, para veterinarians, the farmers and or farmers' neighbors.

(iii) There was no control in the sale to and use of antimicrobials by the farmers.

(iv) Most common diseases in the region were coccidiosis, diarrhea, E. coli infections and gumboro.

(v) All the millers used antibiotics in formulating poultry feeds.

Socio-economic characteristics

(i) Most poultry farmers were females in the area in and around Nairobi.

(ii) Broiler chickens were the dominant type of bird kept.

(iii) Most of the farmers were either secondary or tertiary institution's graduates.

Husbandry practices

(i) Most farmers used antibiotics of various types for various conditions in their farms.

(ii) Agrovets were the main source of antibiotics and instructions on their usage by farmers.

(iii) Some farmers dictated the antibiotic choice based on peer advice or past positive experience. Most farmers preferred combination antibiotics, some containing human antibiotic preparations like Doxycycline, Erythromycin and Streptomycin among others. Resistance in broilers would be a challenge to the humans and must be guarded against.

(iv) Bacterial diseases constituted the majority of diseases reported by the farmers

(v) Most farmers, 72% did not observe antibiotic withdrawal period thus the great danger of residues in meat.

Effects of organic acids on performance of chicken broilers

(i) There was no significant difference in performance of birds on antibiotic and organic acid treatment.

(ii) Addition of organic acids in drinking water resulted in better feed conversion, increase in beneficial gut microflora and decrease in pathogenic microflora.

(iii) Organic acids can thus effectively replace antibiotics in poultry management.

Recommendations

(i) Organic acids should be used to replace antibiotic usage both in poultry feeds and their routine management.

(ii) There is need for a more detailed study involving a large number of birds for longer periods to evaluate the benefits of alternative options to antibiotic use in livestock production.

(iii) There is an urgent need for a policy formulation to regulate sale and use of antibiotics in livestock production to reduce misuse and increase food safety.

(iv) Need for a concerted effort to promote awareness and use of alternatives to antibiotics in production of poultry.

CHAPTER SIX: REFERENCES

Abbas et al. (2011, 2). Anticoccidial effects of acetic acid on performance andpathogenic parameters in broiler chickens challengedwith Eimeria tenella. *Pesquisa Veterinária Brasileira*, *31*(2), 99-103.

ABS TCM LTD. (2013). *Kenya Feed Industry Policy and Regulatory Issues - Study on the Kenyan animal feed and fodder sub-sectors.* Nairobi: SNV/Kenya Netherlands Development Organisation.

Adams, C. (1999). *Nutricines: Food components in health and nutrition.* UK: Nottingham University Press.

AKEFEMA. (2011). *USAID Kenya Dairy Sector competitive program – AKEFEMA capacity building.* Nairobi: AKEFEMA (Kenya Association of Feed Manufacturers).

Alloui et al. (2013, 06 03). The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. *Annals of Animal Science, 13*(1), 17-32. doi:10.2478/v10220-012-0055

Azza M. Kamal & Naela M. Ragaa. (2014, 1 26). Effect of Dietary Supplementation of Organic Acids on Performance and Serum Biochemistry of Broiler chicken. *Nature and science, 12*(2), 38-45.

Baurhoo, B., Littelier, Z., & Ruiz-Feria, C. (2007). Cecal Populations of Lactobacilli and Bifidobacteria and Escherichia coli Populations After In Vivo Escherichia coli Challenge in Birds Fed Diets with Purified Lignin or Mannanoligosaccharides. *Poultry Science, 86*(12), 2509-2516.

Charlotte et al, 2. (2007, October 26). Impact of salinomycin on the intestinal microflora of broiler chickens. *Acta Veterinaria Scandinavica, 49*(30), 1-8. doi:: 10.1186/1751-0147-49-30.

Chaveerach, P., Keuzenkamp, H. A., Urlings, L. J., Lipman, & Van Knapen, F. (2001). In vitro study on the effect of organic acids on Campylobacter jejuni/coli populations in mixtures of water and feed. *Poultry Science, 81*(5), 621-628.

Dalloul et al. (2003, 1). Enhanced Mucosal Immunity Against Eimeria acervulina in Broilers Fed a Lactobacillus-Based Probiotic. *Poultry Science, 82*(1), 62-66.

De Verdal et al, 2. (2010, 6 15). Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. *Poultry science*, *89*(9), 1955-1961.

Dibner and Buttin. (2002). Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research, 11*(4), 453-463.

Dibner, J., & Richards, J. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science, 84*(4), 634-643.

Export Processing Zones Authority. (2005). *Meat Production in Kenya.* Nairobi: Export processing Zones Authority.

Ferkett, P. (2004). Alternatives to antibiotics in poultry production: response, practical experience and recommendations. *Biotechnology in the Feed and Food Industry, In Alltech's 18th Annual Symposium* (pp. 57-66). Nottingham University Press.

Garcia V. et al. (2007). Effect of Formic Acid and Plant Extracts on Growth, Nutrient Digestibility, Intestine Mucosa Morphology, and Meat Yield of Broilers. *Poultry Science Association*, *16*(4), 555-562.

Ghazalah, A. A. (2011). Effect of Dietary Supplementation of Organic Acids on Performance,. *International Journal of Poultry Science 10 (3): 176-184, 2011*, 176-184.

Giannenas I.A. (2006). Organic acids in pig and poultry nutrition. *Journal of the Hellenic veterinary medical society, 57*(1), 51-62.

Hajati et al. (2010). The Application of Prebiotics in Poultry Production. *International Journal of Poultry Science,* 298-304.

http://www.mapsofworld.com/kenya/cities/nairobi.html. (2016, 6 28). Retrieved from Maps of the world.

Ján Kopecký et al. (2012). Effect of Organic Acids Supplement on Performance of broiler chickens. *Animal Sciences and Biotechnologies, 2012, 45 (1)*, 1.

Jeffrey, J. S. (1999, March). *Cooperative extension, University of California, Poultry fact sheet No. 30*. Retrieved from http://animalsciencey.ucdavis.edu/avian/pfs30.htm.

Jensen, B. (2001). The impact of feed additives on the microbial ecology of the gut in young pigs. *Journal of animal and feed sciences, 7*, 45-64.

John and Ricke. (1998). Induction of acid resistance of Salmonella typhimurium by exposure to shortchain fatty acids. *Applied and Environmental Microbiology, 64*(9), 3458-3463.

Karuri, J. (2010). *Overview of kenyan Feedstuff Industry. Challenges and way forward.* Nairobi: Association of Kenya Feed Manufacturers.

Kenya Bureau of standards. (1990). *Poultry feed premixes- Specification.* Nairobi: KEBS.

Kitandu et al. (2006, 3 16). Progress in Control Measures for Chicken Coccidiosis. *Acta Veterinarian, 75*, 265-276.

Korver, D. (2006). Overview of the immune dynamics of the digestive system. *Journal of Applied Poultry Research, 15*, 123-135.

Lan, Y., Verstegen, S., & Williams, B. (2005, 3). The role of the commensal gut microbial community in broiler chicken. *World Poultry Science Journal, 61*(1), 95-104.

Langout, P. (2000). New additives for broiler chickens. Feed Mix Special: Alternatives to antibiotics. *British Journal of Nutrition, 9*(6), 24-27.

M. Nasrin et al, 2. (2012). Gross and histological studies of digestive tract of broilers during postnatal growth and development. *Journal of Bangladesh Agricultural Univ.ersity 10(1): 69–77, 2012* , 69-77.

Marco et al. (2013). Organic Acids Effective to Ameliorate the Negative Impact on Broiler Performance Due to Necrotic Enteritis. *Novus International*.

Masood et al. (2013, 7 21). Role of Natural Antioxidants for the Control of Coccidiosis in Poultry. *Pakistan Veterinary Journal*, *33*(4), 401-407.

Mbugua, W. H. (2010). *Poultry farming.* Nairobi: Association of Kenya Feed Manufacuturers.

Mitema et al,. (2001). An assesment of antimicrobial consumption in food producing animals in Kenya. *Journal of Veterinary Pharmacology and therapeutics*, *24*(6), 385-390.

Mohammadpour, A. A. (2006). Morphological studies on Meckel's diverticulum in the duck (Ansa ansa domesticus) . *Iranian Journal of Veterinary Research, University of Shiraz, Vol. 7, No. 1, Ser. No. 14, 2006* , 14-16.

Montagne, L., Pluske, R., & Hampson, D. (2003). A review of interactions between dietary fibre and the intestinal mucosa and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science Technology, 108*(1), 95-117.

Montonya et al. (2011, 8 29). *Use of an organic acid (Activate® wd) as field intervention to reduce Salmonella spp. in broiler chickens.* (M. Anaya, Ed.) Retrieved from en.engormix.com/MA-poultry-industry.

Naliaka, M. A. (2011). *A comparative study of antibiotic resistance profiles among enteric bacteria in broilers and traditional chicken from selected farms in kericho, kenya.* Kenyatta University, School of Pure and Applied Sciences of Kenyatta University. Nairobi: Kenyata University.

Niba, A., Beal, J., & Kudi, A. (2009). Potential of bacterial fermentation as a biosafe method of improving feeds for pigs and poultry. *African Journal of Biotechnology*, *8*(9), 1758-1767.

Niewold, T. (2007, April). The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action: A hypothesis. *World Poultry Science Journal*, *86*(4), 605-609.

Odhiambo, W., & Manda, D. (2003). *Urban Poverty and Labour Force Participation in Kenya*. . Nairobi: Kenya Institute for Public Policy Research and Analysis (KIPPRA).

Prescott, J., Baggot, J., & Walker, R. (2000). *Antimicrobial therapy in veterinary medicine* (3rd ed.). (J. F. Steeve Giguère, Ed.) Iowa, Iowa, USA: Iowa State University Press.

Ramana et al. (2015, 10 21). A review of the effects of dietary organic acids fed to swine. *Journal of Animal Science and Biotechnology, 6*(45). doi:10.1186/s40104-015-0042-z

Ravindran, V. (2013). Feed enzymes: The science, practice and metabolic realities. *Poultry Science Association, Inc, 22*(3), 628–636.

Revolledo et al, 2. (2006). Prospects in Salmonella Control: Competitive Exclusion, Probiotics, and Enhancement of Avian Intestinal Immunity. *Applied poultry research*, 341-351.

Richards, J., Gong, J., & De-Lange, C. (2005). The gastrointestinal microbiota and its role in monogastric nutritionand health with an emphasis on pigs: Current understanding, possible modulations, and new technologies for ecological studies. *Canadian Journal of Animal Science, 85*(4), 421-435.

Rusel et al. (1998). The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology, 39*, 205-234.

Scott, C. (2011, December 1). Antibiotic use under threat. *PoultryWorld*, p. 6.

Sheikh Adil et al, 2. (2010, 6 14). Effect of Dietary Supplementation of Organic Acids on Performance, Intestinal Histomorphology, and Serum Biochemistry of Broiler Chicken. *Veterinary Medicine International. 2010; 2010: 479485., 2010*, 1-8.

Sheikh et al. (2010). Effect of Dietary Supplementation of Organic Acids on Performance, Intestinal Histomorphology, and Serum Biochemistry of Broiler Chicken. *Veterinary Medicine International, vol. 2010, Article ID 479485, 7 pages, 2010. doi:10.4061/2010/479485*, 7.

Sheikh et al. (2011). Alternative Strategies to Antibiotic Growth Promoters - A review. (M.S.Mir, Ed.) *Vetscan, 6*(1), Article 76.

Shobha and Ravindranath, 1. (2012). Options for Integrated Strategies for the Control of Avian coccidiosis. *International Journal of agriculture & biology, 14*(6), 1014 - 1020.

Sirdar, M. e. (2012, 5 21). A questionnaire survey of poultry layer farmers in Khartoum State, Sudan, to study their antimicrobial awareness and usage patterns. *Onderstepoort Journal of Veterinary Research, 79*(1), Art. 361.

Taylor-Packard et al. (2008, 1 1). Gut Efficiency: The Key Ingredient in Pig and Poultry Production—Elevating Animal Performance and Health. In J. A.-P. P. Spring, & J. A.-P. P. Spring (Ed.), *Elevating animal performance and health* (p. 192). Wageningen Academic Publishers. doi:http://dx.doi.org/10.3920/978-90-8686-636-6

USAID. (2011). *Partnership for safe poultry in Kenya (PSPK) Program.* Winrock international, USAID. Washington DC: USAID. Retrieved 2015 June

Yegani, K., & Korver, D. (2008, 10). Factors Affecting Intestinal Health in poultry. *Poultry Science, 87*(10), 2052-2063.

Yukio et al. (1974, November). Salinimycin, A new polyether antibiotic. *The Journal of Antibiotics,* 814-821.

APPENDICES

Appendix 1: ANOVA (Analysis of Variance) tables used in determining variations between the treatments in the study.

A: Effects of adding organic acids and antibiotics to feeds fed to broiler chickens on their performance

Gross parameters

Total feed intake

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		318591	106197	0.38	0.768
Residual	20	-2	5576951	278848		
Total	23	-2	5887317			

SEM 103.3

Average daily gain

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		62.49	20.83	1.84	0.173
Residual	20	-2	226.92	11.35		
Total	23	-2	289.26			

SEM 0.724

Feed conversion ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.09473	0.03158	0.98	0.422
Residual	20	-2	0.6452	0.03226		
Total	23	-2	0.73528			

SEM 0.0365

Final body weight

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		120983	40328	1.84	0.173
Residual	20	-2	439323	21966		
Total	23	-2	560010			

SEM 31.85

Gut morphometry

Glandular area thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	52051.1	17350.4	76.72	<.001
Residual	28	6332.1	226.1		
Total	31	58383.1			

SEM 7.672

Tunic thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	34920	11640	69.48	<.001
Residual	28	4691.1	167.5		
Total	31	39611.1			

SEM 6.319

Villi breadth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	19895.4	6631.8	48.23	<.001
Residual	28	3849.9	137.5		
Total	31	23745.2			

SEM 4.893

Villi height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	573549	191183	58.9	<.001
Residual	28	90884	3246		
Total	31	664433			

SEM 25.88

Gut bacteriology

Total bacteria count

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		98297	32766	1.17	0.347
Residual	20	-2	561515	28076		
Total	23	-2	652164			

SEM 34.37

Lactobacilli and yeast counts

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		438245	146082	5.01	0.009
Residual	20	-2	582796	29140		
Total	23	-2	993550			

SEM 11.70

Coliforms counts

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		32.08	10.69	0.74	0.542
Residual	19	-3	274.76	14.46		
Total	22	-3	305.22			

SEM 0.777

Newcastle antibody titres

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	65730.	21910.	1.62	0.216
Residual	20	269976.	13499.		
Total	23	335706.			

SEM 24.66

B: Effects of treating drinking water for broiler chickens with organic acids on broiler chicken performance.

Average daily gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	54.057	18.019	3.37	0.035
Residual	24	128.493	5.354		
Total	27	182.55			
SEM 0.491					

Feed conversion ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.05451	0.01817	0.64	0.597
Residual	24	0.68225	0.02843		
Total	27	0.73676			
SEM 0.0312					

Feed intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	257598	85866	0.53	0.666
Residual	24	3893204	162217		
Total	27	4150802			
SEM 74.10					

Final body weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	104654	34885	3.37	0.035
Residual	24	248763	10365		
Total	27	353417			
SEM 21.62					

Gut morphometry

Glandular area thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	42472	14157.3	65.29	<.001
Residual	28	6071.7	216.8		
Total	31	48543.7			
SEM	6.995				

Tunic thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	38639	12879.7	63.56	<.001
Residual	28	5673.6	202.6		
Total	31	44312.6			
SEM 6.684					

Villi breadth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	18648.9	6216.3	37.82	<.001
Residual	28	4601.6	164.3		
Total	31	23250.6			

SEM 4.893

Villi height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1746616	582205.4	1203.68	<.001
Residual	28	13543.2	483.7		
Total	31	1760160			

SEM 25.88

Gut bacteriology

Coliforms counts

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	311.4	103.8	0.84	0.487
Residual	24	2978.8	124.1		
Total	27	3290.2			

SEM 3.653

Lactobacilli and yeast counts

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	34241	11414	3.96	0.02
Residual	24	69237	2885		
Total	27	103478			

SEM 207.481

Total bacteria counts

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	290579	96860	0.75	0.531
Residual	24	3086460	128603		
Total	27	3377039			

SEM 21.124

Newcastle antibody titers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	28181.	9394.	0.70	0.565
Residual	20	269632.	13482.		
Total	23	297813.			

SEM 60.428

Appendix 2: Questionnaire administered to agrovets in the study.

Survey of use of antibiotics and other growth promoters from the agrovets

General

Respondent No. ____________Date:____

Enumerator:____

Name of Agrovet (Agrovets)_____________________________

Location (town/city) ____________________GPRS coordinates ________________________

Name of respondent _____________________________Title/Position ____________________________

Gender (Male/Female) ____`

Mobile telephone number ____

Email ____

Please indicate the highest level of education you have attained (tick one)

University ____

Diploma ____

Secondary ____

Primary ____

None ____

Feed Production

For how long has the company been in the Agrovet (Agrovets) business (years) _

Please do you sell any medicines for use in poultry? Yes/No_________________

Antibiotics (Yes/No) ____

Anticoccidial (Yes/No) ____

Vaccines (Yes/No) ____

Any other (specify)____

If Yes to Qn 11 please kindly indicate the name of poultry antibiotics and anticoccidials that you sell?

Ranking them on a scale of 1 -4, with 1 being most preferred and 4 least preferred.

Antibiotic /Anticoccidial	Indication/target disease	Preference (1 -4)

Please indicate the reasons why farmers the farmers use the medicines (Yes/NO)

To control diseases/treat diseases ___________________________________

It is a standard practice ____

To improve performance such as growth rate, reduce mortality etc. __

Others specify

Who decides on the medicine to be given?

Please indicate the five most preferred antibiotics?

Rank	Antibiotic
1	
2	

Please indicate the five most common poultry diseases you encounter?

Rank	Disease
1	
2	
3	

Appendix 3: Questionnaire administered to feed millers in the study area.

General

Respondent No. __________Date:_________

Enumerator:____

Name of feed compound company ____________________________________

Location (town/city) ____

GPRS coordinates ____

Name of respondent ____

Title/Position ____

Gender (Male/Female) ____

Mobile telephone number ____

Email ____

Please indicate the highest level of education you have attained (tick one)

University ____

Diploma ____

Secondary ____

Primary ____

None ____

Feed Production

For how long has the company been in the feed industry business (years)

Do you produce any of the following feeds?

Poultry feed (Yes/No) ____

Pig feed (Yes/No) ____

Dairy cattle feed (Yes/No) _______________________________________

Do you add antibiotics (Salinomycin, Virginiamycin, Olaquindox, Zinc bacitracin, monensin) in any of the feeds you have mentioned in the question above? If yes, please indicate in table below.

Antibiotic use in various types of feed

Animal	Feed type	Do you include antibiotics (Yes/No)
Poultry	Broiler starter	
	Broiler finisher	
	Chick starter (mash)	

	Growers mash	
	Layers mash	
Pig feed	Sow and weaner	
	Pig finishing	
Dairy cattle feed	Dairy meal	
	Calf feed	

Please indicate the antibiotics your company uses in the following types of feeds.

If you use the antibiotics rank them such 1= mostly used, 2 = used, 3 used sometimes and 4 =least used

Type of feed	Name of antibiotic (Please write 1, 2, 3 or 4 depending on usage)			
	1	2	3	4
Broiler starter				
Broiler finisher				
Chick starter (mash)				
Growers mash				
Layers mash				
Sow and weaner				
Pig finishing				
Calf feed				

Please indicate the rate of inclusion of the antibiotic in various types of feeds (g/tonne of feed)

Broiler feeds

Name of antibiotic	Level of inclusion (g/tonne of feed)	
	Starter	Finisher

Layer feed

Name of antibiotic	Level of inclusion (g/tonne of feed)		
	Chick	Grower	Layers

Please indicate the volume of antibiotics used on a monthly or annual basis

Total volume of antibiotics used poultry feeds

Name of antibiotic	Total volume per month (kg)	
	Broiler feeds	Layer feeds

Please indicate the reasons why you include antibiotics in feed that you compound (Yes/NO)

To control diseases/treat diseases ____

It is a standard practice ____

To improve performance such as growth rate, reduce mortality etc. _____

Others specify _____________________________________

Please indicate the source of information on the use of antibiotics in your company (tick appropriate ones). Source of information:

Antibiotic manufacturer or supplier _______________________________________

By consulting a veterinarian ____

By consulting a nutritionist ____

From literature ____

From fellow feed millers ____

Other source (specify) ____

Where do you get the antibiotics?

Name

Location

Please indicate other feed additives you use in compounding poultry feeds.

Other substances used in compounding poultry feeds

Substance	Feed type	Reason(s) for using the substance

Appendix 4: Questionnaire administered to farmers in the study area.

Survey of use of antibiotics and other growth promoters in poultry management at farm level

General

House hold number________________________Date_____________________________________

Enumerator____

Name of respondent ____

Owner Employee

Gender of Respondent Male______________________Female __________________________

Age ____

Occupation

Total land size in acres

County ____

District ____

Location ____

GPRS coordinates ____

Telephone number ____

How long have you been in poultry business (years)? ______________________________

Please indicate the highest level of education you have attained (tick one)

University ____

Diploma ____

Secondary ____

Primary ____

None ____

Please indicate if you presently have these types of chickens in your farm and show numbers

Type of bird	Presence (Tick)	Number
Indigenous chickens or local chickens		
Layers		
Broilers		

Do you keep records? Yes/No_________

What type of records do you keep?

Type of Record	Tick if yes	remarks
Feeding		
Vaccination		
Daily egg production		
Weights record		
Medication		
Sales		
Death records		
Visitors		

Do you keep the following production data for broilers?

Parameter	Tick/value	Remarks
Source of chicks		
Number of birds purchased		
Age at slaughter or sale		
Weight at slaughter		
Number sold		
Total feed consumed (70kg) bags) up to slaughter		
Number dead		
Number consumed at home		
Where sold		
Selling price per bird (Ksh)		

Do you keep the following production data for your layers?

Parameter		
Source of chicks		
Number purchased		
Date placed/bought		
Age at point of lay/first egg		
Number of birds at point of lay		
Duration of laying		
Number of eggs currently collecting per day		
Number of birds currently		
Current Feed consumed per day (kg)		
Selling price per tray (Ksh)		
Selling price per egg (Ksh)		

Please kindly provide following feeding data

Layers

Parameter	Chick mash	Grower mash	Layers mash
Source of feed (Company)			
What's the age of birds you are currently having (weeks)			
Type of feed used			
Price of feed per 70kg bag (Ksh)			

Broilers

Parameter	Starter	Finisher
Source of feed		
Age of birds (weeks)		
Type of feed used		
Price of feed per 70kg bag (Ksh)		

Please indicate the source of your extension (advisory) services

From government services ____

From the feed companies ____

From Agrovet (Agrovets) dealers ______________________________

From your dairy or other farming cooperative ___________________________

Others specify ____

Please indicate what you do when the birds are sick

Call a vet to treat them (YES/NO) ____

Buy drugs over the counter and give the birds (YES/NO) _________________________

Do nothing (YES/NO) ____

Any other (specify) ____

Do you give medicine to your birds? (YES/NO) _____________

State the reasons why you give the medicines to your birds and indicate the product used

Reasons for giving	Tick one	Product/s used
Treatment when they are sick		
To prevent disease		
As a routine		
To increase egg production		
To increase growth rate		
Advise by other farmers		
Others (specify)		

Are you familiar with antibiotics such as?

If yes, do you give antibiotics to your birds (YES/NO?) __________________________

If yes, please indicate the age at which you give the antibiotics for these birds

Broilers

Give medicine at this age (tick appropriate one)

When medicine given	Tick	Name of antibiotic given
Day old – 1 week		
1 – 2 weeks		
2 - 3 weeks		
3 - 4 weeks		
4 - 5 weeks		
5 - 8 weeks		
Any other time (specify)		

Layers

Give antibiotics at this age (tick appropriate one)

When antibiotic given	Tick	Name of antibiotic given
Day old to 8 weeks		
8 to 16 weeks		
From 16 weeks to end of production		

What is your source of instructions on how to use antibiotics? Tick appropriate

Government extension agents

Veterinarians

Animal production officers

Agricultural extension officers

Private Veterinarians

Animal health assistants

Feed suppliers ____

Agrovets dealers ____

Instructions on the package ____

Others specify ____

Where do you buy your antibiotics?

Name of Agrovet (Agrovets)/Shop

Location (Town)

When you give antibiotics how long, do you take before you kill your broilers?

Indicate the number of days

When you give antibiotics to your laying birds how long, do you take before you sell your eggs.

State the number of days

Have you vaccinated your broilers? Yes No

If the answer to on 33 is yes, indicate the age and vaccine

Age	Vaccine	Method

Have you vaccinated your layers? Yes No

If the answer to Qn 34 is yes, indicate the age and vaccine

Age	Vaccine	Method

In a scale of 1 – 4, indicate the most important challenges that you experience in your poultry farming

(Where one is most challenging and 4 is least challenging)?

Issue	RANK			
	1	2	3	4
Market				
High feed prices				
High chick prices				
Input costs				
Diseases outbreaks				
Slow growth rate				
High death rate				
Management				
Knowledge				
Low egg production				
Treatment not working				
Birds falling sick after vaccinating				
Lack of technical support				
Getting capital				

Weak shelled eggs				
Egg eating				
Cannibalism				
Chick quality				
Feed quality				

Appendix 5: Questionnaire administered to focused group poultry discussion.

Focused group poultry discussion

1. Do you have a problem of poultry diseases in your farms?

Yes (no. of respondents)	No (no. of respondents)

2. Which are the main disease challenges in your farms and at what age?
    a.
    b.
    c.
    d.

3. How do you handle these diseases when they occur?
    i. Treat them
    ii. Seek advice from the Agrovets
    iii. Call a vet
    iv. Take to the lab
    v. Ask fellow farmers
    vi. Others (specify)

4. Which antibiotics do you use when your birds are sick and at what age?
    a.
    b.
    c.
    d.

5. What withdrawal period do we give to our birds before slaughter?
    a.
    b.
    c.

Appendix 6: Commonly sold medicines brands by agrovets within the study area

Antimicrobial/ compound n = 235	Frequency	%	Category
Trimethoprim/sulphadimethoxine	24	10	Anticoccidial
Amprolium	18	8	Anticoccidial
Oxytetracyclines	18	8	Antibiotic
Diveridine+sulphadimidine	18	8	Anticoccidial
Tylosin	16	7	Antibiotic
Tylosin+Doxycycline	16	7	Antibiotic
Piperazine	16	7	dewormer
Levamisole	16	7	dewormer
Doxycycline	15	6	Antibiotic
Aliseryl	14	6	Antibiotic
Fosbac plus t	14	6	Antibiotic
Oxytet/vit	13	6	Antibiotic
Miramed	11	5	Anticoccidial/ antibiotic
Livergen plus	11	5	Herbal extract
Vigosine	6	3	Supplement
Colivet	5	2	Antibiotic
Probiotic	4	2	Probiotic

Appendix 7: Composition of experimental diets used in the study.

Parameter	**Value (starter)**	**Value (finisher)**
ME Kcal/kg	2650	2750
Crude Protein %	22	18
Calcium %	1	1
AV. Phosphorous%	0.4	0.4
Lysine %	1.1	0.9
Methionine %	0.44	0.36
Salt %	0.3	0.5
Acidomix % (TMT 3)	0.3	0.3
Avimatrix % (TMT 4)	0.05	0.05
Salinomycin % (TMT 2)	0.05	0.05

Appendix 8: Kind of antibiotic molecules or brands poultry farmers use in the study area

Type of antibiotic used	**Frequency**	%	Category
Fosbac	42	17	Antibiotic
Aliseryl	24	10	Antibiotic
Broiler booster	20	8	Probiotic
Miramed	20	8	Antibiotic/Anticoccidial
Biotrim	19	8	Antibiotic/Anticoccidial
Vetacox	11	4	Anticoccidial
Oxytet+vit	11	4	Antibiotic
Anticox	9	4	Anticoccidial
Livergen	9	4	Herbal extract
Herbs	9	4	Herbal
Hipradoxy	8	3	Antibiotic
Supermed	7	3	Antibiotic
Tylodoxy	5	2	Antibiotic
Doxycycline	5	2	Antibiotic
Agracox	5	2	Anticoccidial/antibiotic
Tylosin	4	2	Antibiotic
Quinocol	4	2	Antibiotic
Agraryl	4	2	Antibiotic
Bedgen	4	2	Herbal extract
Vetoxy	4	2	Antibiotic
0xytet	4	2	Antibiotic
Esb3	4	2	Anticoccidial
Levacide	3	1	Dewormer
Trimethosal	3	1	Anticoccidial
Fuzol	2	1	Anticoccidial
Amprocox	2	1	Anticoccidial
Amprosul	2	1	Anticoccidial
Piperazine	2	1	Dewormer
Tetracolivet	2	1	Antibiotic
Olaquindox	1	0	Growth promoter
Coscof	1	0	Antibiotic
Amprolium	1	0	Anticoccidial
Biosol	1	0	Anticoccidial/antibiotic

Appendix 9: Active ingredients in the antibiotics used by farmers in the study area

Product	**Active Ingredients.**			
Quinocol	Enrofloxacin	Colistin		
Aliseryl	Oxytetracyclines	Colistine	Erythromycin	Streptomycin
Tetracolivet	Oxytetracyclines	Colistine		
Agraryl	Oxytetracyclines	Erythromycin	Streptomycin	Colistin
Agracox	Pyrimethamine	Sulfadiazine	Sulfadimerazine	Furaltadone
Miramed	Trimethoprim	Sulfadiazine	Erythromycin	
Biotrim	Trimethoprim	Sulfamethoxazole		
Amprosul	Amprolium	Sulfaquinoxaline		
Trimethasol	Trimethoprim	Sulphadimethoxine		
Amprocox	Amprolium	Sulphaquinoxaline		
Vetacox	Diaveridine	Suphadimidine		
Anticox	Diaveridine	Suphadimidine		
Fosbac plus t	Fosfomycin	Tylosin		
Tylodoxy	Doxycycline	Tylosin		
Levamicide	Levamisole			
Booster	Probiotic			
Hipradoxy	Doxycycline			
Doxycycline	Doxycycline			
Oxytet+vit	Oxytetracyline			
Livergen	Herbal extract			
Fuzol	Furazolidone			
Bedgen	Herbal extract			
Vetoxy	Oxytetracyline			
Amprolium	Amprolium			
Oxytet	Oxytetracyclines			
Herbs	Various			
Esb3	Sulfachloropyrazine			
Piperazine	Piperazine			

Appendix 10: Broiler vaccination program

Age (days)	Vaccine	Route of administration
8	Gumboro	Drinking water
10	Newcastle + infectious bronchitis	Drinking water
15	Gumboro	Drinking water
24	Newcastle + infectious bronchitis	Drinking water

Appendix 11: Important challenges

Important challenges n=182	**Frequency**	**%**
Input costs	48	26
Market	29	16
Capital	25	14
Input quality	21	12
Diseases	21	12
Deaths	15	8
Growth	8	4
Technical support	7	4
Knowledge	5	3
Conmen	2	1
Treatment not working	1	1
Total	182	100