EFFECT OF SUPPLEMENTING DIETS WITH ANTIMICROBIALS AND EFFECTIVE MICROORGANISMS ON PRODUCTIVITY AND MEAT QUALITY OF ROSS 308 BROILER CHICKENS

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BY

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DECLARATION

I declare that the mini-dissertation for the degree of Master of Science in Agriculture (Animal Production) hereby submitted by me to the University of Limpopo has not been previously submitted to this or another University. It is my work in design and execution, and that all material contained herein has been duly acknowledged.

Signature……. Date…….

Mogotlane Pontsho Minah

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Above all, I am thankful to the Almighty God, for this work was possible because of Him.

DEDICATION

This work is dedicated to my parents Mr Frans Mogotlane and Mrs Rosina Mogotlane and my brother Emmanuel Mogotlane who have been very patient and supportive throughout this study. It is, also, dedicated to my only daughter Koketso Mogotlane for her presence in my life and to my late brother Tumelo Mogotlane.

ABSTRACT

This study was conducted to determine the effect of supplementing diets with antimicrobials and effective microorganisms on productivity and carcass characteristics of Ross 308 broiler chickens. The study consisted of two parts. The first part determined the effect of antimicrobial and effective microorganism (EM) supplementations on growth performance of unsexed Ross 308 broiler chickens aged one to 21 days. A complete randomized design was used and 150 unsexed day-old chicks with an initial weight of 42 ± 2g were randomly assigned to five different treatments which were replicated 3 times with each replicate having 10 chicks. The five grower diets had the same nutrients (20% CP and 12MJ/kg) but different supplementation levels of 0g oxytetracycline and 0ml EMs/l of water (UAM_0EM_0), 0.01g oxytetracycline ($UAM_{0.01}EM_0$), 30ml EMs/l of water (UAM_0EM_{30}), 50ml EMs/l of water (UAM_0EM_{50}) and 100ml EMs/l of water (UAM_0EM_{100}). A quadratic regression model was used to determine dietary effective microorganism supplementation levels for optimal feed intake and live weight of Ross 308 broiler chickens. A linear model was used to determine the relationship between dietary effective microorganism supplementation levels and metabolisable energy intakes. Antimicrobial and effective microorganism supplementations did not have any effect ($P>0.05$) on growth rate, feed conversion ratio and mortality. Antimicrobial supplementation improved ($P<0.05$) feed intake and live weight of the chickens. Supplementation with 50ml of EMs/l of water improved ($P<0.05$) feed intake. Supplementation with 50 or 100ml of EMs per litre of water increased ($P<0.05$) ME intake of the chickens. Effective microorganism supplementation levels of 72.25 and 48.29ml of drinking water optimized feed intake and live weight, respectively.

The second part of the experiment determined the effect of antimicrobials and effective microorganisms on productivity, blood, carcass characteristics and meat quality of male Ross 308 broiler chickens aged 22 to 42 days. The chickens were randomly allocated to five treatments with three replications, each having six chickens. A total of 90 male chickens, with the initial live weight of 452 ± 3g were allocated to the treatments in a complete randomized design. The chickens were fed a grower diet supplemented with 0g oxytetracycline and 0ml EMs/l of water (MAM_0EM_0), 0.01g oxytetracycline ($MAM_{0.01}EM_0$), 30ml EMs/l of water (MAM_0EM_{30}),

50ml EMs/l of water (MAM_0EM_{50}) and 100ml EMs/l of water (MAM_0EM_{100}). Antimicrobial and effective microorganism supplementation did not have effect (P>0.05) on feed intake, growth rate, live weight, ME intake, blood glucose and mortality. Poorer (P<0.05) feed conversion ratio was observed with the supplementation of antimicrobial. Blood glucose levels were optimized at an effective microorganism supplementation level of 29.00ml of EM/l of drinking water (Figure 4.05).

Supplementing diets with antimicrobials and effective microorganisms did not have effect (P>0.05) on crop, gizzard, proventriculus and large intestine pH values of male chickens. However, supplementation with of 100ml of EMs per litre of drinking water reduced (P<.0.05) the pH of ileum. Effective microorganism supplementation level of 85.00ml per litre of drinking water optimized the crop pH value. Antimicrobial and effective microorganism supplementations did not have influence (P>0.05) on gizzard, proventriculus, small intestine, caecum, large intestine, liver and heart weights of male chickens at 42 days. Effective microorganism supplementation level of 50ml per litre of drinking water reduced (P<.0.05) crop weight. Antimicrobial and effective microorganism supplementations did not have effect (P>0.05) on whole gastro-intestinal tract (GIT), small intestine and caecum length of the chickens. Antimicrobial supplementation reduced (P<0.05) the length of large intestine. Effective microorganism supplementation levels of 41.00, 45.50 and 85.00ml per litre of drinking water optimized crop weights and caecum and large intestine lengths, respectively. Antimicrobial and effective microorganism supplementations did not have any influence (P>0.05) on live weight, carcass weight, breast weight, drumstick weight ad thigh weight. Similarly, antimicrobial and effective microorganism supplementations did not have influence on meat tenderness, juiciness and flavour. There were no antibiotic and effective microbe residues in the meat.

It is, therefore, concluded that effective microorganism supplementation did not have much effect on production parameters, carcass characteristics and meat quality of Ross 308 broiler chickens.

TABLE OF ONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

Poultry production plays an important role in the livelihoods of the majority of people in South Africa as a source of income and food (Gueye, 2000). Additives are normally used in poultry feeds to promote growth and improve overall performance, with antibiotics being one of the frequently used additives. Antibiotics have been used in animal feeds since the 1950's when they were discovered as growth-promoting agents (Fuller, 1989). Positive effects that have been associated with the use of antibiotics in poultry production include a more efficient conversion of feed to animal products, an increased growth rate and a lower mortality rate (Engberg *et al*., 2000). However, there is evidence of some negative effects associated with the use of antibiotics in poultry feeds. The widespread use of antibiotics encourages the growth of antibiotic resistant pathogens (Agunos *et al*., 2012; Graham *et al*., 2007). Antibiotics are, also, found in the meat as residues and such residues have adverse effects on consumers (Janardhana *et al*., 2009; Threlfall *et al*., 2000). Thus, research has been focusing on the probiotics as possible alternatives to antibiotics (Maiorano *et al*., 2012; Anadón *et al.*, 2006).

1.2 Problem statement

Feed additives such as antibiotics are included in diets for poultry in order to increase production by improving nutrient availability and hence productivity. Experiments have shown that low, sub-therapeutic levels of antibiotics increase feed efficiency and growth in animals (Castanon, 2007; Dibner and Richards, 2005). However, some scientific reviews (IFT Expert Report, 2006; Phillips *et al*., 2004) acknowledge that feeding low levels of antibiotics to food-producing animals can result in the development of antibiotic resistant bacteria and, therefore, a risk to humans. Based on the current available knowledge on feed additives, probiotics seem to be the alternative to antibiotics. In poultry, benefits of probiotic supplementation (live yeast, bacteria, etc.) are reported in broiler chickens' performance and health, with evidence of increased resistance of chickens to *Salmonella, Escherichia coli* (*E. coli*) or *Clostridium perfringens* (*C. perfringens*) infections (Higgins *et al*., 2008; and Pradhan, 2006; La Ragione *et al*., 2004). However, other studies reported that supplementing probiotic additives had no significant effects on performance of broiler chickens (Willis *et al*., 2007; Gunal *et al*., 2006; Zhang *et al*., 2005). Salin *et al*. (2013) indicated that effective microorganism

(EM) supplementation does not help chickens develop resistance to diseases. Thus, the effects of supplementing feeds with probiotics on productivity of broiler chickens are not conclusive. Therefore, more research is needed to ascertain the role probiotics can play as additives in poultry feeds.

1.3 Motivation

This study produced information on the effects of antimicrobial and effective microorganism supplementations on productivity and carcass characteristics of broiler chickens aged one to 42 days. This information will help in indicating whether antibiotics' use in poultry can be replaced by effective microorganisms. Such a replacement would help reduce production of antibiotic resistant pathogens in chickens. Antibiotic resistant pathogens in meat may cause adverse effects in consumers of such meat (Bertrand *et al*., 2006).

1.4 Objectives

The objectives of the study were to determine:

i. the effects of supplementing diets with antimicrobials and effective microorganisms on dietary intake, growth rate, digestibility, feed conversion ratio, live weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.
ii. effective microorganism supplementation levels for optimal responses in dietary intake, growth rate, digestibility, feed conversion ratio, live weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

1.5 Hypotheses

The hypotheses of the study were:

i. Supplementing diets with antimicrobials and effective microorganisms have no effect on dietary intake, growth rate, digestibility, feed conversion ratio, live weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.
ii. There are no effective microorganism supplementation levels for optimal responses in dietary intake, growth rate, digestibility, feed conversion ratio, live weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The digestive tract of animals is host to an abundant and diverse micro-biota that play an important role in the health and nutrition of the animals (Callaway *et al*., 2008; Ley *et al*., 2008), but the gastro-intestinal micro-biota can also have detrimental effects on host health and nutrition. The relationship between the host animal and its gut micro-biota can, therefore, be viewed as a balance between mutualism and pathogenicity (Farthing, 2004). Thus, a common approach to maintain health and good performance of the host animal is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens. The composition and activity of intestinal micro-biota can be altered by diet composition and dietary manipulations such as the use of feed additives (Guo *et al*., 2004). For decades, it has been reported in poultry that the routine inclusion of antibiotic growth promoters (AGPs) in diets has a beneficial effect on the health, growth and efficiency of feed conversion (Frost and Woolcock, 1991), probably by beneficially modulating the gastro-intestinal micro-biota and suppressing the growth of pathogens (Gaskins *et al*., 2002). However, the extensive use of antibiotics has caused an antibiotic residue problem in poultry meat and increased proportion and persistence of antibiotic resistant faecal bacteria (Turnidge, 2004; Fuller, 1989). Many studies (Ganan *et al*., 2012; Saleha *et al*., 2009; Roe and Pillai, 2003; Aarestrup, 2000) have reported antibiotic residues in chicken meat products and development of bacterial resistance to antibiotics used in both human medicine and poultry production.

2.2 Gut micro-flora

Intestinal bacteria can be divided into species that exert either harmful (pathogenic) or beneficial effects. Whilst pathogenic bacteria are always present in the gut, the balance of non-pathogenic and pathogenic bacteria will strongly influence the disease and performance status of the chicken. The intestinal tract contains many micro-organisms like bacteria or viruses (La Ragione *et al*., 2004). Some of these organisms are harmless and aid in digestion. Others cause tremendous problems, for example *Salmonella enteritidis*, and are difficult to eliminate. Other organisms do not actually cause disease, but impair the functioning of the digestive enzymes (Guo *et al*., 2004). The micro-flora impose a variety of costs that include competition for nutrients and the production of toxic amino acid catabolites, decreased fat digestibility, and the requirement for increased mucus secretion and gut epithelial

cell turnover. These and other bacterial-induced effects exert a large toll on animal health and performance (Gaskins *et al*., 2002). Gastro-intestinal normal flora plays an important role in the health and performance of poultry (Thongsong *et al*., 2008).

2.3 Use of antibiotics at sub-therapeutic rate in poultry feed

Poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella species*, *Clostridium perfringens* and *Campylobacter sputorum*. Pathogenic microbial flora in the small intestines compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al*., 2000). With respect to animal production, an important goal is to manipulate the micro-flora through diets, supplements, etc. to obtain the desired micro-flora (Dibner and Richards, 2005). The use of feed supplements to achieve better animal health and productivity through manipulation of the gastro-intestinal tract (GIT) microbial ecosystem has gained considerable attention for many years. Feed additives have been the major intervention used to improve performance of commercial poultry enterprises (Mandal *et al*., 2000).

Worldwide, growth-promoting antimicrobials (AGPs), such as antibiotics, have been widely distributed (Chaucheyras-Durand and Durand, 2010) and have been used for decades in animal production. At low levels of inclusion, dietary antibiotics are reported to have beneficial effects on poultry growth, feed conversion efficiency (FCR) (Engberg *et al*., 2000) and the inhibition of pathogen growth (Gaskins *et al*., 2002). Antibiotic growth promoters modify the intestinal flora to improve digestion, metabolism and absorption of a variety of essential nutrients (Wenk, 2003; Van Immerseel *et al*., 2002; ACVM Group, 2000). Antibiotics may achieve this by controlling and limiting the growth and colonization of a variety of pathogenic and non-pathogenic species of bacteria in the guts of chickens (Ferket, 2004), presumably by altering the composition and activities of micro-flora (Collier *et al*., 2003, Knarreborg *et al*., 2002). Sub-therapeutic antibiotics result in a reduction in the microbial load in the gut, thus resulting in more nutrient portioning towards growth and production rather than for disease control (Shane, 2005). A more balanced biota population in the gut can lead to a greater efficiency in digestibility and utilization of nutrients, resulting in an enhanced growth and improved FCR (Bedford, 2000).

2.4 Impacts of non-therapeutic antibiotics use

Any extended AGPs applications, which are supplied for continuous and low-dose application, select for increasing resistance to the agent (Diarra *et al*., 2007; Emborg *et al*., 2003). Thus, their usefulness has seldom been contested, with their relatedness to similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that cause concerns (Phillips, 1999) and drug residues in the body of the chickens (Burgat, 1999). Bacteria developing resistance to these drugs in animals may be transmitted to humans or spread their mechanisms of resistance, which may eventually be found in human pathogens. Such a situation may lead to the loss of therapeutic efficacy in both veterinary and human medicines (Castanon, 2007). Antimicrobial agents can change the bacterial environment by eliminating susceptible strains, and only allowing antibiotic resistant bacteria (i.e., those with higher fitness) to survive (O'Brien, 2002). They may, thus, modify the intestinal micro-flora and create a favourable environment for establishment of resistant and pathogenic bacteria (Johnson *et al*., 2012).

Concerns about the routine use of antibiotics resistance development and transference gene from animal to human micro-biota make it unsafe for use (Castanon, 2007). There are studies that clearly demonstrated the selective nature of low-dose, non-therapeutic AGPs on both the pathogenic and commensal flora of food animals such as chickens (Van den Bogaard *et al*., 2002). Several studies (St. Amand *et al*., 2013; Slavic *et al*., 2011; Diarra *et al*., 2010) have shown the presence of antibiotic resistant bacteria (*Escherichia coli, Salmonella serovars*, *Enterococcus spp.* and *Clostridium perfringens*) in poultry. It was demonstrated that multi-antibiotic-resistant *E. coli* can colonize and persist in the broiler chicken gut (Diarra *et al*., 2007). Today, the non-therapeutic use of antibiotics in poultry feeds has been severely limited in many countries because of concerns related to development of antibiotic-resistant human pathogenic bacteria and legislative action to limit their use is probable in many others (Michard, 2008; Cervantes, 2006; Nollet, 2005). Consequently, the poultry industry must develop alternatives to antibiotic growth promoters (Ferket *et al*., 2002). As a result, it has become necessary to develop alternatives such as beneficial microorganisms that enhance microbial growth.

2.5 Alternatives for antibiotics use

A successful alternative to AGP's should comply with certain characteristics. It should be able to mimic the mode of action or effect of the antimicrobial, and therefore, have a significant beneficial impact on animal production and health which can be reflected in improved digestion, nutrient metabolism and absorption, as well as a decrease in incidence of diseases. It should also be, generally, regarded as safe to both the animals and humans (Collett *et al*., 2001).

2.6 Use of effective microorganisms as an alternative to antibiotics

The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance of the animals are improved (Ravindran, 2006). Probiotics have emerged as most preferred and effective alternative to antibiotics in animal nutrition (Patterson and Burkholder, 2003; Ghadban, 2002), hence they can be used to achieve the goal. Huang *et al*. (2004) defined probiotics as feed additives that contain live microorganisms which promote beneficial effects to the host by favouring the balance of the intestinal micro-biota. Reid *et al*. (2003) defined probiotics as a group of non-pathogenic organisms that when administered in sufficient numbers are known to have beneficial effects on health of the host. Most efficient probiotic microorganisms will likely be strains that are fit enough to survive in the gastro-intestinal environment after application and, furthermore, these microorganisms must be able to reach their targets and colonize throughout the gastrointestinal tract (Karimi- Torshizi *et al*., 2008). Cyberhorse (1999) stated that probiotics can be used in a wide range of circumstances to improve the general health of animals, address specific problems and maximize the performance of the animal.

The principal organisms of EMs are usually five: photosynthetic bacteria (phototrophic bacteria), lactic acid bacteria, yeasts, actinomycetes and fermenting fungi (Mroz, 2001), although the photosynthetic bacteria are not common in animal production. Lactic acid bacteria increase the acidity of the intestine, which inhibits the multiplication of harmful bacteria (Rahimi *et al*., 2010). The importance of yeast is mostly on their cell wall. The yeast cell wall is a complex matrix containing a mixture of carbohydrates and proteins that can provide specific adsorptive capacity (Dawson, 2001). Actinomycetes have the ability to produce antimicrobial secondary

metabolites and extracellular enzymes that decompose organic macromolecules. They, also, possess antimicrobial activity against pathogenic microbes (Bernal *et al*., 2015). A variety of microbial species have been used as probiotics and it has been reported that probiotic products belonging to single or multi-species of *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida* and *Saccharomyces* have a potential effect modulating the intestinal micro-flora and pathogen inhibition (Ohh, 2011; Kabir, 2009; Simon *et al*., 2001).

Due to the resistance caused by the use of antibiotics as growth promoters in poultry diets, investigations evaluating the potential use of dietary probiotics as substitutes for antibiotics are receiving high priority. Probiotics effects can affect the microbial stabilization in gastro-intestinal system like antibiotics (Medici *et al*., 2004; Choct 2001; Rial *et al*., 2000). In poultry industry, probiotics applications have widely been shown to improve the barrier function of intestine and reduce pathogenic problems in gastro-intestinal tracts, thus leading to the enhancement of immune response and replacement of sub-therapeutic antibiotics (Galdeano and Perdigón, 2006; Soderholm *et al*., 2001). The intestine has a mucosa which works as a selective barrier allowing the passage of useful substances and preventing the entering of undesirable agents into the bloodstream. Therefore, the health of this mucosa is essential for efficient feed conversion, maintenance and growth, and, thus, for the well-being of the animal. Healthy chickens are generally considered as having a well-functioning intestinal tract, and an important characteristic of a healthy and well-functioning intestinal tract is the balance of its microbial population. When the microorganism load in the gut is unbalanced, beneficial results could be achieved through the use of dietary probiotics (Cencic *et al*., 2006).

The aim of probiotics is to maintain the gut population balance in favour of beneficial bacteria. It has been reported by Cencic *et al*. (2006) that continuous probiotic supplementation aids in maintaining that balance. Probiotics provide nutrients, effectively stimulating the growth of beneficial micro-flora in the small and large intestines and, hence, resulting in the better balance of bacterium population (Abdel-Rahman and Nafea, 2013; Capcarová *et al*., 2011; Midilli *et al*., 2008). Kabir (2009) stated that probiotics have positive effects on intestinal micro-flora and pathogen inhibition, intestinal histological changes, immunomodulation, some haemato-

biochemical parameters and growth of broiler chickens. Important characteristics of probiotics are the increase of animal resistance to diseases and the improvement of feed efficiency without any residual in the meat (Silva *et al*., 2000).

Mountzoris *et al*. (2010) and Vila *et al*. (2009) reported that feeding probiotics helps maintain a beneficial intestinal micro-flora, enhances the host's resistance to enteric pathogens such as *Salmonella* and *Campylobacter* species and results in a healthy gastrointestinal environment with an improved intestinal function, feed conversion, weight gain and performance of chickens. According to Traldi *et al*. (2007), probiotics can improve the utilization of food and thereby reduce nutrient excretion. In addition, probiotics are used not only as a growth promoter, but also they enhance the immune system and have protective effects against many diseases (Gibson and Fuller, 2000). Several possible mechanisms have been suggested such as altering of the gut pH, maintaining protective gut mucins, selecting beneficial intestinal organisms or ones antagonistic to pathogens, enhancing fermentation acids, enhancing nutrient uptake or increasing immune response (Inboor, 2000).

Under general conditions, probiotics have been promoted to: improve health naturally, stimulate appetite, aid in establishment of gut flora in immature animals like one day old chicks, re-establish gut micro-flora after antibiotic treatment, optimize digestion of feed and reduce stress (Corrêa *et al*., 2003). Moreover, some probiotic strains are able to reduce absorption of bile acids from intestine (Doncheva *et al*., 2002). On the other hand, probiotics produce short-chain fatty acids and reduce cholesterol synthesis in the liver whereby reducing host blood cholesterol (Denli *et al*., 2003). Furthermore, there is a tendency to increase the use of probiotics in diets for animals, which is a more reasonable option, since they do not leave residues in the environment, in the animal body and do not cause cross-resistance in humans compared with antibiotics. However, it is reported that the main effect of probiotics is in the gastro-intestinal tract and it is associated with its capacity to stimulate the immune response and to control the growth of pathogenic bacteria (Kabir, 2009; Higgins *et al*., 2007; Huang *et al*., 2004).

One of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for growth promotion in poultry. All micro-

organisms in the probiotics are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources (Rahimi, 2009). Thus, it is possible to promote growth of broiler chickens and achieve both enhanced performance and good health by using probiotics as alternatives (Ohimain *et al*., 2012). Probiotics might lead to beneficial effects for the host animal due to an improvement of the intestinal microbial balance or of the properties of the indigenous micro-flora (Huang, *et al*., 2004). Moreover, probiotics are reported to be safe, non-toxic and residual free (El-Hammady *et al*., 2014). Microbial probiotics are commonly administered to animals orally either through the feed or drinking water. According to International Animal Health (1999), there are no risks due to overdosing of probiotics since they are compatible with all feeds, feed ingredients like vitamins and minerals and some antibiotics.

2.7 Responses to dietary effective microorganism supplementation

It was reported by Mountzouris *et al*. (2007) and Koenen *et al*. (2004) that probiotics have a good impact on the poultry performance. Several studies reported beneficial effects of probiotics on growth performance (Shim *et al*., 2010; Awad *et al*., 2009), nutrient retention (Mountzouris *et al*., 2010; Li *et al*., 2008), gut health (Awad *et al*., 2009) and intestinal micro-flora of chickens (Mountzouris *et al*., 2010; Teo and Tan, 2007). The beneficial effects of probiotic supplementation to broiler diets in terms of increased body weight and feed conversion are documented in studies of several researchers (Bansal *et al*., 2011; Onderci *et al*., 2008; O'Dea *et al*., 2006). The study of Shim *et al*. (2010) revealed the improvement of feed intake when probiotics were administered to the chickens. Verschuere *et al*. (2000) showed that probiotic supplementation in chicken diets improved digestion and Rolfe (2000) reported decreased pH when diets were supplemented with probiotics.

The study of Gibson and Fuller (2000) reported the ability of probiotics to change the type and number of the micro-flora in the digestive tract. In the study done by Brzóska *et al*. (2012), probiotics significantly reduced chicken mortality and increased dressing percentage compared to the control group that received no bacteria in their diet. Kabir (2009) indicated that probiotics improved sensory characteristics of dressed broiler meat and microbiological meat quality of broilers.

The inclusion of probiotics in the diet has shown to produce contradictory results on broiler performance. Researchers have reported positive (Corrêa *et al*., 2003), none or negative effects (Gunal *et al*., 2006; Flemming and Freitas, 2005; Pelicano *et al*., 2004; Lima *et al*., 2003; Vargas *et al*., 2001) on broiler performance attributed to the action of probiotics. There are conflicting reports on the effects of application of probiotics in the poultry industry because probiotic efficacy can be affected by different factors such as microbial species composition, viability, hydrophobicity of the bacterial cell surface, dosage of bacteria provided to an animal and concentration of bacteria used, frequency of application and methods of using probiotics, the combination of probiotics and synergistically acting components, bird age, overall farm hygiene, and environmental stress factors (Mountzouris *et al*., 2010; Flint and Garner, 2009; Awad *et al*., 2009). However, Yang *et al*. (2009) did not find such positive effects.

The efficacy of probiotic has been reported by Timmerman *et al*. (2004) that the probiotic activity could be related to genera, species or strains. Dose, timing and duration of probiotics may be a factor affecting efficacy. For example, age of animal: during early life, colonization patterns are unstable and new-born animals are then susceptible to environmental pathogens. Initial colonization is of great importance to the host because the bacteria can modulate expressions of genes in epithelial cells thus creating a favourable habitat for themselves (Siggers *et al*., 2007).

2.8 Mode of action of effective microorganisms

There are a number of modes of actions which have been reported by researchers on how they believe the probiotics work. These are as follows:

i. Blocking of adhesion sites

Competitive inhibition for bacterial adhesion sites on intestinal epithelial surface is one of the mechanisms of action for probiotics. Consequently, some probiotic strains have been chosen for their ability to adhere to epithelial cells. Gut bacteria prevent intestinal colonization by pathogenic organisms directly by competing more successfully for epithelial attachment sites (La Ragione *et al*., 2004; Rolfe *et al*., 1996).

ii. Production of inhibitory substances

Probiotic bacteria can produce a variety of substances that are inhibitory to both gram-positive and gram-negative bacteria. These inhibitory substances include organic acids, hydrogen peroxide and bacteriocins. These compounds may reduce not only the number of viable cells but may also affect bacterial metabolism or toxin production (Panda *et al*., 2006; Patterson and Burkholder, 2003)

iii. Competition for nutrients

Competition for nutrients has been proposed as a mechanism of probiotics. Probiotics may utilize nutrients otherwise consumed by pathogenic microorganisms (Delia *et al*., 2012; Angel *et al*., 2005).

iv. Influence on the immune system

The intestinal micro-flora is an important component of the host animal. A critical review of the literature indicates that probiotic supplementation of the intestinal micro-flora may enhance defence, primarily by preventing colonization by pathogens and by indirect, adjuvant-like stimulation of innate and acquired immune functions (McCraken and Gaskins, 1999). Intestinal bacteria provide the host with several nutrients, including short-chain fatty acids, vitamin K, some B vitamins and amino acids (Delcenserie *et al.,* 2008; Fuller, 2001)

2.9 Efficiency of probiotics in farm animals

Potential beneficial effects of probiotics for farm animals by Fuller (1999):

i. Greater resistance to infectious diseases.
ii. Increased growth rate.
iii. Improved feed conversion.
iv. Improved digestion.
v. Better absorption of nutrients.
vi. Provision of essential nutrients.
vii. Improved carcass quality and less contamination.

2.10 Conclusion

Chicken meat is consumed all over the world. However, antimicrobials or antibiotics are very much used to increase productivity of the chickens. The extensive use of antibiotics results in the development of antibiotic resistant microbes which find their

way into humans. This is a health hazard to humans. There is evidence that effective microorganism supplementation, as an alternative to antibiotics, can improve growth performance and health status of the chickens. However, it is also reported by other studies that these microorganisms do not have any influence on the productivity and health of the chickens. Thus, studies on EMs are not conclusive. Therefore, there is need to do more research on the use of effective microorganisms to ascertain whether they can be used as an alternative to antibiotics use in poultry or not. Thus, the aim of this study was to determine the effect of probiotic supplementation on productivity of broiler chickens.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the University of Limpopo Animal unit. The latitude of the area is 23°54'00"S and 29°27'00"E. The ambient temperatures around the study area range between 20°C and 36°C during summer and between 10°C and 25°C during winter (Shiringani, 2007).

3.2 Materials

Materials in Table 3.01 were used in this study.

Table 3.01 Materials

Experimental house	Divided into 15 pens, each measuring $2m^2$.
Household disinfectant	Containing the following ingredients: 4-chloro-m-cresol (5 - < 10%), tar acids, (poly) alkylphenol fraction (5 - < 10%), propan-2-ol (1 - < 2.5%), terpineol (2.5 - < 5%).
Saw dust	Made of wood shavings from blue gum trees and it was obtained from Hearnetzburg, Limpopo, South Africa.
Feeders and drinkers	Plastic feeders and drinkers for feeding and drinking purposes, respectively. They were obtained from NTK, Polokwane, South Africa.
Grower mash (20% CP, 16 MJ ME/kg DM)	Three forms:- Grower mash containing antibiotics (oxytetracycline and coccidiostat) - Grower mash without antibiotics - Grower mash supplemented with effective microorganisms. .
Effective microorganisms (EM) material	Containing lactic acid bacteria, yeasts, actinomycetes and fungi mixture and it was obtained from ZZ2, Limpopo, South Africa.
Ross 308 broiler chicks	150 Day-old Ross 308 broiler chicks from Lufafa hatchery, Tzaneen, Limpopo, South Africa.

Digestibility cages	For digestibility trials
Electronic weighing balance	Used to weigh the chicks, chickens, feeds, carcasses and organs.
pH meter	Crison, Basic 20 digital pH meter (South Africa) for pH measurements
Measuring tape	For gastro-intestinal tract length measurements

3.3 Preparation of the house

The experimental house was cleaned and disinfected with a disinfectant specified in Section 3.2, Table 3.01. The house was then left to dry for a period of one week before being used, in order to eliminate or to reduce the population of infectious microorganisms. The experimental house was divided into 15 floor pens of $2m^2$ per pen. Saw dust was spread on the floor with a thickness of 7cm, measured by a ruler. Feeders and drinkers were also cleaned and disinfected thoroughly before use with the same disinfectant used above.

3.4 Experimental procedures, dietary treatments and design

This study consisted of two parts.

3.4.1 Part 1

One hundred and fifty day-old unsexed Ross 308 broiler chicks (obtained from Lufafa hatchery, Tzaneen) were used in the first part. The chicks were vaccinated at the hatchery against New Castle and Infectious bronchitis (Gumboro) with Vitabron when they were a day old, before being delivered to the experimental site. The experiment was carried out for a period of 21 days. The initial live weights of the chickens were taken using an electronic weighing balance and their initial mean live weight from each replicate was 42 ± 2g. Thereafter, the chicks were weighed weekly until they were 21 days old. The experimental chicks were fed a grower mash formulated by Voorslagvoere Milling Company at Mokopane, South Africa. Feed intake was measured every day. The grower mash was fed to chicks in three different forms, the one containing antibiotics (oxytetracycline and coccidiostat),

without any of the antibiotics and EMs or having effective microorganism. The ingredients of the experimental diets are presented in Table 3.02.

Chicks were assigned to 5 different treatments (Table 3.03) in a complete randomized design manner, with 3 replicates and 10 chicks in each replicate. The effective microorganisms (EM) used were supplied by ZZ2 (Mooketsi, South Africa). The material contained a mixture of lactic acid bacteria with 8.3 x 10^6 CFU/ml (*Lactobacillus planetarium* species), yeasts with 1.8 x 10^5 CFU/ml (*Candida valida* species), actinomycetes with 3 x 10^3 CFU/ml (*Streptomyces albus* species) and fermenting fungi with 1.1 x 10^5 CFU/ml (*Aspergillus oryzae* species). Effective microorganisms were added daily to the drinking water in the chick fountains (drinkers) with the amount offered per litre specified in Table 3.03.

Chicks were allowed to feed and drink water *ad libitum*. Light was provided for 24 hours per day throughout the experiment and deaths were observed everyday throughout the study.

3.4.2 Part 2

In the second part of the experiment, ninety male Ross 308 broiler chickens aged 22 days were used. The chickens were weighed when the study commenced and the initial mean live weight of the chickens was 452 ± 3g. Like in the first experiment, the chickens were weighed weekly until they were 42 days old.The chickens were fed the same grower mash used in the first experiment with feed intake being measured every day. The EM amounts were administered the same way as in the first part. The chickens were randomly assigned to five treatments as in the first experimental part. Each treatment had 3 replicates with 6 chickens per replicate. The dietary treatments are presented in Table 3.04. The chickens were still allowed to feed and drink water *ad libitum* for this part of the experiment.

Table 3.02 Ingredients of grower mash for the study

	Treatment				
	AM_0EM_0	$AM_{0.01}EM_0$	AM_0EM_{30}	AM_0EM_{50}	AM_0EM_{100}
Feed Ingredient (%)					
Yellow maize	39.83	39.83	39.83	39.83	39.83
Soybean full fat	17.73	17.73	17.73	17.73	17.73
Wheat	15.00	15.00	15.00	15.00	15.00
Sunflower	12.39	12.39	12.39	12.39	12.39
Fishmeal	5.66	5.66	5.66	5.66	5.66
Vitamin + minerals premix	3.00	3.00	3.00	3.00	3.00
Oil - sunflower	2.50	2.50	2.50	2.50	2.50
Sodium bicarbonate	1.50	1.50	1.50	1.50	1.50
Limestone	1.50	1.50	1.50	1.50	1.50
Salt	0.30	0.30	0.30	0.30	0.30
Monocalcium phosphate	0.20	0.20	0.20	0.20	0.20
DL methionine	0.15	0.15	0.15	0.15	0.15
L threonine	0.15	0.15	0.15	0.15	0.15
L lysine	0.10	0.10	0.10	0.10	0.10
Effective microorganisms*	0	0	30	50	100
Terramycin	0	0.01	0	0	0
Total	100	100	100	100	100
Nutrients					
Crude Protein (%)	20	20	20	20	20
Energy (MJ/kg DM)	12	12	12	12	12
Lysine (%)	1.08	1.08	1.08	1.08	1.08
Methionine (%)	0.53	0.53	0.53	0.53	0.53
Threonine (%)	0.89	0.89	0.89	0.89	0.89

* : Units are ml of EMs/litre of drinking water.

Table 3.03 Dietary treatments for Part 1

Treatment code	Treatment description
UAM_0EM_0	Unsexed broiler chickens fed a grower diet (20% CP)
$UAM_{0.01}EM_0$	Unsexed broiler chickens fed a grower diet (20% CP) containing antibiotics
UAM_0EM_{30}	Unsexed broiler chickens fed a grower diet (20% CP) supplemented with 30ml of effective microorganisms/litre of drinking water
UAM_0EM_{50}	Unsexed broiler chickens fed a grower diet (20% CP) supplemented with 50ml of effective microorganisms/litre of drinking water
UAM_0EM_{100}	Unsexed broiler chickens fed a grower diet (20% CP) supplemented with 100ml of effective microorganisms/litre of drinking water

Table 3.04 Dietary treatments for part 2

Treatment code	Treatment description
MAM_0EM_0	Male broiler chickens fed a grower diet (20% CP)
$MAM_{0.01}EM_0$	Male broiler chickens fed a grower diet (20% CP) containing antibiotics
MAM_0EM_{30}	Male broiler chickens fed a grower diet (20% CP) supplemented with 30ml of effective microorganisms/litre of water
MAM_0EM_{50}	Male broiler chickens fed a grower diet (20% CP) supplemented with 50ml of effective microorganisms/litre of water
MAM_0EM_{100}	Male broiler chickens fed a grower diet (20% CP) supplemented with 100ml of effective microorganisms/litre of water

3.5 Live weight measurements

Mean live weights were calculated from the weekly measurements by dividing the total weight with the number of chickens in that pen.

3.6 Growth rate measurements

Average daily gains were calculated by subtracting the initial weight of the chicken from the final weight and the answer was divided by the number of days.

3.7 Feed intake measurements

The voluntary feed intake was measured by subtracting the difference in weight of leftovers from that offered per day and the total was divided by the total number of chickens per pen. The feed offered per day and leftovers were measured using the electronic weighing balance used.

3.8 Feed convention ratio (FCR) measurements

Daily average feed intake and weight gain were used to calculate feed conversion ratio. Average feed intake was divided by average weight gain to find the FCR value (McDonald *et al.*, 2010).

$$\text{Feed conversion ratio (g DM feed/g live weight gain)} = \frac{\text{Average feed intake}}{\text{Average weight gain}}$$

3.9 Digestibility measurements

Digestibility measurements were carried out when the chicks were between the ages of 16 and 21 days of age and when the chickens were between 37 and 42 days of age for the first and second parts, respectively. Digestibility was conducted in digestibility cages equipped with separate feed and water troughs. Two birds were randomly selected from each pen and transferred to the cage for the measurement of apparent digestibility for the first part. For the second part one chicken from each pen was transferred to the cage for apparent digestibility measurements. The digestibility trials were carried out for 6 days for both the experiments. The 6-day digestibility trial was divided into two phases: a three-day period for acclimatization and a three-day collection period. Faeces voided by chickens were collected daily at 10.00 hours. Care was taken to avoid contamination from feathers, scales, debris and feeds. Apparent digestibility (AD) of the nutrients was calculated according to the procedures of McDonald *et al*. (2010) using the following formula:

$$\text{AD (\%)} = \frac{\text{(Amount of nutrient ingested – amount of nutrient excreted)}}{\text{amount of nutrient ingested}} \times 100$$

3.10 Blood sample collection

At Day 43, one chicken from each pen was weighed and blood was taken from the wing vein for glucose analysis. The chickens were held by the side and the large vein on the wing was chosen to draw the blood so that enough blood could be drawn for the required analysis. The blood was collected in EDTA collection tubes and the tubes were stored until used.The blood samples were sent to the Lancet Laboratory in Pretoria, South Africa for the analysis.

3.11 Slaughtering and defeathering

All the 15 chickens that were used for drawing blood were slaughtered for the determination of carcass characteristics (carcass and organ weights, organ pH and gastro-intestinal length measurements). The chickens were killed by decapitation as advised by the Animal Research Ethics Committee of the University of Limpopo. The carcasses were then put inside a bucket containing hot water for few seconds and they were then taken out. The carcasses were then put on a table for defeathering with hands.

3.12 pH measurements

Crison, Basic 20 pH meter was used to measure the pH. The carcasses were cut open at the abdominal site and the digestive tracts were removed from the abdominal cavities of the chickens. The pH of gizzard, crop, proventiculus, ileum (section of the small intestine), caecum and large intestines were measured. The digesta pH was measured at each segment using an electonic pH meter prior to the emptying of the digesta for weight measurement.

3.13 Gastro-intestinal tract measurements

The whole gastro-intestinal tract's length was measured. In addition, the length of small intestines, large intestines and caecum were measured separately. The proventriculus, gizzard, crop, small intestines, caecum, and large intestines were cleaned and weighed using an electronic weighing balance.

3.14 Chemical analysis

Dry matter contents of feeds were determined in order to calculate the dry matter intake (DMI) of the feeds. The dry matter contents were determined by drying the sample in the oven over night at a temperature of 105°C. Dry matter contents of the

faeces were determined the same way as those of the feeds for the determination of dry matter digestibility (DMD). Gross energy values for feeds and faeces were determined using an adiabatic bomb calorimeter according to the method previously described by Association of Analytical Chemists (AOAC) (2000) at the University of Limpopo Animal Nutrition Laboratory. A full analysis for faeces and feeds (diet composition) was performed at the Pietermartizburg laboratory, Kwa-Zulu Natal, South Africa.

3.15 Meat sample preparation

Meat samples which were previously frozen at -40°C for 4 days were thawed for 7 hours at room temperature prior to cooking. Only thighs and drumsticks were prepared and the skin was left on the meat samples. Nothing was added to the meat samples to add taste. The method adopted by Pavelková *et al*. (2013) was used to cook the meat samples. An oven set at 180ºC was allowed to preheat prior to cooking. The meat samples were put in trays and they were covered with aluminium foil to prevent water loss. Thereafter, the trays with meat were put in an oven for approximately 60 minutes and the meat samples were turned after every 10 minutes. Samples were cut into small pieces and served immediately after cooking.

3.16 Sensory evaluation

The method adopted by Pribela (2001) was used for sensory evaluation of the meat. The following attributes: meat tenderness, juiciness and flavour, were evaluated using a five-point scale. The five-point ranking scale scores used are as indicated in Table 3.05. The sensory tasting panel consisted of 21 female students to evaluate the sensory attributes. The students were never subjected to any tasting training before and they were from the University of Limpopo. There was no special selection method that was used, the students were just picked randomly for tasting. Each panel member was given a chance to taste all samples from the 5 treatments. Each member was offered to drink lemon juice after tasting meat from each treatment before proceeding to the next treatment as to wash out the taste of the previous treatment to avoid confusion of tastes (Pribela, 2001).

Table 3.05 Sensory evaluation scores used

	Sensory attribute		
Score	Tenderness	Juiciness	Flavour
1	Too tough	Too dry	Very bad flavour
2	Tough	Dry	Poor flavour
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good flavour
4	Tender	Juicy	Good flavour
5	Too tender	Too juicy	Very good flavour

3.17 Meat analysis

Male breast meat samples were sent to the CSIR in Johannesburg for analysis (Pavlov *et al*., 2008). The analysis was done to check on whether there were any antibiotic residues in the tissues of the meat. The breast meat was also compared to breast meat from Woolworths. Breast meat samples from 17 chickens were analyzed. In the residue laboratory, chicken breasts purchased from Woolworth's stores were included because of the retailer's reputation for good sanitary practices. The concentration of tetracycline in breast meat was analysed using the MaxSignal® Tetracycline (TET) Elisa Test Kit (Bioo Scientific). The plates were read using the GMDS Micro-plate Reader (Inqaba Biotech). MaxSignal® Tetracycline (TET) Elisa Test Kit is a competitive enzyme immunoassay for quantitative analysis of tetracycline in meat and other matrices (Bioo Scientific application note). The plate wells had been coated with tetracycline. During the analysis, a sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The resulting colour intensity, after addition of substrate has an inverse relationship with the target concentration in the sample. Samples were kept at -80^{0}C for long term storage and thawed to room temperature overnight prior to homogenizing for test. Once thawed the breast was homogenized after removal of the bones using a blender. The minced meat was then extracted following the instructions in the Bioo Scientific application note. In brief, about 1g of meat was extracted with 3ml of buffer from the kit and 1ml n-hexane by centrifuging for 10 minutes. An aliquot of 200µl of the supernatant was then transferred to a new

vortexed microfuge tube and then 75µl offloaded in duplicate into the 96 well plate. Tetracycline standards (75µl) ranging in concentration from 0 -1.6ppb were added to separate wells. The ELISA testing was then done according to the testing protocol with the plate being read on a plate reader at 450nm. The tetracycline concentration was calculated using a standard curve constructed from plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/ml on a logarithmic curve using the following formula:

$$\text{Relative absorbance (\%)} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100$$

The data was loaded onto Microsoft Excel worksheet which calculated the concentrations of tetracycline in the meat.

3.18 Data analysis

Data on feed intake, digestibility, growth rate, feed conversion ratio, live weight, carcass characteristics, digestive organ pH, weight and length of digestive organs, antibiotic residues and meat quality of broiler chickens were analysed using General Linear Model (GLM) procedures of the statistical analysis system. Least Significant Difference (LSD) test was applied for mean separation where there were significant differences ($P<0.05$) between treatment means (SAS, 2012). Regression analysis was used to determine the dose-related optimal responses for significantly different variables in feed intake, digestibility, live weight, feed conversion ratio, growth rate, carcass characteristics and meat quality to effective microorganism supplementation levels.

Responses in feed intake, digestibility, growth rate, live weight, feed conversion ratio, carcass characteristics, blood glucose, digestive organ traits and meat quality were modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2$$

Where:

Y = feed intake, live weight, blood glucose, carcass characteristics, digestive tract organ size and digestive organ pH values.

a = intercept;

b_1 and b_2 = coefficients of the quadratic equation;

x = dietary effective microorganism supplementation level and

$-b_1/2b_2$ = x value for optimal response. The quadratic model was used because it gave the best fit.

The relationship between effective microorganism supplementation level and metabolisable energy and feed conversion ratio were modelled using a linear regression equation (SAS, 2012) of the form:

$Y = a + bx$

Where Y = metabolisable energy, feed conversion ratio or ileum pH; a = intercept; b = coefficient of the linear equation; and x = effective microorganism supplementation level.

CHAPTER 4

RESULTS

4.1 Nutrient composition of the diets

Results of the nutrient composition of the diets used in Experiments 1 and 2 are presented in Table 4.01. The diets had similar protein and energy contents of 20% and 12MJ/kg DM, respectively. However, the diets had different levels of antimicrobial (AM) and effective microorganism (EM) supplementations. The supplementation levels were a diet not supplemented with either antimicrobials or effective microorganisms (AM_0EM_0), a diet supplemented with 0.01g of antimicrobials per kg ($AM_{0.01}EM_0$), a diet supplemented with 30ml of effective microorganisms per litre of water (AM_0EM_{30}), a diet supplemented with 50ml of effective microorganisms per litre of water (AM_0EM_{50}) and a diet supplemented with 100ml of effective microorganisms per litre of water (AM_0EM_{100}).

4.2 Part 1

Results of the effects of antimicrobial and effective microorganism supplementations on feed intake, metabolisable energy (ME) intake, growth rate, feed conversion ratio (FCR) and live weight of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.02. Unsexed Ross 308 broiler chickens on a diet supplemented with antimicrobials ($UAM_{0.01}EM_0$) and those on a diet supplemented with 50ml of EMs per litre of drinking water (UAM_0EM_{50}) had higher ($P<0.05$) dry matter (DM) intakes than those on a diet not supplemented with either antimicrobials or effective microorganisms (UAM_0EM_0). However, chickens on $UAM_{0.01}EM_0$, UAM_0EM_{30}, UAM_0EM_{50} or UAM_0EM_{100} diets had similar ($P>0.05$) DM intakes. Similarly, chickens on AM_0EM_0, AM_0EM_{30} or AM_0EM_{100} diets had the same ($P>0.05$) intakes. Supplementing diets with antimicrobials or effective microorganisms did not affect ($P>0.05$) growth rates and feed conversion ratios of the chickens.

Unsexed Ross 308 broiler chickens fed a diet supplemented with antimicrobials only had higher ($P<0.05$) live weights than those on a diet not supplemented with either antimicrobials or effective microorganisms (UAM_0EM_0) and those on a diet supplemented with 100ml of EMs per litre of drinking water (UAM_0EM_{100}). However, chickens fed $UAM_{0.01}EM_0$, UAM_0EM_{30}, UAM_0EM_{50} or UAM_0EM_{100} diets had the same ($P>0.05$) live weights. Similarly, chickens fed UAM_0EM_0, UAM_0EM_{30}, UAM_0EM_{50} or UAM_0EM_{100} diets the same ($P>0.05$) live weights. Unsexed broiler chickens fed on diets supplemented with 50ml or 100ml of EMs per litre of drinking water (UAM_0EM_{50}

or UAM_0EM_{100}, respectively) had higher (P<0.05) ME intakes than those offered UAM_0EM_0, $UAM_{0.01}EM_0$ or UAM_0EM_{30} diets. Chickens on UAM_0EM_0, $UAM_{0.01}EM_0$ or UAM_0EM_{30} diets had similar (P>0.05) ME intakes. There were no deaths of chickens during this part of the study.

Table 4.01 Diet composition (% except MJ/kg DM for energy and mg/kg DM for Zn, Cu, MN and Fe)

	Treatment				
Feed Nutrient	AM_0EM_0	$AM_{0.01}EM_0$	AM_0EM_{30}	AM_0EM_{50}	AM_0EM_{100}
DM	91	91	91	91	91
CP	20	20	20	20	20
Energy	16.92	16.92	16.92	16.92	16.92
ADF	7.58	7.19	7.58	7.58	7.58
NDF	21.13	31.91	21.13	21.13	21.13
Fat	3.47	3.16	3.47	3.47	3.47
Ash	6.57	6.46	6.57	6.57	6.57
Ca	0.70	0.90	0.70	0.70	0.70
Mg	0.24	0.25	0.24	0.24	0.24
K	1.00	0.99	1.00	1.00	1.00
Na	0.14	0.19	0.14	0.14	0.14
K/Ca+Mg	0.47	0.39	0.47	0.47	0.47
P	0.81	0.82	0.81	0.81	0.81
Zn	54	117	54	54	54
Cu	12	24	12	12	12
Mn	144	189	144	144	144
Fe	327	478	327	327	327
Antimicrobial*	0	0.01	0	0	0
EM**	0	0	30	50	100

* : Antimicrobial inclusion is in g/kg DM.

** : Effective microorganism inclusion is in ml/litre of drinking water.

Feed intakes and live weights of unsexed Ross 308 broiler chickens were optimized at effective microorganism supplementation levels of 72.25 (r^2 = 0.941) and 48.29 (r^2 = 0.953) ml of EM/l of drinking water, respectively (Figures 4.01 and 4.02, respectively and Table 4.03). A positive relationship was observed between effective microorganism supplementation level and ME intakes of unsexed Ross 308 broiler chickens aged one to 21 days (Figure 4.03).

Table 4.02 Effect of supplementing diets with antimicrobials and effective microorganisms on DM feed intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g live weight gain), live weight (g/bird aged 21 days) and ME intake (MJ/kg DM) of unsexed Ross 308 broiler chickens aged one to 21 days

Variable	Treatment					SEM
	UAM_0EM_0	$UAM_{0.01}EM_0$	UAM_0EM_{30}	UAM_0EM_{50}	UAM_0EM_{100}	
DM intake	52^{b}	60^{a}	57^{ab}	61^{a}	56^{ab}	3.78
Growth rate	19.8	21.8	21.4	20.3	20.3	1.14
FCR	2.6	2.8	2.7	3.0	2.8	0.03
Live weight	434^{b}	499^{a}	469^{ab}	464^{ab}	433^{b}	30.92
ME	11.3^{bc}	11.4^{bc}	11.2^{c}	11.8^{a}	11.8^{a}	0.17

a, b, c : Means in the same row not sharing a common superscript are significantly different (P<0.05).

SEM : Standard error of the means.

$Y = 51.676 + 0.289x - 0.002x^2$

$r^2 = 0.941$

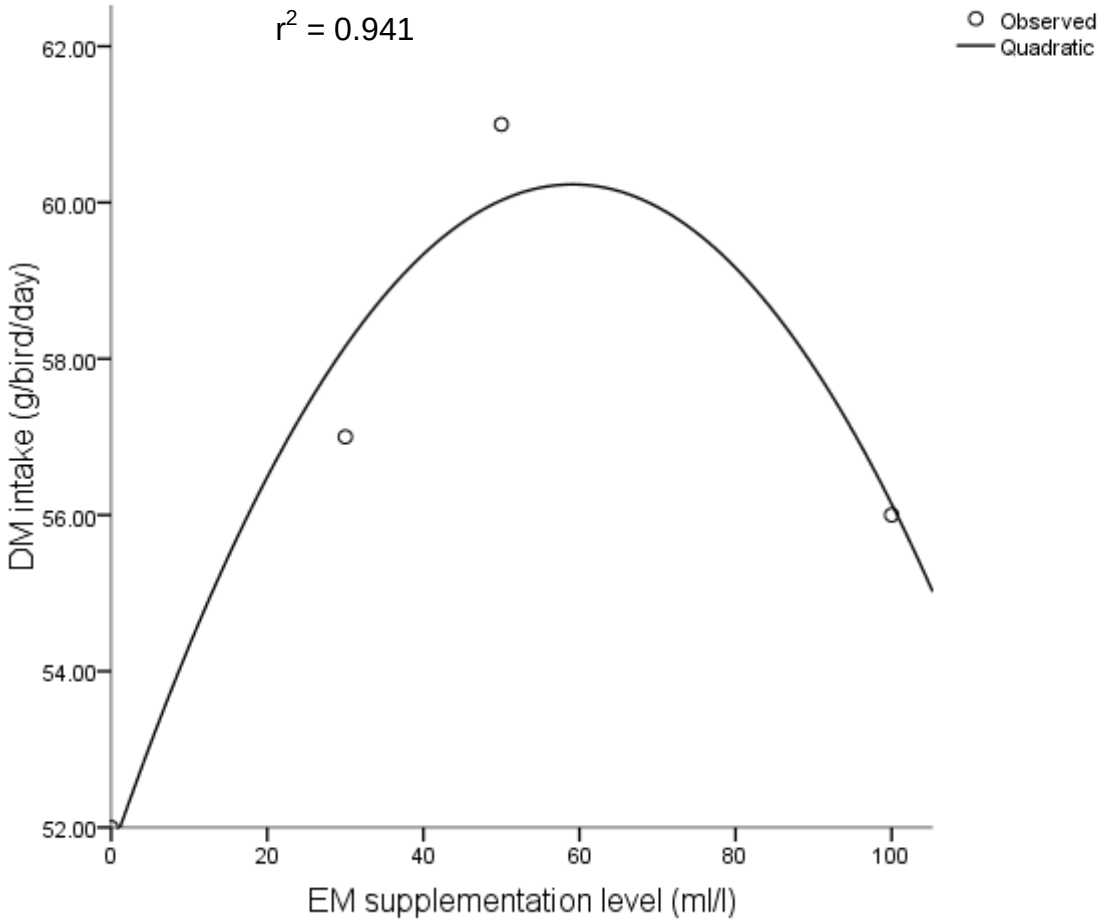

Figure 4.01 Effect of effective microorganism supplementation level on dry matter intake of unsexed Ross 308 broiler chickens aged one to 21 days

$$Y = 435.507 + 1.352x - 0.014x^2$$

$$r^2 = 0.953$$

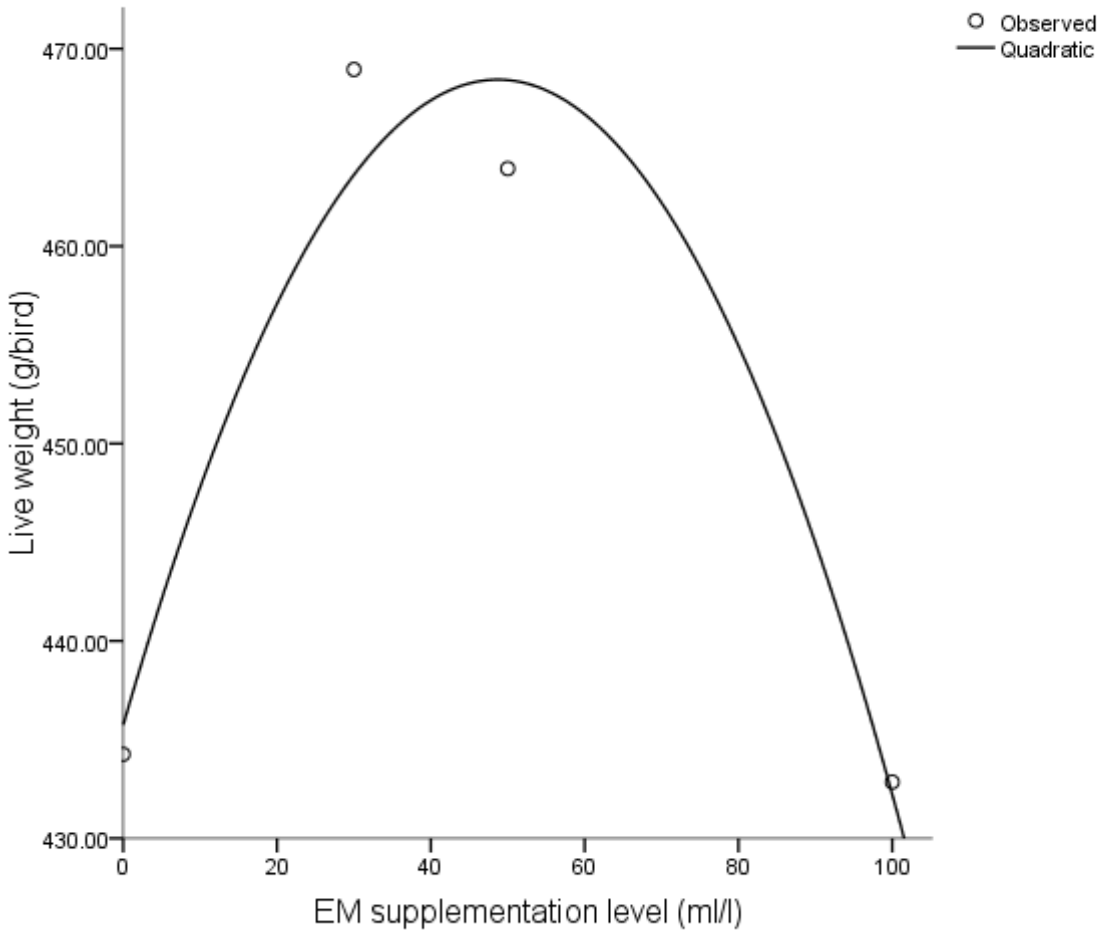

Figure 4.02 Effect of effective microorganism supplementation level on live weights of unsexed Ross 308 broiler chickens aged 21 days

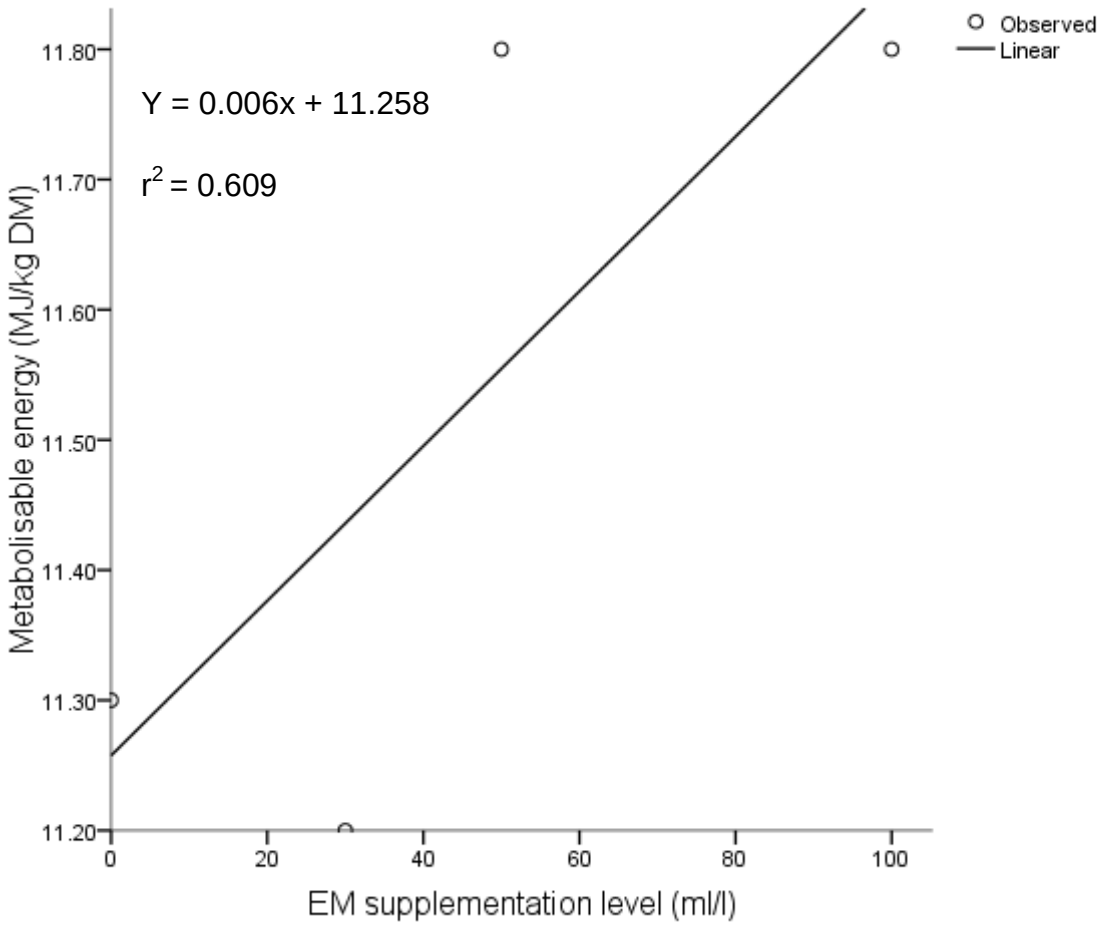

Figure 4.03 Relationship between effective microorganism supplementation level and ME intake of unsexed Ross 308 broiler chickens aged one to 21 days

Table 4.03 Effective microorganism supplementation levels for optimal DM feed intake (g/bird/day) and live weight (g/bird aged 21 days) of unsexed Ross 308 broiler chickens aged one to 21 days

Trait	Formula	EM level	Optimal Y-value	r^2
DM Intake	$Y = 51.676 + 0.289x - 0.002x^2$	72.25	62.12	0.941
Live weight	$Y = 435.507 + 1.352x - 0.014x^2$	48.29	465.15	0.953

EM level : Effective microorganism supplementation level for optimal Y-value.

r^2 : Regression coefficient.

4.3 Part 2

Results of the effects of antimicrobial and effective microorganism supplementations on DM feed intake, ME intake, growth rate, FCR and live weight of male Ross 308 broiler chickens aged 22 to 42 days are presented in Table 4.04. Supplementing diets with antimicrobials and effective microorganisms had no ($P>0.05$) effect on DM intake, growth rate, live weight and ME intake of male Ross 308 broiler chickens. Male broiler chickens fed a diet supplemented with antimicrobials only had poorer ($P<0.05$) FCR values than those fed a diet not supplemented with either antimicrobials or effective microorganisms (MAM_0EM_0). However, male broiler chickens fed $MAM_{0.01}EM_0$, MAM_0EM_{30}, MAM_0EM_{50} or MAM_0EM_{100} diets had similar ($P>0.05$) FCR values. Similarly, male broiler chickens on MAM_0EM_0, MAM_0EM_{30}, MAM_0EM_{50} or MAM_0EM_{100} had the same ($P>0.05$) FCR values. There were no deaths of chickens during this part of the study.

Male Ross 308 broiler chickens fed diets supplemented with antimicrobials or 100ml of EMs per litre of drinking water (MAM_0EM_{100}) had higher ($P<0.05$) blood glucose levels than those fed a diet supplemented with 30ml of EMs per litre of drinking water (MAM_0EM_{30}) (Table 4.04). However, chickens fed MAM_0EM_0, $MAM_{0.01}EM_0$, MAM_0EM_{50} or MAM_0EM_{100} diets had similar ($P>0.05$) blood glucose levels. Similarly, broiler chickens fed MAM_0EM_0, MAM_0EM_{30} or MAM_0EM_{50} diets had the same ($P>0.05$) blood glucose levels.

A positive relationship was observed between effective microorganism supplementation level and FCR of male Ross 308 broiler chickens aged 21 to 42

days, with a probability value of 0.16 and an r^2 value of 0.708 (Figure 4.04). Blood glucose levels of male Ross 308 broiler chickens were optimized at an effective microorganism supplementation level of 29.00ml per litre of drinking water (r^2 = 0.619) (Figure 4.05).

Table 4.04 Effect of supplementing diets with antimicrobials and effective microorganisms on DM feed intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g live weight gain), live weight (g/bird aged 42 days), ME intake (MJ/kg DM) and blood glucose level (mmol/l) of male Ross 308 broiler chickens aged 22 to 42 days

Variable	Treatment					SEM
	UAM_0EM_0	$UAM_{0.01}EM_0$	UAM_0EM_{30}	UAM_0EM_{50}	UAM_0EM_{100}	
Intake	111	130	114	120	116	15.64
Growth rate	61.9	58.9	58.1	58.1	55.4	6.97
FCR	1.8^{b}	2.2^{a}	2.0^{ab}	2.1^{ab}	2.1^{ab}	0.18
Live weight	1703	1681	1685	1677	1626	161.48
ME	11.8	11.3	11.6	11.4	11.5	0.34
Glucose	11.4^{ab}	12.3^{a}	8.9^{b}	10.8^{ab}	12.0^{a}	1.45

a, b, : Means in the same row not sharing a common superscript are significantly different (P<0.05).

SEM : Standard error of the means.

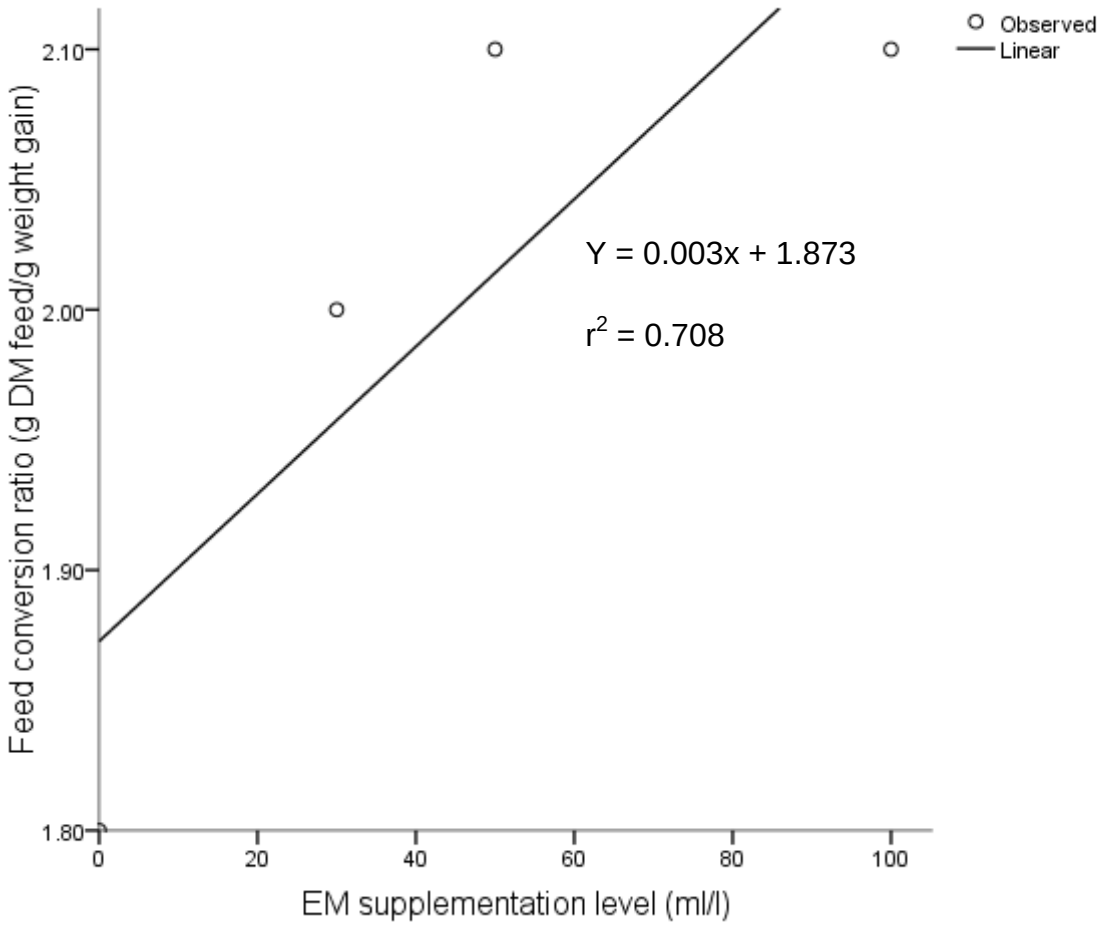

Figure 4.04 Relationship between effective microorganism supplementation level and FCR of male Ross 308 broiler chickens aged 22 to 42 days

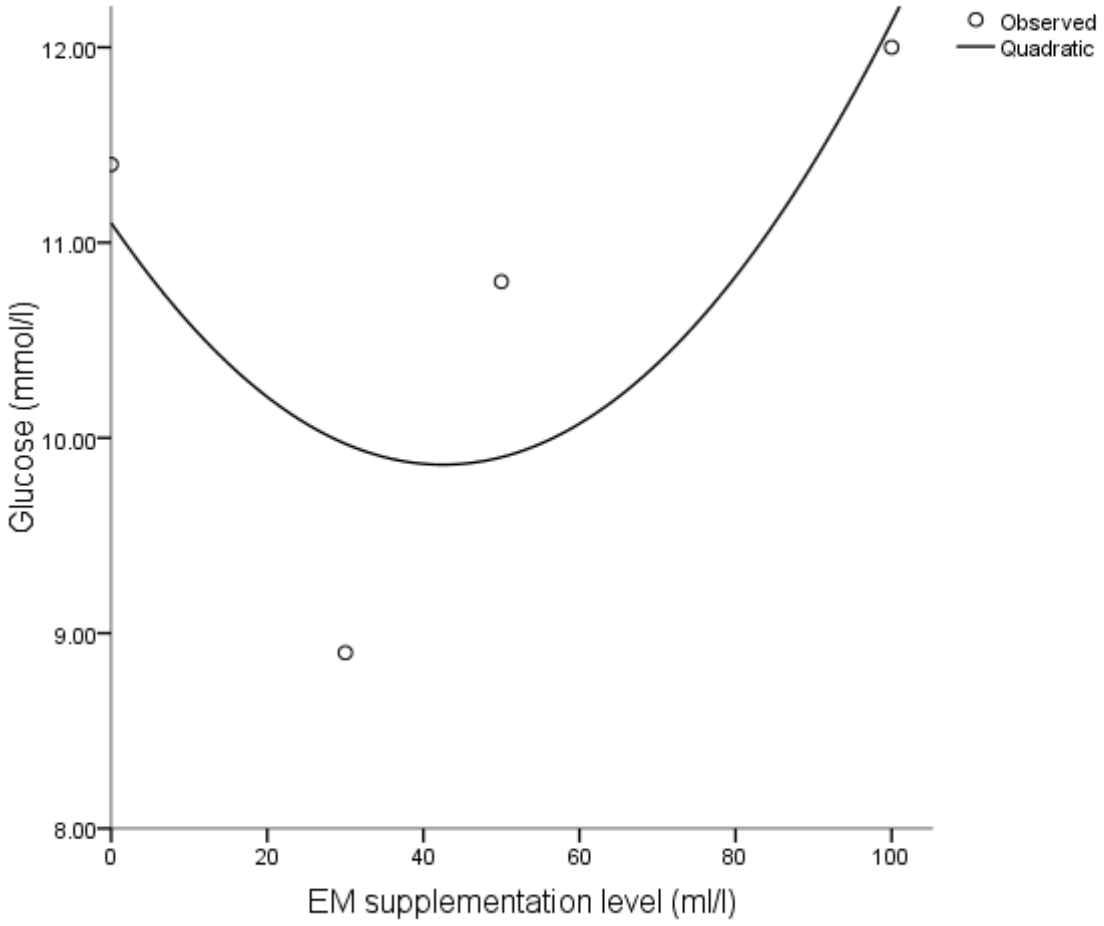

Figure 4.05 Effect of effective microorganism supplementation level on blood glucose values of male Ross 308 broiler chickens aged 42 days

Results of the effects of antimicrobial and effective microorganism supplementations on crop, gizzard, proventriculus, ileum and large intestine pH values of male Ross 308 broiler chickens aged 42 days are presented in Table 4.05. Male broiler chickens fed a diet supplemented with 50 ml of EMs per litre of drinking water (MAM_0EM_{50}) had higher ($P<0.05$) crop pH values than those fed a diet supplemented with antimicrobials only ($MAM_{0.01}EM_0$). However, broiler chickens on MAM_0EM_0, $MAM_0EM_{30,}$ MAM_0EM_{50} or MAM_0EM_{100} diets had similar ($P>0.05$) crop pH values. Similarly, chickens fed MAM_0EM_0, $MAM_{0.01}EM_0$, MAM_0EM_{30} or MAM_0EM_{100} diets had the same ($P>0.05$) crop pH values. Supplementing diets with antimicrobials or effective microorganisms did not affect ($P>0.05$) gizzard, proventriculus and large intestine pH values of male Ross 308 broiler chickens.

Male Ross 308 broiler chickens fed a diet not supplemented with either antimicrobials or effective microorganisms (MAM_0EM_0) had higher ($P<0.05$) ileum pH values than those fed diets supplemented with 30ml or 100ml of EMs per litre of drinking water (MAM_0EM_{30} or MAM_0EM_{100}, respectively). However, chickens on MAM_0EM_0, $MAM_{0.01}EM_0$ or MAM_0EM_{50} diets had similar ($P>0.05$) ileum pH values. Male broiler chickens fed $MAM_{0.01}EM_0$, MAM_0EM_{30} or MAM_0EM_{50} diets had similar ($P>0.05$) ileum pH values. Similarly, chickens on MAM_0EM_{30} or MAM_0EM_{100} diets had the same ($P>0.05$) ileum pH values.

Crop pH values of male Ross 308 broiler chickens were optimized at an effective microorganism supplementation level of 85.00ml per litre of drinking water (r^2 = 0.586) (Figure 4.06). A negative relationship was observed between effective microorganism supplementation levels and pH values of ileums of male Ross 308 broiler chickens aged 42 days, with a probability value of 0.77 and an r^2 value of 0.853 (Figure 4.07).

Table 4.05 Effect of supplementing diets with antimicrobials and effective microorganisms on pH values of gut organs of male Ross 308 broiler chickens aged 42 days

Variable	Treatment					SEM
	MAM_0EM_0	$MAM_{0.01}EM_0$	MAM_0EM_{30}	MAM_0EM_{50}	MAM_0EM_{100}	
Crop	5.45^{ab}	5.18^{b}	5.46^{ab}	6.01^{a}	5.26^{ab}	0.41
Gizzard	3.95	3.42	3.31	3.22	2.70	0.98
Proventriculus	3.90	4.25	4.30	4.14	3.69	0.46
Ileum	6.22^{a}	5.92^{ab}	5.85^{bc}	5.96^{ab}	5.59^{c}	0.19
Large intestines	5.89	5.66	5.62	5.71	5.47	0.40

a, b, : Means in the same row not sharing a common superscript are significantly different ($P<0.05$).

SEM : Standard error of the means.

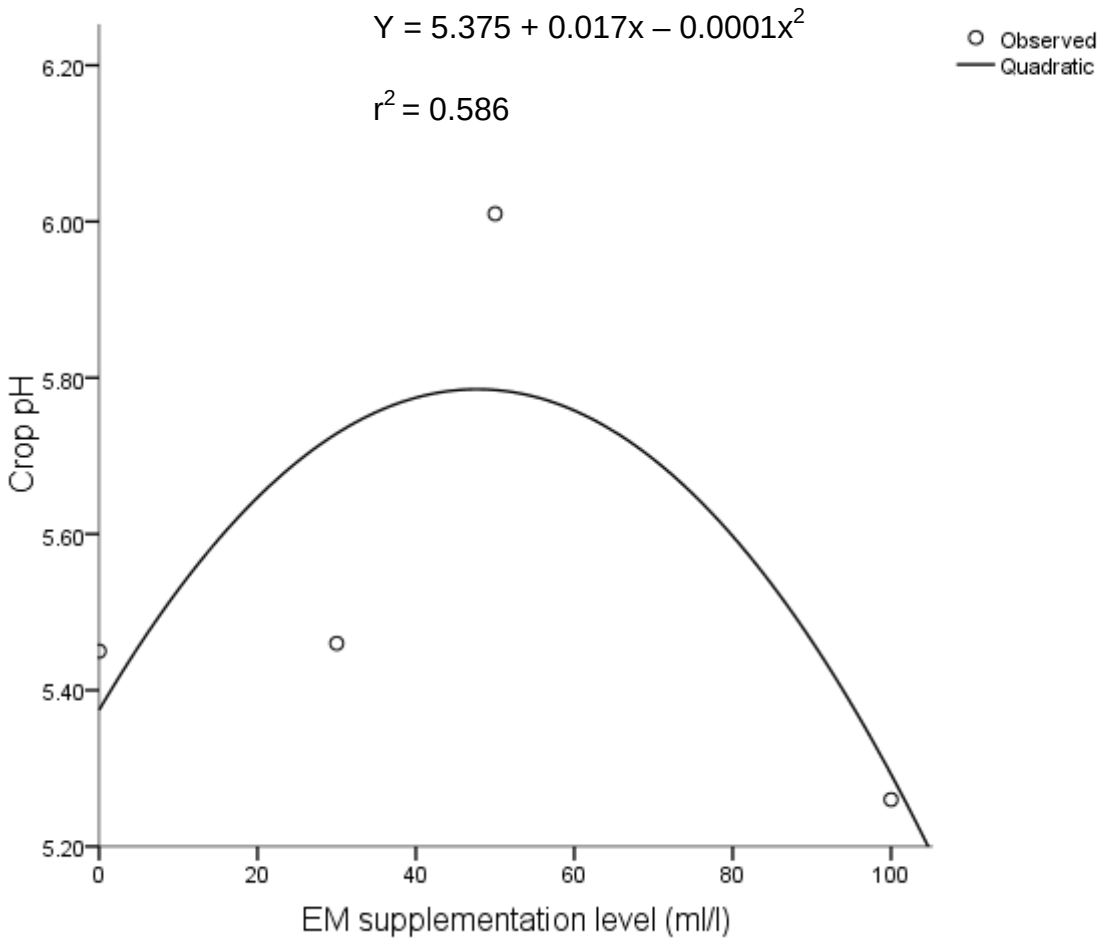

Figure 4.06 Effect of effective microorganism supplementation level on pH values of crops of male Ross 308 broiler chickens aged 42 days

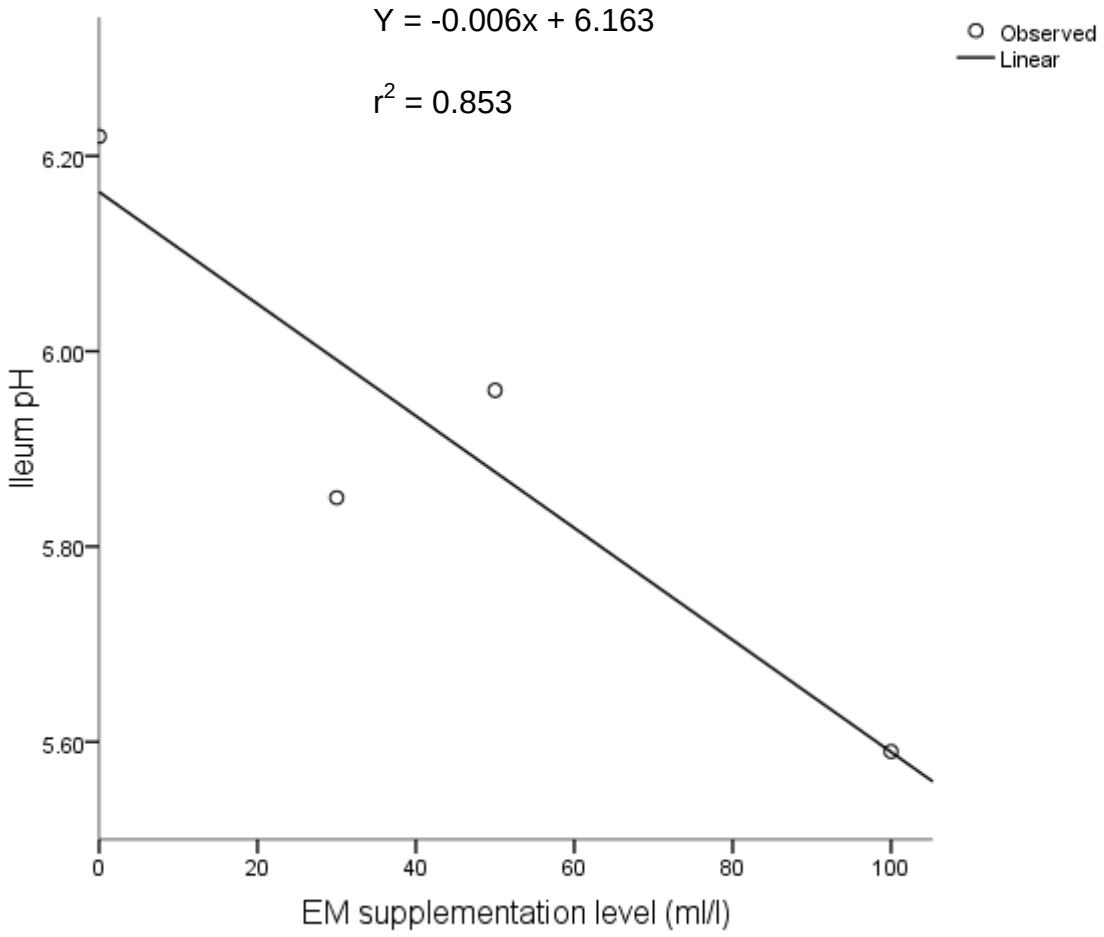

Figure 4.07 Relationship between effective microorganism supplementation level and ileum pH values of male Ross 308 broiler chickens aged 42 days

Results of the effects of antimicrobial and effective microorganism supplementations on weights and lengths of gastro-intestinal tract organs of male Ross 308 broiler chickens aged 42 days are presented in Table 4.06. Male broiler chickens fed a diet not supplemented with antimicrobials or effective microorganisms (MAM_0EM_0) had higher (P<0.05) crop weights than those fed a diet supplemented with 50ml of EMs per litre of drinking water (MAM_0EM_{50}). However, male chickens on MAM_0EM_0, $MAM_{0.01}EM_0$, MAM_0EM_{30} or MAM_0EM_{100} diets had similar (P>0.05) crop weights. Similarly, male chickens on $MAM_{0.01}EM_0$, MAM_0EM_{30} or MAM_0EM_{100} diets had the same (P>0.05) crop weights. Supplementing diets with antimicrobials or effective microorganisms had no effect (P>0.05) on gizzard, proventriculus, small intestine, caecum, large intestine, liver and heart weights of male broiler chickens.

Antimicrobial and effective microorganism supplementations had no (P>0.05) effect on gastro-intestinal tract and small intestine length of male broiler chickens (Table 4.06). Male chickens fed a diet supplemented with 30ml of EMs per litre of drinking water (MAM_0EM_{30}) had longer (P<0.05) caecum values than those fed a diet supplemented with 50ml of EMs per litre of drinking water. However, male chickens on MAM_0EM_0, $MAM_{0.01}EM_0$, MAM_0EM_{30} or MAM_0EM_{100} diets had similar (P>0.05) caecum lengths. Similarly, broiler chickens on MAM_0EM_0, $MAM_{0.01}EM_0$, MAM_0EM_{50} or MAM_0EM_{100} diets had the same (P>0.05) caecum lengths.

Male chickens fed a diet not supplemented with either antimicrobials or effective microorganisms had longer (P<0.05) large intestines than those of chickens fed a diet supplemented with antimicrobials only (Table 4.06). However, broiler chickens on MAM_0EM_0, MAM_0EM_{30}, MAM_0EM_{50} or MAM_0EM_{100} diets had similar (P>0.05) large intestine lengths. Similarly, chickens fed $MAM_{0.01}EM_0$, MAM_0EM_{30}, MAM_0EM_{50} or MAM_0EM_{100} diets had the same (P>0.05) large intestine lengths.

Male broiler chicken crop weights and caecum and large intestine lengths were optimized at effective microorganism supplementation levels of 41.00 (r^2 = 1.000), 45.50 (r^2 = 0.206) and 85.00 (r^2 = 0.994) ml per litre of drinking water, respectively (Figures 4.08, 4.09 and 4.10, respectively and Table 4.07).

Table 4.06 Effect of supplementing diets with antimicrobials and effective microorganisms on weights (g) and lengths (cm) of gastro-intestinal tract (GIT) organs of male Ross 308 broiler chickens aged 42 days

Variable	Treatment					SEM
	MAM_0EM_0	$MAM_{0.01}EM_0$	MAM_0EM_{30}	MAM_0EM_{50}	MAM_0EM_{100}	
Organ weight						
Crop	6.58[a]	5.22[ab]	4.75[ab]	4.11[b]	4.95[ab]	1.01
Gizzard	36.94	35.28	29.17	37.09	34.58	7.76
Proventriculus	11.24	9.21	8.58	9.29	9.44	2.30
Small intestine	75.17	64.63	69.24	65.14	54.20	12.33
Caecum	3.02	3.05	3.13	3.23	3.40	0.79
Large intestine	5.31	6.60	5.59	6.26	4.64	1.69
Liver	39.16	38.81	37.60	33.17	35.23	7.64
Heart	9.60	7.42	6.29	7.78	6.61	1.91
Organ length						
Whole GIT	232.67	215.67	230.67	209.67	226.50	30.53
Small intestine	218.33	205.00	218.00	197.00	214.50	29.37
Caecum	18.50[ab]	18.33[ab]	20.50[a]	14.50[b]	19.67[ab]	2.75
Large intestine	14.33[a]	10.67[b]	12.67[ab]	12.67[ab]	12.00[ab]	1.69

a, b, : Means in the same row not sharing a common superscript are significantly different (P<0.05).

SEM : Standard error of the means.

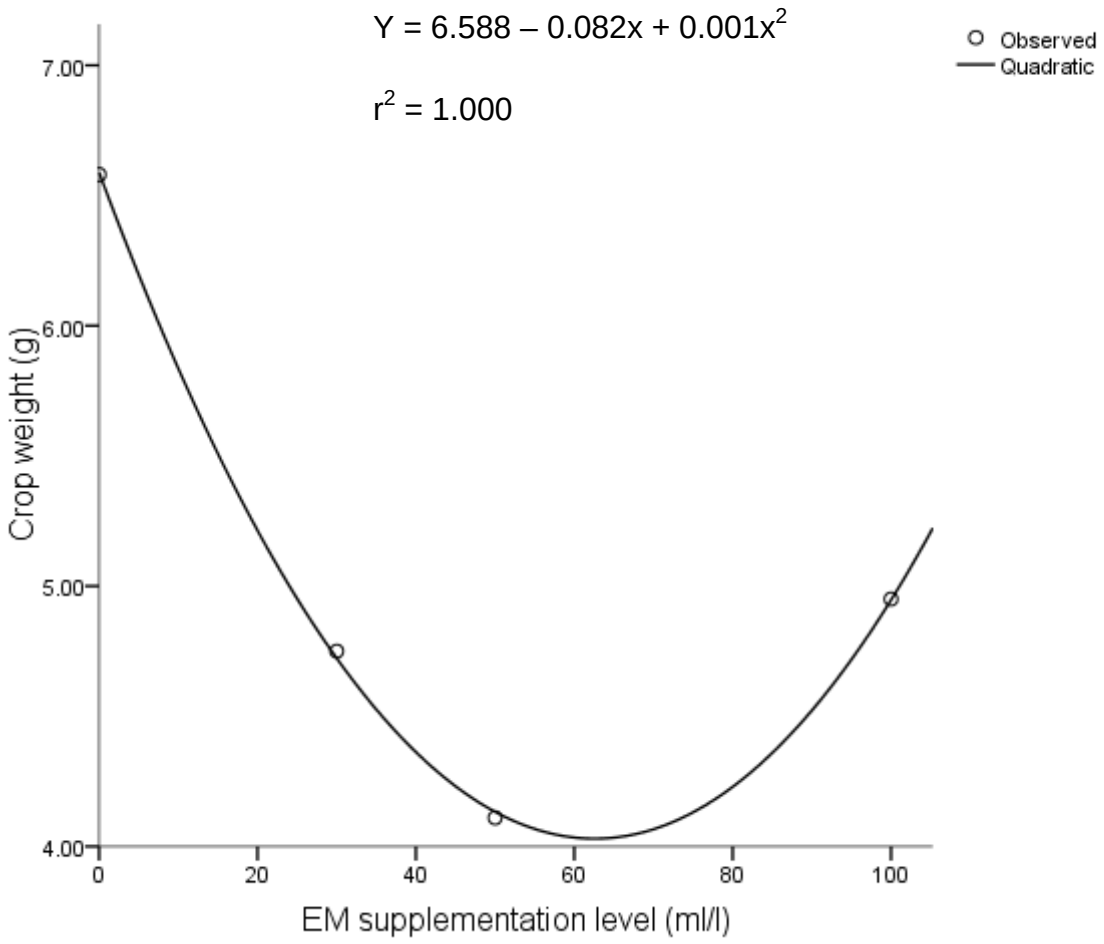

Figure 4.08 Effect of effective microorganism supplementation level on crop weights of male Ross 308 broiler chickens aged 42 days

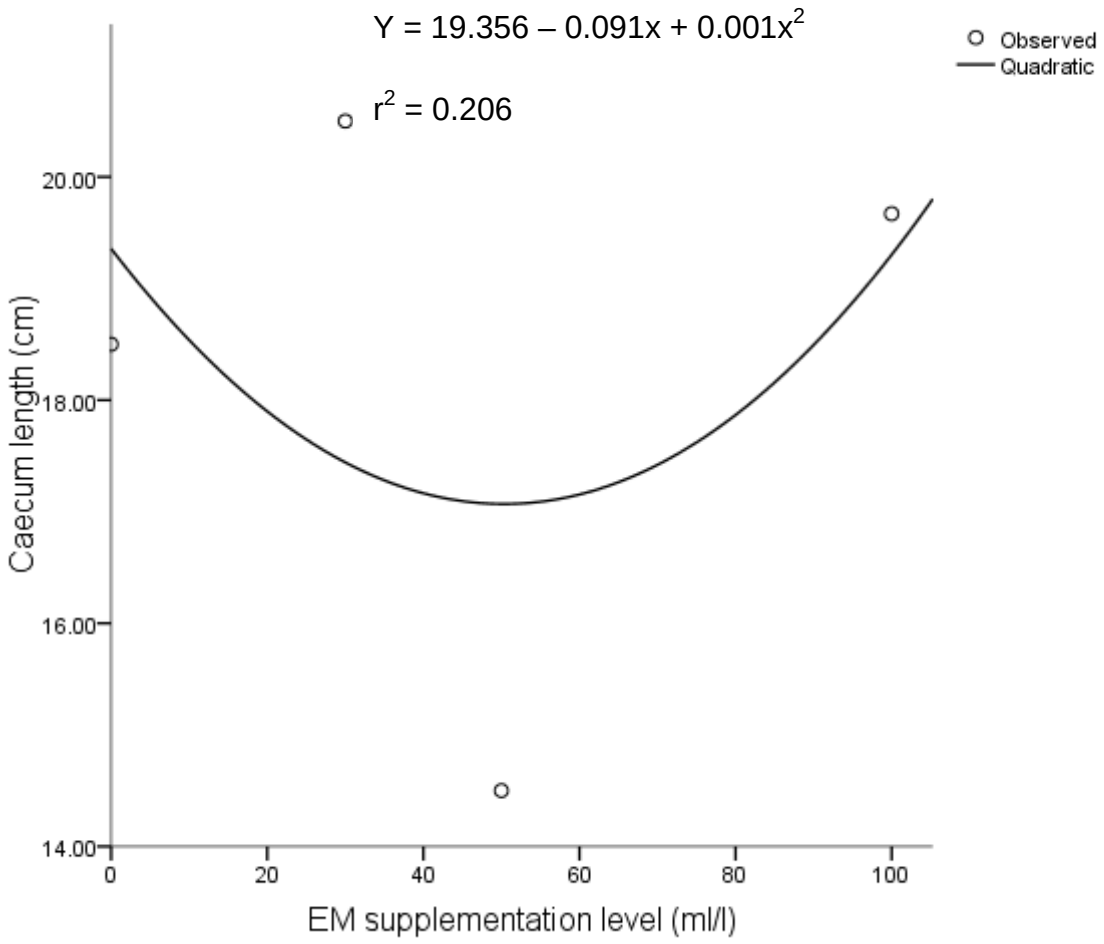

Figure 4.09 Effect of effective microorganism supplementation level on caecum lengths of male Ross 308 broiler chickens aged 42 days

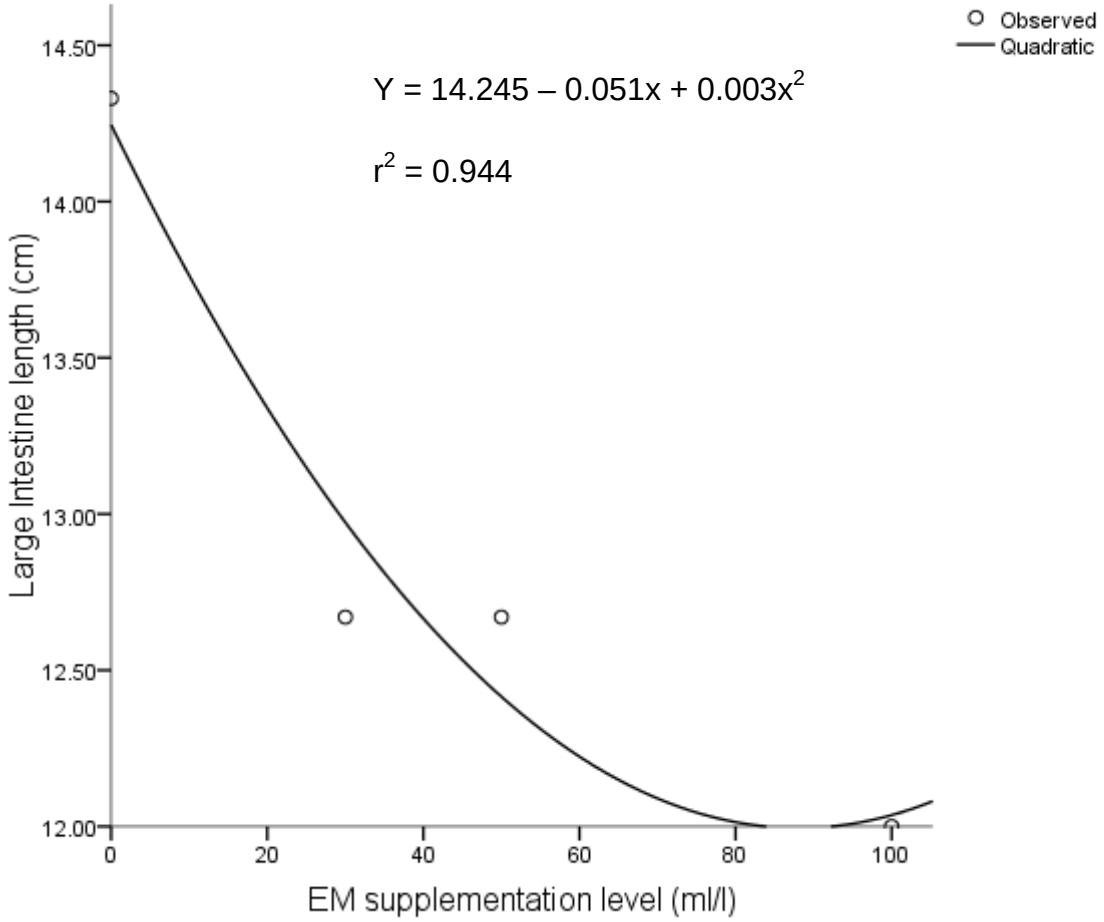

Figure 4.10 Effect of effective microorganism supplementation level on large intestine lengths of male Ross 308 broiler chickens aged 42 days

Table 4.07 Effective microorganism supplementation levels for optimal crop weights (g), caecum lengths (cm) and large intestine lengths (cm) of male Ross 308 broiler chickens aged 42 days

Gut organ	Formula	EM level	Optimal Y-value	r^2
Crop weight	$Y = 6.588 – 0.082x + 0.001x^2$	41.00	4.90	1.000
Caecum length	$Y = 19.356 – 0.091x + 0.001x^2$	45.50	8.19	0.206
Large intestine length	$Y = 14.245 – 0.051x + 0.003x^2$	85.00	12.08	0.944

EM level : Effective microorganism supplementation level for optimal Y-value.
r^2 : Regression coefficient.

Results of the effects of antimicrobial and effective microorganism supplementations on live and carcass weights of male Ross 308 broiler chickens aged 42 days are presented in Table 4.08. Dietary antimicrobial and effective microorganism supplementations had no (P>0.05) effect on live, carcass, breast, drumstick and thigh weights of male Ross 308 broiler chickens.

Table 4.08 Effect of supplementing diets with antimicrobials and effective microorganisms on live and carcass weights (g) of male Ross 308 broiler chickens aged 42 days

Variable	Treatment					SEM
	MAM_0EM_0	$MAM_{0.01}EM_0$	MAM_0EM_{30}	MAM_0EM_{50}	MAM_0EM_{100}	
Live weight	1621.9	1630.2	1462.9	1518.1	1401.3	244.45
Carcass weight	1055.8	1092.9	966.1	993.0	965.6	181.88
Breast weight	312.6	324.4	276.6	277.2	290.1	56.77
Drumstick weight	149.3	151.5	142.1	149.3	131.0	24.84
Thigh weight	174.8	166.5	145.4	157.3	157.3	31.28

SEM : Standard error of the means.

Results of the effects of antimicrobial and effective microorganism supplementations on tenderness, juiciness and flavour of male Ross 308 broiler chickens meat are presented in Table 4.09. Supplementing diets with antimicrobials and effective microorganisms did not affect (P>0.05) tenderness, juiciness and flavour of male Ross 308 broiler chicken meat.

Table 4.09 Effect of supplementing diets with antimicrobials and effective microorganisms on meat tenderness, juiciness and flavour of male Ross 308 broiler chickens aged 42 days

Variable	Treatment					SEM
	MAM_0EM_0	$MAM_{0.01}EM_0$	MAM_0EM_{30}	MAM_0EM_{50}	MAM_0EM_{100}	
Tenderness	3.0	3.7	3.3	3.7	3.7	0.52
Juiciness	3.3	3.7	3.0	3.0	3.3	0.48
Flavour	2.3	3.0	3.7	3.7	3.7	0.75

SEM : Standard error of the means.

Results of the effects of antimicrobial and effective microorganism supplementations on their residues in the meat of male Ross 308 broiler chickens aged 42 days are presented in Table 4.10. Meat from treated male broiler chickens did not (P>0.05) have antimicrobial and effective microorganism residues.

Table 4.10 Effect of supplementing diets with antimicrobial and effective microorganisms on their residues in the meat of male Ross 308 broiler chickens aged 42 days

Variable	Treatment						SEM
	MAM_0 EM_0	$MAM_{0.01}$ EM_0	MAM_0 EM_{30}	MAM_0 EM_{50}	MAM_0 EM_{100}	Woolworths	
OD-1	2.29	2.62	2.14	2.,43	2.79	2.72	0.52
B/BO	1.30	1.07	0.99	0.96	1.14	1.54	0.21
Results	0.0	.0.0	0.0.	0.0.	0.0.	0.0	0.0
Pass/Fail	Pass	Pass	Pass	Pass	Pass	Pass	

SEM : Standard error of the means; OD-1: Optical density;
B/BO : Absorbance standard (or sample)/absorbance zero standard

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The diets, in the present study, were formulated to have crude protein and energy levels of 20% and 12MJ/kg, respectively. The diets contained the same nutrients except for the effective microorganism (EM) and antimicrobial supplementations. Effective microorganism and antimicrobial supplementations did not change the nutrient composition of the diets. The diets met the nutrient requirements for broiler chickens as specified by McDonald *et al*. (2010).

Supplementing the diets with antimicrobials and effective microorganisms did not affect growth rate and feed conversion ratio of unsexed Ross 308 broiler chickens aged one to 21 days. Similar results have been reported elsewhere (Lorençon *et al*., 2007; Gunal *et al*., 2006; Pelicano *et al*., 2004; Gunes *et al*., 2001). However, Datta (2013) and Ashayerizadeh *et al*. (2009) observed that antimicrobial and effective microorganism supplementations improved feed conversion ratio and growth rate of broiler chickens. The authors indicated that improvements in FCR and growth rate of the chickens were due to improved feed intake. Other studies reported poorer FCR (Shabani *et al*., 2012; Falaki *et al.*, 2011; Aftahi *et al.*, 2006) and reductions in growth rate (Hossain *et al*., 2015) of broiler chickens with probiotic supplementation.

The present study indicates that antimicrobial supplementation improved feed intake of unsexed Ross 308 broiler chickens aged one to 21 days. Similarly, supplementing diets with 50ml of effective microorganisms per litre of drinking water increased feed intake of the chickens. However, chickens supplemented with antimicrobials or effective microorganisms had similar intakes, possibly by indicating that either of them can be used when required. Duwa *et al*. (2013) and Bai *et al*. (2013) reported increased intakes with antimicrobial supplementations to the diets of broiler chickens aged 1 to 21 days. However, Ghahri *et al*. (2013) and Bitterncourt *et al*. (2011) reported no improvements in intake when broiler chickens were supplemented with antimicrobials. Contrearas-Castillo *et al*. (2008) reported similar intakes when broiler chickens were supplemented with either antimicrobials or effective microorganisms. However, Faria *et al*. (2009) observed better intakes in broiler chickens supplemented with antimicrobials than in those supplemented with effective microorganisms. Boratto *et al*. (2004) reported higher intakes in broiler chickens supplemented with effective microorganisms than in those supplemented with

antimicrobials. An effective microorganism supplementation level of 72.25ml per litre of drinking water optimized intake of broiler chickens in the present study. No similar results were found in the literature.

Antimicrobial supplementation improved live weight of unsexed Ross 308 broiler chickens aged 21 days. This was possibly due to improved dry matter intake with antimicrobial supplementation. Yang *et al*. (2009) reported that improvements in live weights of broiler chickens were due to improved intakes with antimicrobial supplementations. In the present study, effective microorganism supplementation did not improve live weight of unsexed broiler chickens aged 21 days. Similarly, live weights of broiler chickens supplemented with effective microorganisms were the same as those of chickens supplemented with antimicrobials. However, results of the present study indicate that during the starter phase an effective microorganism supplementation level of 48.29ml/litre of drinking water optimized live weight of the chickens. A number of studies have reported improved live weights of broiler chickens with antimicrobial and probiotic supplementations (Bonnet *et al*., 2009; Diarra *et al*., 2007; Hosamani *et al*., 2004). Generally, these studies indicate that antimicrobial supplementation to the diets tends to increase feed intake, digestibility and FCR, resulting in improved live weights of the chickens.

Supplementing the diets with antimicrobials did not improve metabolisable energy intake of unsexed Ross 308 broiler chickens aged one to 21 days. However, chickens supplemented with 50 or 100ml of EMs per litre of drinking water had higher ME intakes than those supplemented with antimicrobials or having no any supplementation at all. In fact, there was a positive linear relationship between EM supplementation level and ME of the diet. It is possible that effective microorganisms improved digestibility of the diet with the help of microbial enzymes, as observed by Zhang and Kim (2013) and Li *et al*. (2009). However, improved diet ME intake did not have any positive impact on live weight of the chickens in the present study. This is similar to the observation made by Sinol *et al*. (2012) with broiler chickens. Other studies reported that EM supplementation improved ME (Mohan, *et al*., 1996) and live weight (Taheri *et al*, 2010) of broiler chickens. However, some studies reported no improvement in ME intake (Apata, 2008) and live weight (Aliakbarpour *et al*., 2012) of broiler chickens with EM supplementation.

Effective microorganism and antimicrobial supplementations to the diets of male Ross 308 broiler chickens aged 22 to 42 days had no effect on feed intake, growth rate, live weight and ME intake of the chickens. Other authors have, also, observed that supplementation with antimicrobial or EM had no influence on feed intake (El-Hammady *et al*., 2014; Bai *et al*., 2013), growth rate and live weight (Nunes *et al*., 2012; Gunal *et al*., 2006) of broiler chickens aged 21 to 42 days. The no improvement in live weight of the chickens in the present study might have been brought about by the fact that there were no improvements in feed consumption and growth rate of the chickens with EM and antimicrobial supplementations. However, antimicrobial supplementation improved FCR of male broiler chickens but this did not have any effect on live weight of the chickens at 42 days old. On the other hand, El-Hammady *et al*. (2014) reported some improvements in live weights and body weight gains in antibiotic-fed chickens as compared to those of chickens on probiotics or control. Other studies have showed higher final body weights (Tabidi *et al*., 2013; EL-Nagmy *et al*., 2007; Khaksefidi and Rahimi, 2005) and daily weight gains (Yin-bo Li *et al*., 2014; Kabir *et al*., 2004) with probiotic treatments. Amerah *et al*. (2013) observed no differences in performance of broiler chickens between the antibiotic or probiotic treatments but both antibiotic and probiotic treatments performed better than the unsupplemented treatment. This inconsistency among research reports may be related to differences in probiotic types, management practices and environmental conditions among the experiments. Other authors have suggested that under favourable management and/ or environmental conditions, the effect of such feed additives may be worthless (Boostani *et al*., 2013).

The results of the present study show that supplementing diets with antimicrobials improved feed conversion ratio of broiler chickens aged 22 to 42 days. Antibiotic supplementations improve feed conversion ratio, likely, by altering the composition and activities of gut micro-flora (Collier *et al*., 2003; Knarreborg *et al*., 2002) which tend to improve digestibility. The results of the present study are contradictory to those of Teirlynck *et al*. (2009), Ceylan *et al*. (2003) and Engberg *et al*. (2000) who reported that supplementation with antibiotic growth promoters did not have any effect on feed conversion ratio of broiler chickens. Effective microorganism supplementation, in the present study, did not have any influence on the FCR of the chickens. Similarly, chickens fed EM or antimicrobial supplemented diets had the

same FCR. Amerah *et al*. (2013) and Gunes *et al*. (2001) reported improved FCR with the inclusion of antibiotics in the diets and Aliakbarpour *et al*. (2012), Chumpawadee *et al*. (2008) and Ahmad (2004) reported no effect on FCR with probiotic supplementation. Ghahri *et al*. (2013) reported no significant differences in FCR between probiotic and antibiotic supplemented diets. Jwher *et al*. (2013), Midilli *et al*. (2008) and Mountzouris *et al*. (2007) reported that probiotic supplementations to the diets of broiler chickens improved FCR. However, Awad *et al*. (2009) found that probiotic supplementation did not improve feed conversion ratio of broiler chickens.

Blood glucose of male Ross 308 broiler chickens was not affected by EM and antimicrobial supplementations. However, supplementing diets with 30ml of effective microorganisms improved blood glucose levels of the chickens as compared to those of chickens on antimicrobial supplemented diets. Al-Saad *et al*. (2014) and Ashayerizadeh *et al*. (2009) reported that blood glucose levels of the chickens were not affected by antibiotic or probiotic supplementations. Gheith (2008) and Abd El-Baky (2007) observed no influence of probiotic supplementation on blood glucose levels in broiler chickens. The results of the present study disagree with those of other authors who indicated reductions (Salim *et al*., 2011; Al-Kassie and Abd-Aljaleel, 2007) and increases (Abd, 2014**)** in blood glucose levels in probiotic-supplemented groups compared with the unsupplemented groups. Al-Kassie *et al*. (2008) suggested that the decrease in blood glucose levels in probiotic-treated groups could be due to decreased stress factor on chickens. Hashemzadeh *et al*. (2013) reported increases in blood glucose levels in antibiotic-treated groups compared with the control but no differences when compared with probiotic-treated groups. Azza *et al*. (2012) found improved blood glucose levels in broiler chickens fed antibiotic or probiotic supplemented diets.

The present study shows that dietary antimicrobial and EM supplementations did not affect crop, gizzard, proventriculus and large intestine pH values. Similarly, supplementation with antimicrobials did not affect ileum pH values. However, supplementation with 30 or 100ml of effective microorganisms per litre of drinking water decreased the pH of the ileum. There was a negative linear relationship between EM supplementation level and ileum pH of the digesta. Denli *et al*. (2003)

found that supplementation with antibiotics or probiotics had no effect on intestinal pH. Similarly, Olnood *et al*. (2015) found no effect on gut pH with probiotic supplemenntation. The lower ileum pH recorded in chickens supplemented with EMs could be as a result of the fermentative action of microorganisms on carbohydrates to produce more lactic acid which decreases pH levels (WGO, 2008). Similarly, Biernasiak and Slizewska (2009) reported decreases in broiler crop and ileum pH values with probiotic supplementation. In contrast to the present results, Agboola *et al*. (2015) showed that supplementing feeds with probiotics did not have any effect on ileum pH values.

The results of the present study indicate that supplementation of diets with antimicrobials and effective microorganisms did not affect crop, gizzard, proventriculus, small intestine, caecum, large intestine, liver and heart weights of male broiler chickens aged 42 days. Supplementation with 50ml of effective microorganisms per litre of drinking water decreased crop weight of the chickens. However, antibiotic and EM supplementations had similar effect on crop pH values of the chickens. It is possible that EM helped with feed fermentation in the crops of the chickens. This reduced the necessity of the crops to build muscles for efficient and effective fermentation of the diets (McDonald *et al*., 2010). Agboola *et al*. (2015), Hossain *et al*. (2015) and Kamruzzaman *et al*. (2005) reported no influence of antibiotic or probiotic supplementations on broiler chicken heart, liver and gizzards weights. Yakhkeshi *et al*. (2012) and Zhang *et al.* (2005) reported that probiotic or antibiotic supplementations did not induce increases in organ weights of the chickens. On the other hand, when diets were supplemented with probiotics increases in gizzard, heart, liver (Olatoye *et al*., 2014; Fallah *et al*., 2013; Paryad and Mahmoudi 2008) and intestine weights (Çelik *et al*., 2007) of the chickens were observed. Beiki *et al*. (2013) reported decreases in organ weights when the chickens were supplemented with antibiotics as compared to probiotics. In the present study an effective microorganism supplementation level of 41.00ml per litre of drinking water optimized the crop weight of male broiler chickens at 4.90g.

Whole gastrointestinal tract, small intestines and caecum of male broiler chickens aged 42 days were not affected by antimicrobial or effective microorganism supplementations. Similarly, antibiotic supplementation did not affect caecum lengths

of male broiler chickens. However, supplementation with EMs reduced the caecum lengths of the chickens; the caecum length being optimized at the supplementation level of 45.5ml of EM/l of water. Effective microorganism supplementation had no effect on large intestine lengths of the chickens. Similarly, there were no differences in large intestine lengths between the chickens supplemented with antibiotics and those supplemented with EMs. However, antibiotic supplementation reduced large intestine lengths of the chickens. El-Hammady *et al*. (2014) and Yakhkeshi *et al*. (2012) observed no significant differences in intestinal and caecum lengths among control, antibiotics and probiotic supplemented chicken group. Also, Pani *et al*. (2014) and Ledezma-Torres *et al*. (2015) reported no effect of probiotic supplementation on intestinal lengths of broiler chickens. Beike *et al*. (2013) and Denli *et al*. (2003) indicated reductions in lengths of the intestines with sub-therapeutic levels of antibiotic supplementation. Farhoomand and Dadvend (2007) observed shorter intestinal lengths when broiler chickens were supplemented with probiotics.

Inclusion of antimicrobials and effective microorganisms did not affect the live, carcass, breast, drumstick and thigh weights of male Ross 308 broiler chickens. Kamruzzaman *et al*. (2005) reported no significant differences in carcass characteristics of the control, antibiotic and probiotic supplemented broiler chickens. Other authors have observed no influence of probiotic supplementation on carcass characters of broiler chickens (Mazaheri *et al*., 2014, Willis *et al*., 2007). The results of the present study are contradictory to those of Olatoye *et al*. (2014), Habibi *et al*. (2013), Aluwong *et al.* (2013) and Shabani *et al*. (2012) who reported positive effects of probiotic supplementation on carcass characteristics of broiler chickens. Ashayerizadeh *et al*. (2009) reported lowest carcass yield with the control than the antibiotic or probiotic supplemented broiler chickens. Datta (2013) recorded higher broiler carcass weights with antibiotic or EM supplementations.

Supplementing the diets with antimicrobials or EMs had no effect on tenderness, juiciness and flavour of the chicken meat. Mathivanan *et al*. (2006), Pelicano *et al*. (2005) and Loddi *et al*. (2000) reported that neither probiotic nor antibiotic supplementations affected flavour, tenderness and juiciness of the chicken meat. Similarly, Abdel-Raheem and Abd-Allah (2011) and Brzóska *et al*. (2010) found no

differences in broiler chicken meat tenderness and juiciness with probiotic supplementation. However, Liu *et al*. (2012) reported lower chicken meat juiciness with probiotic supplementation. Liu *et al*. (2012) and Pelicano *et al*. (2003) reported improved chickens meat flavour with antibiotic and probiotic supplementation. It is not clear how antibiotic or probiotic supplementations affect sensory attributes of the meat (Brzóska *et al*., 2010).

The results of the present study indicate that supplementation of diets with antimicrobials and effective microorganisms did not show any residues in the breast meat of male broiler chickens aged 42 days. The results are in agreement with those of Pavlov *et al*. (2008), Al-Mustafa and Al-Ghamdi (2000) and Al-Ghamdi *et al*. (2000) who reported no antibiotic residues in the breast meat muscles of tested chickens. The results are contrary to those of Rutherford *et al*. (2000) and Atef *et al*. (1993) who found antibiotic residues in the meat of tested broiler chickens.

5.2 Conclusion and recommendations

All the diets had similar nutrient contents and met the nutrient requirements of the broiler chickens. Thus, any differences in responses must have been due to antibiotic or EM supplementations. Antibiotic and EM supplementations had no effect on growth rate and FCR of unsexed broiler chickens aged one to 21 days. However, antibiotic supplementation improved live weights of unsexed broiler chickens aged 21 days. This might have been due to improved diet intake with antibiotic supplementation, even though antibiotic supplementation did not improve ME intake of the chickens. Unsexed broiler chickens on diets with EM supplementations of 30 or 50ml per litre of drinking water had similar live weights with those supplemented with antibiotics, suggesting that antibiotic supplementation can be substituted with EM supplementation without reducing live weight of the chickens. However, on a higher EM supplementation level of 100ml per litre of drinking water the chickens had lower live weights than those on antibiotic supplemented diets. This was despite the fact that broiler chickens supplemented with 50 or 100ml/l of water had higher ME intakes than those supplemented with antibiotics or not supplemented with anything. Further studies are recommended to determine why higher ME intakes did not have positive effects on live weight of the chickens.

Antibiotic and EM supplementations had similar effects on intake, growth and FCR of the chickens at the starter and finisher stages, indicating that either of these can be used in broiler chicken production. An effective microorganism supplementation level of 72.25ml per litre of drinking water is recommended because it optimized intake of unsexed broiler chickens aged one to 21 days.

Effective microorganism and antibiotic supplementations did not have effect on intake, ME intake, growth and live weight of male broiler chickens aged 22 to 42 days. This was regardless of the fact that antibiotic supplementation improved FCR of the chickens. It is, also, important to note that live weight of the chickens was improved with antibiotic supplementation at the starter stage and not at the finisher stage. There might be need to do further studies on this to determine factors that tend to differently affect the responses to antibiotic and EM supplementation, depending on the age of the chicken. Supplementing diets with antibiotics and EMs did not affect male broiler chicken meat tenderness, juiciness and flavour. Thus, supplementing diets with EMs would not affect the sensory attributes of meat, and possibly having no adverse effect on the demand for the meat. It is, thus, recommended that more research be done on the acceptability of meat from chickens supplemented with effective microorganisms. There were no antimicrobial and effective microorganism residues in the meat of the treated broiler chickens. Thus, it is safe to supplement the chickens with antimicrobials and effective microorganisms.

CHAPTER 6

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