Coated butyric acid and C 12 monoglyceride supplementation of broiler feed to improve performance and gut health

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By

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Declaration

I, Bakang Rebaone Letlole declare that the dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

B.R Letlole

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Abstract

Due to the ban of antibiotics across the world there is pressure in finding alternative feed additives that can be used for food animals. The aim of this study was to determine the effect of butyric acid (Novyrate C) and monoglyceride (FRA®C12), with and without the addition of antibiotics (Zinc Bacitracin 15%) on the performance and gut health of broiler chickens. The study was conducted in an environmentally controlled commercial broiler house. The house contained ninety-six pens in total, divided into two rows consisting of forty-eight pens each over the length of the house. A total of two thousand three hundred and four male Ross 308 broiler birds were randomly distributed throughout the pens, 24 birds per pen at a stocking density of 22 kg/m^2. All the birds received a typical South African maize-soya based diet throughout the trial. Dietary treatment combinations were implemented in a 3-phase feeding programme: Starter (0 to 14 d), Grower (14 to 28 d) and Finisher (28 to 35 d). This study was conducted using eight different dietary treatments. Treatment 1 was the positive control (Zinc Bacitracin 15% at 0.5 g/kg) and treatment 2 was the negative control (without the Zinc Bacitracin). Treatments 3, 4 and 5 consisted of Zinc Bacitracin together with either butyric acid (1 g/kg for starter, 0.75 g/kg for grower and 0.25 g/kg for finisher phase), mono-glyceride (1 g/kg) or a combination of the test products (butyric acid and monoglyceride). Treatments 6, 7 and 8 were the same as 3, 4 and 5 without the Zinc Bacitracin. Each treatment was repeated once within a block, resulting in 12 replications per treatment. The birds had *ad libitum* access to water and feed during the duration of the trial.

A weekly numerical difference was recorded for body weight (BW), feed intake (FI) and feed conversion ratio (FCR). Two chicks per pen were sacrificed at the ages 20 and 33 days and duodenum, jejunum and ileum samples were sectioned from the gut. Results showed that growth was not significantly different between the treatments.The feed intake showed no significant difference either, but the birds that were not supplemented with the Zinc Bacitracin showed a lower FI (3747 g) compared to the group with Zinc Bacitracin inclusion (3767 g) at 35 days of age. The group of birds supplemented with Zinc Bacitracin resulted in a significantly lower FCR ($P < 0.05$) compared to the group without AGP at 7 days of age. The cumulative FCR of birds supplemented with both butyric acid and monoglycerides without the Zinc Bacitracin was significantly lower (1.47) compared to the same group with Zinc Bacitracin (1.51) from 0-35 days ($P < 0.05$).

The viscera and wing weights expressed as a percentage of the carcass weight were significantly lower for birds that were supplemented with Zinc Bacitracin compared to the group of birds without AGP ($P < 0.05$), but no significant differences were reported for the thighs, drumsticks and breast weights relative to the carcass weight ($P < 0.05$) at 35 days of age. The supplementation of butyric acid, monoglycerides and their combination without AGP resulted in a significantly longer villi length in the duodenum, jejunum and ileum at 20 and 33 days. The combination of the two products resulted thus in a lower FI, FCR and increased villi height. The recommendation will be to use the products together as an alternative for antibiotic growth promoters in broiler feed.

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Chapter 1

Introduction and motivation

There is a greater need to stop emerging transboundary agriculture and food system threats, such as diseases and pests, in order for the farming systems to improve production (SAPA, 2013; FAO, 2017). The need for accelerated productivity growth is hindered by the deterioration of natural resources, the spread of transboundary pest and disease of plants and animals, some of which are becoming resistant to antimicrobials and the loss of biodiversity (Gullberg *et al.*, 2011; SAPA, 2013; FAO, 2017). In order to achieve sustainable production of nutritious and accessible food for the growing population, a healthy livestock should be maintained (FAO, 2017). In the past 25 years, there has been improvement in agricultural production and food security through implementing policies to promote intensification of agricultural production, and the sustainable management of forest resources (CDDEP, 2016; FAO, 2017). Animal diseases have a potential impact on human health and this is magnified by increasing levels of resistance in viruses, bacteria, parasites and fungi to antimicrobial drugs. Examples of these drugs include antibiotics, antifungals, antivirals, antimalarials and anthelmintics (Gullberg *et al.*, 2011; Llor & Bjerrum, 2014; FAO, 2017). Treatment, prophylaxis and growth promoters are the common uses of antibiotics in food-producing animals and to have a sustainable and economical animal industry (Moyane *et al.*, 2013). The small and large intestines of the chicken are populated by beneficial bacterial, which is referred to as microflora (Jacob & Pescatore, 2013). Gut commensal bacteria, including segmented filamentous bacteria and lactobacilli (Lee *et al.*, 2012), *Enterococcus, Escherichia, Campylobacter, Salmonella* and *Clostridium* (Marshall & Levy, 2011), play a significant role in the growth performance, immune status and health of commercial broiler chickens (Lee *et al.*, 2012).

Antimicrobial resistance (AMR) is a globally issue, and it results in limited ability to treat common infectious diseases which lead to prolonged illness, disability and death (CDDEP, 2016; FAO, 2017). Research indicated that about 700 000 people die of drug-resistant infection annually (FAO, 2017), and it is projected that by 2050 this will go up to 10 million people (O'Neil, 2016). The quantity of antibiotics utilised in animal production is extensive, and includes medication that is vital for humans, for example, 70% of the antibiotics defined as medically important for humans are used in animals (O'Neil, 2016). Many scientists see this as a threat to human health, animal health and food security, given that wide scale use of antibiotics encourages the development of resistance that can spread to affect humans and animals alike (CDDEP, 2016; O'Neil, 2016). The issue of antibiotic use in agriculture and its impact on drug resistance has been recognised by the World Health Organization (WHO), the UN's Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE) as part of its Global Action Plan, requires that countries that are members to develop a National Action Plans that can help tackle AMR which incorporate considerations of animal usage (CDDEP, 2016; O'Neil, 2016). There is growing pressure from consumers and investors on food

companies and restaurant chains to reduce unnecessary use of antibiotics in their supply chains (Marshall & Levy, 2011; O'Neil, 2016). This pressure from investors could play a crucial role in changing behaviours to address AMR (O'Neil, 2016). Antibiotics can reach the environment through three principal channels: animal waste, human waste and manufacturing waste (O'Neil, 2016). This will cause contamination of the soil, crops and water sources and lead to the development of drug resistance amongst the pathogens with which they interact (CDDEP, 2016; O'Neil, 2016). In South Africa, consumers want access to cheap animal protein with little regard to how it was produced and a small section of the population has a high net worth (Roosendaal, 2011).

The negative impact of antibiotic usage on human health may differ between countries and regions, influenced by the interaction between human populations, use of land, contaminated water sources, national and international trade, animal demography, national policies that deal with production, trade, food security and animal health (Moyane *et al.*, 2013; CDDEP, 2016). The antibiotic resistance involves different sectors, such as medicine, veterinary medicine, animal husbandry, agriculture, environment and trade (Moyane *et al.*, 2013). A lot more research is done on the use of antibiotic in developed countries than in developing countries (Marshall & Levy, 2011; Moyane *et al.*, 2013). The utilisation of antibiotics is suspected to be fueling the antibiotic resistance in species of bacteria which are common to humans and animals (Gullberg *et al.*, 2011; CDDEP, 2016). Data on the volume of antibiotics utilised in food animal production in South Africa is limited and not well documented and information regarding the patterns of antibiotic usage in food animals is scarce (Moyane *et al.*, 2013). This is because in most cases overcrowded and unhygienic conditions of industrial animal farming result in the spread and emergence of microbes, so one would suggest to improve these conditions to reduce the need of prophylactic use of antibiotics (Moyane *et al.*, 2013; SAPA, 2013).

The antibiotics used as growth promoters are utilised on animals at sub-therapeutical concentrations without veterinary prescriptions or administered for long periods of time (Carlet *et al.*, 2012). A number of clinical and environmental data suggest that the rate of antimicrobial resistance is high in South Africa (Moyane *et al.*, 2013). Penicillin resistance in South Africa remains mainly intermediate in level, with a low prevalence of fully resistant isolates (Crowther-Gibson *et al.*, 2011). In recent years, improper use of tetracycline antibiotics has resulted in presence of residues in edible animal meat. These residues resulted in harmful effects on consumer's health, such as allergic reactions and gastrointestinal disturbance (Javadi, 2011; Marshall & Levy, 2011). The analysis of the antimicrobial residues in meat was done using screening methods sensitive at antibiotic concentrations close to the maximum residue limit (MRL), the EU established limits for certain antibiotics (Timbermont *et al.*, 2010; Javadi, 2011). Microbial inhibitions assays are widely used today as a method for the detecting antibiotic residues in food (Pikkemaat, 2009; Gondová *et al.*, 2014). Residues of a range of veterinary drugs have varying degrees of stability during cooking and the cooking influences the levels of risk posed by such residues (Pikkemaat, 2009; Gondová *et al.*,

2014). With most of the food-producing animals being cooked before consumption, more research is needed to accurately determine consumer exposure to antibiotics (Javadi, 2011). Communicable diseases are the leading cause of death in African countries in the past 5 years (Essack *et al.*, 2016).

Since 1992 there has been a steady increase in the production of chicken meat and South African poultry industry continues to demonstrate this by providing 64.4% of locally produced animal protein (excluding milk) consumed in the country (Palmer & Ainslie, 2006; SAPA, 2016). The per capita consumption of poultry meat in 2017 was 38.08 kg (SAPA, 2017). This is due to that fact that poultry meat is by far the cheapest form of animal protein. On a rand per kilogramme basis, broiler meat and eggs remain the most affordable form of animal protein sources (SAPA, 2016). The average selling price for class A2, A3 and C2 beef was R46.07, R46.23and R39.95 per kg, respectively (DAFF, 2018; SAPA, 2018). With the South African economy currently in recession it will be difficult for consumers to afford beef, mutton or even pork meat. The economy moved into recession with quarter (Q1) 2017 down by 0.7% following a 0.3% contraction in the Q4 of 2016 (STAT SA, 2017). Despite significant economic growth globally since 2000, the average income of people living in Africa is about 5 percent of the average income of people living in the United States (FAO, 2017). This will have an impact on the food prices and the spending patterns of the South African consumers. The poultry meat consumption results from the broiler industry's response to demands of consumers and food service operators for value-added, branded and convenient products (Roosendaal, 2011). Therefore, it is wise to try and improve the production efficiency of poultry industry, in order to meet demands and feed the nation.

South African poultry industry uses antibiotic growth promoters (AGPs) therapeutically and prophylactically as they are cost effective in improving performance, stabilising intestinal microbial flora and preventing intestinal pathogens (Huyghebaert *et al.*, 2011; El-Ghany *et al.*, 2016). Information on the amount of antibiotics utilised and their patterns of consumption in animal food production is not well documented in South Africa (Henton *et al.*, 2011; Eager *et al.*, 2012). The AGPs ban in the European Union caused re-occurrence of necrotic enteritis (NE) in broilers (Kaczmarek *et al.*, 2016; Al-Baadani *et al.*, 2016; Kaldhusdal *et al.*, 2016; Özcan *et al.*, 2016). The maintenance requirements of the birds increase when the bird faces immune stress (Kelebemang, 2005; Sugiharto, 2016). This is due to the fact that the bird needs to produce antibodies when the immune system is challenged and this process requires protein and energy (Kelebemang, 2005; Sugiharto, 2016; Tallentire *et al.*, 2016). Studies on the impact of nontherapeutic antibiotic use on resistance in land food animals have focused primarily on *Enterococcus*, *Escherichia*, and *Campylobacter* and less on *Salmonella* and *Clostridium* (Marshall & Levy, 2011). The Food and Drug Administration states that antibiotic drugs in food producing animals cause microbes to become resistant, making human diseases harder to treat (Lieberman *et al.*, 2006). Food service companies have policies that prohibit the purchase of meats from farms that use AGPs important in human medication (Wegener, 2003; Dibner & Richard, 2005). The poultry industry needs AGP alternatives

to ensure optimal performances and gut health (Dahiya *et al.*, 2006; Castanon, 2007; Cooper & Songer, 2009; Kaldhusdal *et al.*, 2016; Van Immerseel *et al.*, 2016). Developing countries are faced with the challenge of meeting the high demand of food production that is free of antibiotics and producing safe and sustainable feed (Missaglia, 2016).

Antibiotic growth promoters elicit antibacterial action that improves performance in various ways such as decreasing the occurrence of subclinical infections, improving absorption due to thinning of the intestinal wall and reducing the growth-depressing metabolites produced by Gram-positive bacteria (Dibner & Richard, 2005; Huyghebaert *et al.*, 2011; Khosravinia, 2015). Research indicates at least 10% of the total apparent metabolisable energy (AME) was due to the energy cost of the gut microflora in broilers (Kleyn, 2013), which can be lowered with the inclusion of AGPs in the diet. When the immune system is under stress, nutrients are diverted from growth to facilitate the immune response, and maintenance requirements increase in order to repair damaged tissues (Kleyn, 2013). If the lining of the GIT is damaged, the true genetic potential cannot be exploited due to disease burden which will affect growth and FCR (Kleyn, 2013).

Butyric acid is a short chain aliphatic carboxylic fatty acid, found naturally in plants, fruits, vegetables and dairy products (Dolan *et al.*, 2016). It is also produced in the gastrointestinal tract of mammals from fermentation of fibre or starch by resident bacteria (Dolan *et al.*, 2016). It showed potent anti-inflammatory effect, affected the immune system positively and limited salmonella colonisation in studies involving piglets and poultry (Abdelqader & Al-Fatafah, 2015). Monolaurin is a monoester formed from lauric acid which is a medium-chain fatty acid (Lieberman *et al.*, 2006). Monolauric is more biologically active than lauric acid (Lieberman *et al.*, 2006), and has antiviral, antibacterial and antifungal properties (Lieberman *et al.*, 2006). This is achieved by solubilising the lipids and phospholipids of the enveloped pathogen causing the disintegration of its envelope (Lieberman *et al.*, 2006). Antimicrobial effects related to the interference of signal transduction in cell replication (Lieberman *et al.*, 2006). Lauric acid and 1-monolaurin have antibacterial properties against gram-positive bacteria (Lieberman *et al.*, 2006; Batovska *et al.*, 2009). Monolaurin and butyric acid increased the length and surface area of villi in the ileum and jejunum (Timbermont *et al.*, 2010; Zeitz *et al.*, 2015; Al-Baadani *et al.*, 2016), indicating an increase in cell proliferation (Abdelqader & Al-Fatafah, 2015). Research showed improved final body weights and FCR when supplementing broilers with butyric acid and monolaurin (Timbermont *et al.*, 2010; Qaisrani *et al.*, 2015; Zeitz *et al.*, 2015). Little research is available on butyric acid and monolaurin in South African conditions as AGP alternatives. This trial was done to observe the impact of removing AGPs from broiler feed and replacing the AGPs with butyric acid and monolaurin products, on performances of the birds. It also monitored any incidence ofnecrotic enteritis (NE) and the impact the two alternative products had on gut health and morphology of the broiler chicken.The null hypothesis (H_0) of this study was that the two feed additives (butyric acid and monolaurin) will not improve performance and health of broilers under commercial rearing conditions without the use of AGPs in the feed. The

alternative hypothesis (H_1) was that the two feed additives will improve performance and health of broilers under commercial rearing conditions without the use of AGPs in the feed. A second null hypothesis was that the two feed additives will not reduce the occurrence of necrotic enteritis under commercial rearing conditions without the use of AGPs in the feed. The alternative hypothesis was that the two feed additives will reduce the occurrence of necrotic enteritis under commercial rearing conditions without the use of AGPs in the feed.

Chapter 2
Literature Review

2.1 Introduction

Animal diseases have the potential to impact human health. This potential is magnified by increasing levels of resistance in bacteria, parasites, viruses and fungi to antimicrobial drugs, examples include antibiotics, antifungals, antivirals, antimalarials and anthelmintics (FAO, 2017). The risk concerning the development of antimicrobial resistance led the European Union to ban the application of antibiotics as growth promoters since 2006, which was followed by North America and other parts of the world (Sugiharto, 2016).

Antibiotics are commonly used in food-producing animals for treatment and prophylaxis of disease and as growth promoters, and have played an important role in sustainable and economical animal industry (Moyane *et al.*, 2013). The Food and Drug Administration states that antibiotic drugs in food producing animals cause microbes to become resistant, making human diseases harder to treat (Lieberman *et al.*, 2006). Food service companies have policies that prohibit the purchase of meats from farms that use antibiotic growth promoters (AGPs) that are important in human medication (Wegener, 2003; Dibner & Richard, 2005). The livestock industry needs to find AGP alternatives as the global world is moving away from the use of antibiotics in food producing animals (Castanon, 2007; Cooper & Songer, 2009; Kaldhusdal *et al.*, 2016; Van Immerseel *et al.*, 2016).

2.2 Poultry industry in South Africa

The poultry industry contributes approximately 18% of its share of gross domestic products to the agricultural sector and consists of three branches, namely the day-old chick supply industry, the broiler industry and the egg industry (DAFF, 2016; SAPA, 2016). The gross value of primary agricultural production from poultry meat came up to R44.04 billion, reflecting an annual increase of 20.1% (SAPA, 2017). Broiler meat accounted for more than 70% of the poultry industry (Mkhabela & Nyhodo, 2011) and it remains the most affordable source of animal protein at R22.66 per kg compared to beef average selling prices for classes A2, A3 and C2 were R46.07, R46.23 and R39.95 per kg, respectively (Mkhabela & Nyhodo, 2011; DAFF, 2018; SAPA, 2018). The per capita consumption of poultry meat was 38.08 kg in 2017 compared to 38.38 kg in 2016 (SAPA, 2017). Challenges that the poultry industry are faced with include high feed cost, droughts, imports of frozen broiler meat, new brining regulations, the fluctuation of the South African rand and in the near future, meeting the high demand of sustainable production of safe food free of antibiotics (Missaglia, 2016; SAPA, 2016). South African poultry industry uses antibiotic growth promoters (AGPs) therapeutically and prophylactically as they are cost effective in improving performance, stabilising intestinal microbial flora and preventing intestinal pathogens (Huyghebaert *et al.*, 2011; El-Ghany *et al.*, 2016). However, information on the volume of antibiotics utilised in animal production is limited

in South Africa and is lacking in terms of the patterns of antibiotic consumption in food animals (Henton *et al.*, 2011; Eager *et al.*, 2012). The Department of Health has urged the poultry producers, veterinarians and pharmaceutical industries to join hands with the Department of Agriculture, Forestry and Fishery and to come up with proactive actions that will help address the antimicrobial usage in animal production. The process is said to begin with a review of the use of antimicrobials in animal feeds and additives which will take three years (SAPA, 2016).

2.3 Modern broiler production

The performance of the modern broiler is influenced by a number of factors such as management, nutrition, environment, health and parent flock and in order to achieve the genetic potential of the bird all of these aspects should be correct (Kidd, 2004; Kelebemang, 2005; Sugiharto, 2016; Tallentire *et al.*, 2016). Management of the bird includes lighting programme, temperature monitoring, stocking density, litter quality, air quality and humidity in the broiler house (Kidd, 2004; Tallentire *et al.*, 2016). The nutrition involves the quality of the raw materials used in the feed, formulating a diet that will meet the nutrient requirements of the birds, pellet quality, and reducing anti-nutritional factors in the feed (Kidd, 2004; Kelebemang, 2005; McKay, 2009; Tallentire *et al.*, 2016). The health of the bird can be easily affected by the air quality, which can cause respiratory problems and metabolic diseases while poor litter quality can cause footpad and breast lesions (Kidd, 2004; Skinner-Noble & Teeter, 2004; Tallentire *et al.*, 2016). High loads of pathogens can cause nutrient deficiencies and diseases (Kidd, 2004; Skinner-Noble & Teeter, 2004; Tallentire *et al.*, 2016). Different measures are used to evaluate the performance of a flock of broilers when reared commercially. These include growth rate, days to market, mortality rate and feed efficiency (Kelebemang, 2005). Faster growth rate of modern broilers contributes to the energy efficiency of the birds; their term weight is reached in a shorter period and relatively less energy is required for metabolic heat production and more energy is directed towards growth (Kelebemang, 2005; Tallentire *et al.*, 2016). The efficiency of growth is measured by the feed conversion ratio (FCR), which is a number of units of feed required to produce a unit of chicken or calculated as feed intake divided by weight gain, and is regarded as the most costly expense in broiler production (Skinner-Noble & Teeter, 2004; Kelebemang, 2005). The lower the FCR value the more efficient the flock is with the feed supplied. Some publications use the inverse known as feed conversion efficiency (FCE), which is the unit of chicken produced per kilogram of feed (Skinner-Noble & Teeter, 2004; Kelebemang, 2005). The feed efficiency is better in the first weeks of broiler production and then declines with increased target market weight (Taylor-Pickard & Spring, 2008; Tallentire *et al.*, 2016). Feed efficiency of broiler birds has changed over the years through genetic selection for growth rate (Kidd, 2004; Kelebemang, 2005; Taylor-Pickard & Spring, 2008; McKay, 2009). It is estimated that at least 85% of the improvement in performance is due to genetic changes (McKay, 2009). In order for the birds to grow, energy is required both as a part of the tissue and as fuel to carry out the synthesis

(Sugiharto, 2016). The modern bird will attempt to consume enough feed to meet their metabolic energy requirement, which is dependent on their energy requirements for body maintenance, growth, development and production (Ferket & Gernat, 2006; Lopez & Leeson, 2008). Maintenance requirement is influenced by various factors such as the general health status of the bird, degree of mobility and body heat loss (Sainsbury, 1980; Ferket & Gernat, 2006; Lopez & Leeson, 2008).

Apart from growth rate, another genetic strategy to improve the efficiency of the modern broiler includes improving the digestive efficiency. This involves selection for a larger gizzard and a greater intestinal villi size (McKay, 2009; Tallentire *et al.*, 2016). An increase in the gizzard size is more ideal when the physiological and chemical properties of the grains in the diet make the feed difficult for the bird to break down (McKay, 2009; Tallentire *et al.*, 2016). However, in the gut an increase in the size and length of the villi translates to a larger surface area and improved nutrient absorption (McKay, 2009; Tallentire *et al.*, 2016). Maintaining a good gut health is vital for ensuring the growth, health and welfare of the bird. When gut health is affected, digestion and nutrient absorption are compromised, which has a detrimental impact on feed conversion leading to economic losses and a greater susceptibility to infections. The gut inhabitants include a community of microorganisms, i.e bacteria, fungi, protozoa and viruses. Unfortunately, genetic selection has not led to the ultimate potential of the digestive efficiency broilers are expected to have (Tallentire *et al.*, 2016). Factors such as bird health may limit the extend to which the farmer can reap the benefits of the improved efficiency (Kelebemang, 2005; Taylor-Pickard & Spring, 2008). General health of a flock has a significant impact on the feed conversion of the birds (Collett, 2009; Sugiharto, 2016). This can be due to reduction in feed intake and more feed directed towards maintenance than growth. The bird needs to produce antibodies when the immune system is challenged and this process requires protein and energy (Sugiharto, 2016). The production of immunoglobulins results in a small nutrient cost; good antibody concentration levels indicate a more efficient capacity to resist disease by humoral immune responses instead of an active inflammatory response (Ferket, 2004; Collett, 2009; McDonald *et al.*, 2011). Collett (2009) reported a nutritional cost of approximately 0.5% of body mass from the synthesis of leukocytes when responding to antigen stimulation. Selection for disease resistance is challenging to implement, due to the fact that it involves sib or progeny testing and challenging these birds with pathogens (Kleyn, 2013). Broiler health management is formed by inheriting disease resistant genes from parent flocks, vaccination, medication programmes and biosecurity (Taylor-Pickard & Spring, 2008; McDonald *et al.*, 2011).

Poultry production intensification and the ban of antibiotics have increased the financial risk of diseases such as necrotic enteritis, and these diseases manifest as a decrease in production efficiency (Collett, 2009; Huyghebaert *et al.*, 2011; Kaczmarek *et al.*, 2015; Özcan *et al.*, 2016). Disease causes functional derangement of normal metabolic and homeostatic processes, which results in a decline in production (Sainsbury, 1980; Collett, 2009). Diseases which also result in inflammation are associated with lower feed intake, which causes a greater need to mobilise skeletal muscle protein (Obled, 2003;

Taylor-Pickard & Spring, 2008), leading to rapid transition into negative nitrogen balance (Collett, 2009). The inflammatory response to a disease challenge begins with acute-phase protein synthesis in the liver and is followed by several behavioural, hormonal and metabolic responses (Collett, 2009; Sugiharto, 2016). This causes the redirection of amino acids needed towards pathways involved in the host defense system (Obled, 2003; Taylor-Pickard & Spring, 2008; Sugiharto, 2016). Amino acids that are required for the synthesis of compounds and proteins used in host defense differ from amino acids produced from muscle proteolysis, which leads to unrestricted mobilisation of muscle proteins to supply the required quantity of the most limiting amino acid (Obled, 2003; Collett, 2009).

2.4 Gastrointestinal tract anatomy and digestive physiology in the chicken

2.4.1 Mouth, oesophagus and crop

The digestive tract includes the beak, mouth, salivary glands, tongue, pharynx, oesophagus, crop, proventriculus, gizzard, intestines, caeca, colon and cloaca (Card & Nesheim, 1972; Klasing, 1998; Grist, 2004; Jacob & Pescatore, 2013). The oral cavity or the mouth of the chicken consists of a beak with a palate and a choanal slit on the dorsal palate, with no teeth and lips (Kleyn, 2013). It is lined with stratified squamous epithelium (Klasing, 1998; Aughey & Frye, 2010). The surface of the tongue is covered by thick stratified squamous epithelium (Klasing, 1998; Aughey & Frye, 2010). The tongue has variable shapes with the pharynx beginning caudally to it (Frandson *et al.*, 2009). The oesophagus follows within the neck and it dilates into a feature called the crop or ingluvies (Grist, 2004; Frandson *et al.*, 2009; Jacob & Pescatore, 2013) or diverticulum at its point of entry into the thoracic cavity (Klasing, 1998). The lining of the oesophagus contains stratified squamous non-keratinised epithelium and numerous mucosal glands in the connective tissue lamina propria (Aughey & Frye, 2010; Kleyn, 2013). The crop is a simple diverticulum of the oesophagus (Card & Nesheim, 1972; Klasing, 1998; Aughey & Frye, 2010). Both the oesophagus and the crop are lined by a keratinised stratified squamous epithelium (Frandson *et al.*, 2009). The function of the crop is to provide a temporary storage space after swallowing (Klasing, 1998; Frandson *et al.*, 2009; Jacob & Pescatore, 2013; Svihus, 2014).

Food consumed by birds is taken in via the mouth and mixed with saliva to ensure lubrication (Grist, 2004). Amylase and ptyalin are present in the saliva and in scrapings from the mouth and oesophagus (Turk, 1982; Klasing, 1998; Jacob & Pescatore, 2013). The food is swallowed in a ball called a bolus, and the bolus travels down the oesophagus due to the gravity and wave-like contraction of muscles also known as peristalsis (Grist, 2004). There is little or no conversion of starch into sugar in the crop, so saliva plays a minor role in enzymatic digestion (Card & Nesheim, 1972; Klasing, 1998). Proteolytic and amylolytic enzymes may also be found in the crop contents (McDonald *et al.*, 2011; Kleyn, 2013). Food has been known to stay in the crop for up to 20 hours, and about 25% of the starch in the crop can be hydrolysed to sugar in 2 hours (Turk, 1982; McDonald *et al.*, 2011; Kleyn,

2013). Most of this sugar is absorbed directly from the crop, whereas some is used by the bacteria to produce ethyl alcohol, lactic acid and acetic acid which give the crop a sour smell (Turk, 1982; Klasing, 1998). The acid production contributes about 3 percent of crop content and causes a drop in the pH of the crop content (Turk, 1982; Kleyn, 2013). These organic acids are absorbed and used as a source of energy by the birds (Turk, 1982; McDonald *et al.*, 2011).

2.4.2 Proventriculus, gizzard and small intestine

The oesophagus ends in the proventriculus, also known as the glandular stomach or the true stomach (Card & Nesheim, 1972; Klasing, 1998; Aughey & Frye, 2010). It is oval in shape (Svihus, 2014) and joined to the muscular stomach via a narrowing known as the isthmus (Kleyn, 2013). The gastric epithelium of the proventriculus is simple columnar (Aughey & Frye, 2010). The lining of the proventriculus contains papillae and multi-lobular glands which function to provide pepsin and hydrochloric acid for enzymatic digestion (Card & Nesheim, 1972; Grist, 2004; Frandson *et al.*, 2009; Kleyn, 2013; Svihus, 2014). Immediately upon ingestion of food, there is a reflex stimulation of the vagus nerves to the gastric mucosa, which initiates the secretion of gastric juice into the proventriculus (Klasing, 1998; Grist, 2004). The gastric juice consists of proteinases, hydrochloric acid and mucin (Card & Nesheim, 1972; Grist, 2004; Jacob & Pescatore, 2013). A molecule called pepsinogen is converted to pepsin under acidic conditions, which breaks protein chains through the hydrolysis of the peptide links. The hydrochloric acid also functions to denature the foodstuff ingested by the chicken. Mucus comprises of glycoproteins and mucopolysaccharides. It is a gel that forms a protective layer of epithelial cells of the proventriculus and its primary function is to stabilise the micro-environment of the mucosal surface and protects the gastric mucosa against the effects of acid and pepsin (Grist, 2004; McDonald *et al.*, 2011; Kleyn, 2013).

The second chamber that follows is called the gizzard also known as the muscular stomach or ventriculus muscularis (Klasing, 1998; Grist, 2004; Frandson *et al.*, 2009). The inside of the gizzard is lined with secretory product of the mucosal glands, which solidifies at the surface to form a hard cuticle of koilin (Frandson *et al.*, 2009; Aughey & Frye, 2010). The main function of the gizzard is to grind the food into fine paste and through rhythmic contractions pass it into the duodenum (Card & Nesheim, 1972; McDonald *et al.*, 2011; Kleyn, 2013). The internal surface of the gizzard is covered with a hard, yellowish, ridged lining called the cutica gastrica which functions to grind the food. The development of the gizzard is important because it governs many of the physiological aspects of the GIT. These aspects include the regulation of the GIT motility, the regulation of digesta flow and gastroduodenal refluxes, enhancement of digestive secretions such as HCl, bile acid and endogenous enzymes and synchronisation of the digestive and absorptive processes. The resultant paste is then passed to the small intestine (Grist, 2004; McDonald *et al.*, 2011; Kleyn, 2013).

The small intestine begins with the duodenum which forms a distinctive loop with the pancreas sandwiched between descending and ascending parts (Kleyn, 2013). The longest segment of the small intestine is the jejunum and it retains a remnant of the embryonic connection to the yolk sac,

Meckel's diverticulum (Grist, 2004; Frandson *et al.*, 2009; Svihus, 2014). The short ileum terminates at the large intestine, a point distinguished by the presence of paired caeca (Frandson *et al.*, 2009). The small intestine has diffuse and nodular lymphatic tissue present in the lamina propria and the submucosa (Aughey & Frye, 2010). The duodenum is lined by long villi, whereas the villi in the jejunum are shorter and the wall contains large amounts of lymphoid tissue. A peculiarity of the jejunum is a sphincter found at the jejunal-rectal junction (Kleyn, 2013).

Food from the gizzard to the duodenum causes a rise in the pH and this pH of 6.4 is maintained throughout the gut (McDonald *et al.*, 2011; Kleyn, 2013). The duodenal mucus is suggested to have a buffering effect because the secretions from the pancreas and liver only get added from the junction between the duodenum and small intestine (Grist, 2004; McDonald *et al.*, 2011; Kleyn, 2013). The liver secretes bile, which is added to the ingesta via two bile ducts (Card & Nesheim, 1972). The bile is stored and concentrated in the right enlarged lobe of the liver called the gallbladder (Card & Nesheim, 1972). It is a detergent that is vital in the breakdown of lipids and absorption of fat-solule vitamins (Grist, 2004; Jacob & Pescatore, 2013). The bile contains bile pigments, bile salts and an amylase and lipase factor which accelerates the action of the pancreatic enzyme lipase (McDonald *et al.*, 2011; Kleyn, 2013). The pancreas secretes the inactive enzyme, trypinogen, which will only be able to split protein into peptides after activation by the enterkinase of the intestine (Card & Nesheim, 1972; McDonald *et al.*, 2011; Kleyn, 2013). The pancreatic juice also contains sodium bicarbonate which neutralises the acidity of the food exiting the stomach and secretes insulin, which affects the metabolism of certain compounds and glucogen (Card & Nesheim, 1972; Grist, 2004). The intestine secretes the enzymes saccharases that hydrolyse sucrose to glucose and fructose and maltase, which converts maltose to glucose (McDonald *et al.*, 2011; Kleyn, 2013).

2.4.3 Large intestine, caeca, cloaca and microflora

The large intestine consists of a pair of caeca that are joined to the colon, which continues to the cloaca (Card & Nesheim, 1972; Grist, 2004; Jacob & Pescatore, 2013). The surface of the large intestine has no villi (Kleyn, 2013) and absorbs water and electrolyte, but no organic nutrients (McDonald *et al.*, 2011; Jacob & Pescatore, 2013; Kleyn, 2013). The epithelium in the caeca consists of simple columnar with mucous cells, with abundant lymphatic tissue forming the caecal tonsils in the narrow proximal part of the caecum (Aughey & Frye, 2010). The caeca is the plural of caecum, two blind pouches located at the junction of the small and large intestine (Jacob & Pescatore, 2013). A vital function of the caeca is the fermentation of coarse materials, which produce several fatty acids as well as eight B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid and vitamin B_{12}) (Jacob & Pescatore, 2013). The cloaca is an opening where the large intestine, reproductive and the urinary tract empties into (Card & Nesheim, 1972; Grist, 2004; Jacob & Pescatore, 2013). The cloaca is subdivided by transverse folds into three portions, namely the coprodeum, urodeum and the proctodeum (Grist, 2004; Samuelson, 2007). Tall columnar epithelium

with a variable number of mucus-secreting cells forms the lining of the cloaca (Aughey & Frye, 2010). In the cloaca, waste from the urinary system and the disgestive system mix together due to antiperistaltic movement (Card & Nesheim, 1972; Jacob & Pescatore, 2013). Antiperistalsis is a prominent pattern of motility in the colon of the bird. The histologic features of the liver, gallbladder and exocrine pancreas of the bird are not significantly different from those of the same organs in mammals (Samuelson, 2007).

The chick hatches with a sterile gut (Pelicano *et al.*, 2005; Aranda-Olmedo & Rubio, 2016). Upon hatching, a variety of micro-organisms colonise the intestinal tract of the chicken (Pelicano *et al.*, 2005; Lee *et al.*, 2011; Aranda-Olmedo & Rubio, 2016). The small intestine becomes fully established by microflora at two weeks and by 6-7 weeks the caecum is predominated by the genus *Clostridium* in healthy chickens (Aranda-Olmedo & Rubio, 2016). The small and large intestines are populated by beneficial microorganisms, also referred to as microbiome (Pelicano *et al.*, 2005; Jacob & Pescatore, 2013; Aranda-Olmedo & Rubio, 2016). Chamber & Gong (2011) define the intestinal microflora as a complex mixture of bacterial population that colonises a certain area of the GIT in animal hosts that have not been affected by medical or experimental intervention or disease (Pelicano *et al.*, 2005). The major microbial groups in chickens include *Bacteriodes, Clostridium, Bifidobacterium, Enterobacteriacea, Eubacterium, Lactobacillus, Fusobacterium, Peptostreptococcus, Propionibacterium* and *Streptococcus* (Forder *et al.*, 2007; Gaggia *et al.*, 2010; Torok *et al.*, 2011; Aranda-Olmedo & Rubio, 2016). The intestinal ecosystem consists of an estimate of 100 trillion non-pathogenic bacteria representing upwards of 500-1000 different bacterial species (Heres *et al.*, 2004; Lee *et al.*, 2011; Torok *et al.*, 2011; Aranda-Olmedo & Rubio, 2016). These microbes are essential for the health and the well-being of their host animal (Pelicano *et al.*, 2005; Torok *et al.*, 2011; Stanton, 2013). Intestinal microbiota has a significant influence on the host metabolism and physiology, such as metabolic homeostasis, angiogenesis, obesity, immune function and brain development (Pelicano *et al.*, 2005; Aranda-Olmedo & Rubio, 2016; Han *et al.*, 2016). Gut commensal bacteria play a vital role in the health, immune status and growth performance of commercial broiler chickens (Uni *et al.*, 2003; Heres *et al.*, 2004; Pelicano *et al.*, 2005; Lee *et al.*, 2011; Aranda-Olmedo & Rubio, 2016).

The beneficial bacterial communities in the birds play a significant role in growth and development of the GIT, influencing the production of bile acids and digestive enzymes and consequently affecting nutrient digestion and absorption (Baurhoo *et al.*, 2007; Brümmer *et al.*, 2010; Ranjitkar *et al.*, 2016). Additionally, they stimulate gut immune functions and stop colonisation of the gut with pathogenic bacteria via competitive exclusion and production of bacteriocins (Uni *et al.*, 2003; Sunkara *et al.*, 2011; Zhang & Sunkara, 2014; Ranjitkar *et al.*, 2016; Awad *et al.*, 2017). The GIT is the largest lymphoid tissue in the body and the way in which it develops has a direct impact on the immune system (Pelicano *et al.*, 2005; Allen *et al.*, 2013; Aranda-Olmedo & Rubio, 2016). Shifts in the microbiota can affect morphology of the gut wall and also induce immune reactions that have

effects on energy expenses of the host (Pelicano *et al.*, 2005; Huyghebaert *et al.*, 2011; Aranda-Olmedo & Rubio, 2016). By using 16S rRNA-analysis it was found that the gut bacterial population is influenced by the diet, host phylogeny and gut morphology (Gaggia *et al.*, 2010; Torok *et al.*, 2011; Lee *et al.*, 2012; Aranda-Olmedo & Rubio, 2016). A healthy gut will consist of a balance of beneficial and non-beneficial bacteria and this will maximise bird performance (Pelicano *et al.*, 2005; Sugiharto, 2016). The effects of a disturbance in the GIT microflora cause the bird to be prone to diseases such as diarrhoea, and this will decrease production. The GIT bacteria need nutrients to survive and compete with the host for nutrients (Pelicano *et al.*, 2005; Aranda-Olmedo & Rubio, 2016). Decrease in production involves high mortality rates, lower feed efficiency and poor growth rates (Pelicano *et al.*, 2005; Kabir, 2009; Lee *et al.*, 2012; Aranda-Olmedo & Rubio, 2016).

High numbers of *Lactobacilli* can depress broiler growth because of nutrient uptake competition or impaired fat absorption due to bile acid de-conjugation (Pelicano *et al.*, 2005; Bjerrum *et al.*, 2006; Yin *et al.*, 2010; Torok *et al.*, 2011). *Streptococcus faecium* and *Clostridium perfringes* can also cause growth depression in chickens if high quantities become dominant in the gut (Pelicano *et al.*, 2005; Bjerrum *et al.*, 2006; Yin *et al.*, 2010; Aranda-Olmedo & Rubio, 2016).

2.4.4 Gut health

Gut health depends on the maintenance of the stability between the host, the intestinal microbiota, the intestinal environment and dietary compounds. Factors that affect gut health include enteric diseases, feed form and excessive levels of nutrients, environmental stress, biosecurity, appetite and anti-nutritional factors (Baurboo *et al.*, 2007; Yin *et al.*, 2010). Gut health affects growth through feed digestion, nutrient absorption and protein and energy usage (Collett, 2009). Enteric diseases result in pathogens reducing efficiency of digestion and absorption of nutrients in the gut (Brümmer *et al.*, 2010; Yin *et al.*, 2010; Abid *et al.*, 2016). Due to intensive breeding in the modern chicken for eggs and growth rate in layers and broilers the digestive tract has adapted to tremendous changes. These changes resulted in higher production rate and higher feed intake which could lead to a vulnerable digestive tract to impaired functionality (Yin *et al.*, 2010; Svihus, 2014).

The intestinal barrier contains a variation of cells, of which the enterocytes are the most abundant. The space between epithelial cells is bound by tight junction proteins and this regulates the permeability of the intestinal barrier (Uni *et al.*, 2003; Smimov *et al.*, 2006; Forder *et al.*, 2007; Yin *et al.*, 2010; Lee *et al.*, 2012). Epithelial cells are held tightly together by intercellular junctional complexes that control the passage of ions and molecules via the para-cellular pathway (Chiba *et al.*, 2008; Awad *et al.*, 2017). Awad *et al.* (2017) defines tight junctions (TJs) as multi-protein complexes that join cells of the same tissue together and form channels that allow permeation between the cells, resulting in epithelial surfaces of different tightness. The vital components of tight junction proteins are occluding, tricellulin and claudin (Chiba *et al.*, 2008; Peng *et al.*, 2009; Awad *et al.*, 2017). These transmembrane proteins contribute to the semi-permeable barrier; some examples include junctional adhesion molecules (Jams), the coxsackie virus and adenovirus receptor (CAR) (Awad *et al.*, 2017).

The cytosolic proteins bind membrane components to the actin cytoskeleton and take part in signalling between TJs and cell nucleus (Peng *et al.*, 2009; Awad *et al.*, 2017). The claudin protein family creates a seal that regulates paracellular transport in the intestinal epithelium (Chiba *et al.*, 2008; Awad *et al.*, 2017). Literature indicates that claudin- 1, 3, 4, 5, 7 and 19 are pore-sealing claudins and claudin-2 and 15 are considered as pore-forming claudins (Chiba *et al.*, 2008; Awad *et al.*, 2017). Claudin-2 and 15 can form para-cellular anion/cation pores as well as water channels, allowing them to reduce epithelial tightness and to increase solute permeability by allowing the passage of sodium ions (Chiba *et al.*, 2008; Awad *et al.*, 2017). The tight junctions are controlled in their molecular composition, ultrastructure and function by intracellular proteins and the cytoskeleton (Peng *et al.*, 2009; Awad *et al.*, 2017). They are involved in the physiological function of epithelial cells (Chiba *et al.*, 2008). The permeability of the gut can be induced by modulation of TJs (up or down regulation), relocation of TJs or cytokine and hydrogen peroxide-induced decrease in trans-epithelial tissue resistance (Chiba *et al.*, 2008; Peng *et al.*, 2009; Awad *et al.*, 2017). Permeability of tight junction is determined by measuring the trans-epithelial electrical resistance (Baurhoo *et al.*, 2007; Awad *et al.*, 2017). The gastro intestinal tract is an integral part of the immune system due to its size (Baurhoo *et al.*, 2007; Yegani & Korver, 2008; Yin *et al.*, 2010).Tight junctions consist of proteins that mediate adhesion and form a paracellular diffusion barrier (Lee *et al.*, 2012; Awad *et al.*, 2017). The intestine has been shown to have a number of functions, such as absorption of nutrients and acting as a barrier that stops the invasion of antigens and pathogenic bacteria into the mucosal tissue (Deplancke & Gaskins, 2001; Smimov *et al.*, 2006; Yin *et al.*, 2010; Lee *et al.*, 2012). Stress to the modern birds from systemic changes leads to loss of gut barrier integrity (Baurhoo *et al.*, 2007; Brümmer *et al.*, 2010; Sunkara *et al.*, 2011). Decreased tight junction integrity greatly increases ion conductance across the para-cellular route compared to the transcellular route, which leads to a concept termed leaky gut. This gives pathogens and endotoxins the ability to access the whole body including vital organs (Awad *et al.*, 2017). When the integrity is affected it results in reduced digestive capacity, less absorption surface area for nutrients, dysbacteriosis (microbial imbalance), loss of intestinal barrier function, poor growth, low feed efficiency and economical losses (Baurhoo *et al.*, 2007; Brümmer *et al.*, 2010; Yin *et al.*, 2010; Sunkara *et al.*, 2011; Zhang & Sunkara, 2014). When the function of the barrier is reduced this is shown in auto-immune, inflammatory and atopic diseases (Baurhoo *et al.*, 2007; Yin *et al.*, 2010; Sunkara *et al.*, 2011; Lee *et al.*, 2012; Zhang & Sunkara, 2014). Chronic inflammatory diseases are associated with leaky gut where fluid from the host leaks into the lumen and this reduces performance through wet litter, bacterial enteritis and diarrhoea (Baurhoo *et al.*, 2007; Sunkara *et al.*, 2011; Lee *et al.*, 2012; Zhang & Sunkara, 2014). This can result in a systematic inflammatory response syndrome which means the entire body goes into an inflammatory state and organs fail to function (Sunkara *et al.*, 2011; Zhang & Sunkara, 2014; Sugiharto, 2016). Damaging the intestinal epithelium may result in intestinal barrier dysfunction

which permits endotoxins passage from gut lumen to blood circulation resulting in multi-organ dysfunction (Brümmer *et al.*, 2010; Abdelqader & Al-Fatafah, 2015; Majidi-Mosleh *et al.*, 2016).

About 500 different species are found in the intestinal microbiota and live in direct symbiosis with the host, suppling energy to the intestinal wall (Uni *et al.*, 2003; Smimov *et al.*, 2006; Forder *et al.*, 2007; Yin *et al.*, 2010). These species also prevent colonisation by pathogenic bacteria and help to maintain the intestinal immune system (Lee *et al.*, 2012; Sugiharto, 2016; Awad *et al.*, 2017). The composition of the microbial population is affected by age, diet and gut location of the animal (Baurhoo *et al.*, 2007; Yin *et al.*, 2010; Ranjitkar *et al.*, 2016). However, the genetics of the animal, the rearing conditions, degree of stress the animal is facing, immune status and interactions of the different bacterial communities also have an important impact on the microbial population composition (Baurhoo *et al.*, 2007; Ranjitkar *et al.*, 2016).

The intestinal lumen is folded into villi and microvilli and this ensures an increased absorption area for nutrients. The key to optimal feed usage depends on the length and structure of these villi. Lengthened villi are regarded as a good sign of superior gut health and improved nutrient absorption due to increased surface area (Awad *et al.*, 2008; Brümmer *et al.*, 2010). At hatch the enterocytes of the small intestine are round and immature and access to nutrients within the first 24 hours post hatch initiates rapid development of the intestine (Forder *et al.*, 2007; Kleyn, 2013). After a couple of days after hatch the crypts become well defined, which increases the surface area (Beyer & Barondes, 1982; Deplancke & Gaskins, 2001; Uni *et al.*, 2003; Smimov *et al.*, 2006; Forder *et al.*, 2007; Brümmer *et al.*, 2010; Majidi-Mosleh *et al.*, 2016). Whereas, some literature states that rapid increases in villous height and surface area happen at different rates in the intestinal segments, reaching a plateau at 6 to 8 days in the duodenum, but in the jejunum and ileum it occurs after 10 days (Nkukwana *et al.*, 2015). When there is a disturbance in the gut or the intestinal epithelium, there is a decrease in the villus size, increase in the cell turnover and decrease in the digestive and absorptive (Pelicano *et al.*, 2005). The increased proliferation of crypt cells in both the small and the large intestine in rats is suggested that it may reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity (Leeson *et al.*, 2005).

Goblet cells are produced in the crypts of the intestinal tract and over 3 days they migrate up to the villi side towards the villi tip (Deplancke & Gaskins, 2001; Uni *et al.*, 2003; Smimov *et al.*, 2006; Forder *et al.*, 2007). This is where the goblet cells become sloughed and released into the intestinal lumen (Beyer & Barondes, 1982; Forder *et al.*, 2007; Brümmer *et al.*, 2010; Majidi-Mosleh *et al.*, 2016). The goblet cells are then replaced in a continuous form. Goblet cells function to produce mucus, this mucus comprises of mucin glycoproteins which assist with transportation between the lumen and the epithelial cells (Smimov *et al.*, 2006; Forder *et al.*, 2007; Brümmer *et al.*, 2010; Majidi-Mosleh *et al.*, 2016). The mucus protects the lining in the intestine from damage caused by gut microflora, enteropathogens, digestive processes and coarse dietary components (Deplancke & Gaskins, 2001; Forder *et al.*, 2007; Brümmer *et al.*, 2010; Majidi-Mosleh *et al.*, 2016). Mucus is also

believed to facilitate the absorption of certain minerals, but for this to happen the nutrients must cross the mucus layer to reach the enterocytes for absorption to take place (Brümmer *et al.*, 2010; Abdelqader & Al-Fatafah, 2016). The single cell layer of intestinal epithelium is involved in nutrient digestion and absorption and it forms the most vital barrier between the internal and external environment (Deplancke & Gaskins, 2001; Brümmer *et al.*, 2010; Abdelqader & Al-Fatafah, 2016; Majidi-Mosleh *et al.*, 2016). It limits both transcellular and paracellular passage of luminal antigens and other noxious substances to circulation (Abdelqader & Al-Fatafah, 2016).

2.5 Immunity and performance in broilers

In the modern broiler production the hatchling is exposed to a range of pathogens at a time when its immune system is least able to mount protective responses (Butter & Walter, 2009; Cserep, 2009). The immune system is made up of the innate response and the adaptive response (Erf, 2004). The innate response is a non-specific, quick response and is regarded as being similar each time, whereas the adaptive response depends on the activation from the innate immune system and is specific (Erf, 2004; Butter & Walter, 2009; Cserep, 2009). The adaptive response includes specific responses such as antibody formation to individual micro-organisms. The innate response makes use of physical epithelial barriers, such as the intestinal cells, sentinel cells like macrophages and chemical messengers (Erf, 2004; Remus *et al.*, 2014). The innate immune system acts as a sensory organ to the response of pathogens in the body by communicating through a series of behavioural, cellular and metabolic changes that influence growth and nutrient requirements (Erf, 2004; Butter & Walter, 2009; Cserep, 2009). The response is initiated by the release of cytokines that activate the cellular (phagocytic) and humoral (antibody) responses and increase body heat production through rapid basal metabolic rate (Butter & Walter, 2009; Cserep, 2009; Remus *et al.*, 2014). The gut-associated lymphoid tissue (GALT) represents a fraction of the mucosal-associated lymphoid tissue (MALT), which incorporates the bronchial, salivary, nasopharyngeal, and genitourinary lymphoid tissues (Yegani & Korver, 2008; Butter & Walter, 2009). The GALT involves the bursa of Fabricius, caecal tonsils, Peyer's patches, and lymphoid aggregates in the urodenum and proctodeum (Yegani & Korver, 2008).The MALT is the first line of defence on mucosal surface (Yegani & Korver, 2008; Butter & Walter, 2009; Remus *et al.*, 2014). Lymphoid organs are regarded as the most important structural contributors of the avian immune system, together with the bursa of Fabricius (a site of B-lymphocyte development and differentiation) and the thymus (site of T-lymphocyte development and differentiation) which are regarded as the primary lymphoid organs (Chen *et al.*, 1996; Funk & Thompson, 1996; Erf, 2004; Yegani & Korver, 2008). The bursa of Fabricius is involved in antibody production and its production starts during late embryogenesis together with other GALT (Yegani & Korver, 2008; Cserep, 2009). There are several classes of antibodies or immunoglobulins (Ig) produced by plasma cells, the terminally differentiated end products of the B lymphocyte lineage.

The bursa of Fabricius becomes colonised by precursor cells during embryogenesis and is essential for the normal progression of avian B lymphocyte development (Davison *et al.*, 1996). Within the bursa, lymphocytes divide and differentiate prior to their migration to the periphery as mature B lymphocytes where the lymphocytes function as the antigen reactive precursors to antibody producing plasma cells (Davison *et al.*, 1996). The bursa contains substantial numbers of macrophages whose functions include phagocytosis of apoptotic cells (Davison *et al.*, 1996). It is also responsible for the establishment and maintenance of the peripheral B cell repertoire, and introduction of antigens into the bursa results in increased B cell responses in the periphery following subsequent systemic challenge with antigen (Davison *et al.*, 1996; Funk & Thompson, 1996).

The immune cells that are fucntional evacuate the primary lymphoid organs, whereas the secondary lymphoid organs are scattered throughout the body (Yegani & Korver, 2008; Cserep, 2009). These secondary lymphoid organs include spleen, bone marrow, gland of Harder, bronchial-associated (BALT) and gut-associated (GALT) lymphoid tissues which are characterised by aggregates of lymphocytes and antigen-presenting cells (Yegani & Korver, 2008). The lining of the gut forms the interface between foreign material such as feed and microflora and the bird (Korver, 2006; Yegani & Korver, 2008). Regardless of the pathogenic capacity of the foreign material ingested by the bird, an immune response can be stimulated and this may have negative impacts on feed efficiency of the bird (Yegani & Korver, 2008; Collett, 2009). This is because the response costs energy and nutrients are diverted away from production (Yegani & Korver, 2008) and towards the immune system (Collett, 2009; Kleyn, 2013). This is due to the fact that antibodies require protein in order to be synthesised and energy to fuel the process. The energy requirement of the bird is increased due to the increase in the basal metabolic rate (Kleyn, 2013). Glucose is a 6 carbon (hexoses) monosaccharide (sugar) used by animals as a substrate for cellular oxidation (Kleyn, 2013). During an immune response, glucose is derived from the peripheral tissues and towards cell populations and tissues responsible for creating an immune response (Kleyn, 2013). Metabolic costs of barrier defences are mainly due to maintenance of that barrier (Korver, 2006). The first line of defence causes systemic metabolic changes. These changes non-specifically inhibit the colonisation and growth of the pathogens in the broiler bird (Korver, 2006). The changes include metabolic inefficiencies, fever, skeletal muscle catabolism and acute phase protein synthesis (Korver, 2006; Collett, 2009). The fever causes a disruption in the growth of bacteria by increasing the body temperature above the optimal range for pathogens to survive (Korver, 2006). This, however, also leads to a decrease in tissue growth rate and body protein synthesis (Collett, 2009; Kleyn, 2013). Immune challenges decrease protein synthesis and increase protein degradation, which leads to reduced feed intake and poor performance (Collett, 2009; Kleyn, 2013). Reduction in appetite causes degradation of body tissues and fat reserves which are utilised as energy. The skeletal muscle is taken apart to provide the liver with amino acids for acute-phase protein synthesis (Korver, 2006; Collett, 2009). The proteins synthesised are involved in an array of mechanisms that are there to limit microbial growth (Korver,

2006). Some nutrients like minerals and vitamins are removed from circulation and stored in tissues, which limits their availability for usage by invading microbes (Korver, 2006). Unfortunately, these effects not only limit the growth of the pathogen, but the growth of the bird as well (Korver, 2006).

Physical barriers such as antimicrobial secretions, which include lysozyme, mucocilliary clearance, acid environment of the gizzard and proventriculus and tight cellular junctions at epithelial layers, may prevent bacterial invasions or infections (Wigley, 2013). Pathogens recognise patterns via receptors such as Toll-like receptors (TLR) and this is vital in innate immune activation in chickens (Chen *et al.*, 1996; Wigley, 2013). The key to recognition of bacteria includes two TLR-2 that identify peptidoglycan, TLR-4 that attaches lipopolysaccharide (LPS), TLR-5 that identifies flagellin (large component of bacterial flagella), and TLR-21, which identifies unmethylated CpG DNA found in bacteria (Wigley, 2013). Most invasions induce a strong inflammatory response that is caused by identification of flagellin through TLR-5, resulting to the production of CXCL chemokines and pro-inflammatory cytokines including IL-IB and IL-6 (Butter & Walter, 2009; Wigley, 2013). When the ileum is infected by *Clostridium perfringens* the activation of the innate immune system occurs via TLRs- 1, 2 and 15 leading to the expression of a TNF-α ortholog (Wigley, 2013; Vidya *et al.*, 2017). This leads to the recruitment of phagocytic heterophils and macrophages (Wigley, 2013). Heterophils form the polymorphonucleur cell population of the avian; these phagocytic cells recognise bacteria through an array of TLRs including TLR-2 variants, TLR-4, TLR-5, TLR-7 and TLR-21 (Wigley, 2013; Vidya *et al.*, 2017). The invasion also causes the polymononucleur cells to be attracted to the intestine resulting in enteritis (Wigley, 2013). The largest surface of contact in the GIT is the mucosal surface contact and pathogens are trapped by secreted products such as immunoglobulin (IgA), lysozymes and other antimicrobial compounds (Korver, 2006). This result in their inactivation and prevention of colonisation and proliferation and the body excretes them via the digestive tract (Korver, 2006).

Host defense peptides (HDPs) are also called antimicrobial peptides, and play a role in the innate immunity (Sunkara *et al.*, 2011). Defensins and cathelicidins represent two major families of HDPs in vertebrates, and B-defensins are expressed throughout the digestive, respiratory and reproductive tracts (Sunkara *et al.*, 2011). HDPs consist of broad-spectrum antimicrobial activities against bacteria, protozoa, enveloped virus and fungi mainly through direct binding and lysis of microbial membranes (Sunkara *et al.*, 2011). Natural HDPs are positively charged and contain less than 100 amino acid residues with amphipathic properties (Zhang & Sunkara, 2014). The majority of HDPs are synthesised in a strategic way in the host phagocytic and mucosal epithelial cells that regularly encounter the micro-organisms from the environment, hence it is hard for pathogens to develop resistance to them (Sunkara *et al.*, 2011; Zhang & Sunkara, 2014). They are processed by host proteases to release mature peptides upon infection and inflammation, where the matured peptides are broadly active against Gram-positive and -negative bacteria, mycobacteria, fungi, viruses and even cancerous cells (Zhang & Sunkara, 2014). They rely on the physical membrane-lytic

mechanisms as they kill bacteria with a low risk of triggering resistance (Zhang & Sunkara, 2014). HDPs help to control the host immune response to infections; this is seen by inducing chemotaxis and activation of immune cells, induction of angiogenesis, regulation of dendritic cell differentiation and re-epithelialisation, modulation of cytokine and chemokine gene expression and potentiation of antigen-specific adaptive immune response (Zhang & Sunkara, 2014). They bind directly to and neutralise bacterial membrane components such as lipopolysaccharides (LPS), lipotechoic acids and peptidoglycan and inhibit the production of pro-inflammatory cytokines induced by bacteria and membrane components (Zhang & Sunkara, 2014).

2.6 Use of antibiotics as growth promoters in livestock

When antibiotics are produced naturally, they are small organic molecules and majority are produced by the genus *Streptomyces*, which is within the filamentous bacterial group (*Antinomycetes*) and by filamentous fungi (Dwoling *et al.*, 2013). Antibiotics are utilised in intensive swine, poultry and feedlot cattle systems, with limited use in dairy cows, sheep and companion animals (Ferket, 2004; Teillant & Laxminarayan, 2015). Antibiotics used in animal feed elicit benefits such as enhanced weight gain and improved feed efficiency (Donoghue, 2003; Ferket, 2004; Teillant & Laxminarayan, 2015). They control certain populations of microorganisms to the improvement of the host (Miles *et al.*, 2006; Lee *et al.*, 2012). Reduction in the size of the gut is due to loss of mucosa cell proliferation in the absence of luminal short chain fatty acids derived from microbial fermentation causing a thinning of the intestinal villi and gut wall (Ferket, 2004; Miles *et al.*, 2006; Lee *et al.*, 2012; Teillant & Laxminarayan, 2015). Antibiotics therefore enhance nutrient digestibility as a result of the reduced gut wall and villus lamina propria (Miles *et al.*, 2006; Lee *et al.*, 2012). Antibiotics also alter certain properties of bacterial cellular metabolism causing impaired growth or death of the cell (Ferket, 2004). Whereas other antibiotics affect the building and maintenance of the bacterial cell wall or interrupt protein translation at the ribosomal level (Ferket, 2004). Below are some examples of antibiotics used in the animal industry:

1. Tetracycline antibiotics show a wide range of activity against Gram-positive and Gram-negative bacteria and are cheap (Javadi, 2011). This group of antibiotics include tetracycline, oxytetracycline, chlortetracycline and doxycycline (Javadi, 2011; Mungroo & Neethirajan, 2014). These antibiotics are widely utilised in veterinary medicine for treating and preventing diseases and also promoting growth in poultry and cattle (Javadi, 2011). These products are easy to administer and it is regarded as effective through oral dosing via water and feed (Marshall & Levy, 2011; Javadi, 2011).
2. Doxycycline is known as a broad-spectrum antibiotic and is used extensively in the treatment of infectious or respiratory tract infectious diseases caused by rickettsiae, mycoplasmas and Chlamydia in various species (Javadi, 2011).

3. Antibiotics such as avoparcin, bacitracin, bambermycins, tylosin and virginiamycin are popular as narrow-spectrum and have a smaller impact on the broad range of gut flora (Marshall & Levy, 2011). However, some of these antibiotics have structural relationships with agents used clinically for humans and could lead to cross-resistance (Engster *et al.*, 2002; Marshall & Levy, 2011).
4. Zinc bacitracin is a mixture of high molecular weight polypeptides. It has activity against Gram-positive organisms. Bacitracin acts bactericidally by binding to isoprenyl pyrophosphate, the lipid carrier that transfers *N*-acetyl-muramyl-*N*-acetylglucisamyl-amino acid cell wall building blocks across the cytoplasmic membrane (Phillips, 1999). It also has an impact on the quantity of *C. perfringens* and *Lactobacillus salivarius* (Engberg *et al.*, 2000; Miles *et al.*, 2006).
5. Virginiamycin contains two synergistic components; virginiamycin M1 and S1. Each component inhibits cell growth of Gram-positive and less so Gram-negative bacteria by inhibiting protein synthesis. It produces lasting damage to ribosomes through a catalytic type of inactivation of the 50-S subunit (Cocito, 1969; Chinali *et al.*, 1981; Miles *et al.*, 2006).

The precise mechanism of growth promotion triggered by AGPs is said to be unclear (Huyghebaert *et al.*, 2011). AGPs have antibacterial properties that improve performance in different ways such as decreasing the incidence of subclinical infections and microbial use of nutrients, improving absorption due to thinning of the intestinal wall and reducing the growth-depressing metabolites produced by Gram-positive bacteria (Dibner & Richard, 2005; Huyghebaert *et al.*, 2011; Lee *et al.*, 2012; Lee *et al.*, 2013; Khosravinia, 2015). AGPs' mode of action is through their effect on the intestinal microflora of the chicken (Dibner & Richard, 2005; Castanon, 2007; Lee *et al.*, 2012; Khosravinia, 2015). The intestinal microflora reduces the animal's efficiency through the following mechanisms: most AGPs target Gram-positive organisms (*Clostridium* and *Streptococcal* bacteria), which cause poor health and performance of the animal. AGPs lead to a decrease of the microbial destruction of vital nutrients and enhance the absorption and utilisation of nutrients (Ferket, 2004). Some literature reasons that the growth-inhibitory action of AGPs is unclear because antibiotics are used in sub-therapeutic or sub-minimum inhibitor concentration (MIC) doses (Huyghebaert *et al.*, 2011; Lee *et al.*, 2012; Lee *et al.*, 2013). Certain literature hypothesises that AGPs permit growth by inhibiting the production and excretion of cytokines by immune cells, after AGPs accumulate in these cells (macrophages) (Ferket, 2004; Huyghebaert *et al.*, 2011; Lee *et al.*, 2012). Cytokine release lead to an acute phase response resulting in decrease in appetite and muscle tissue catabolism (Ferket, 2004; Butter & Walter, 2009; Huyghebaert *et al.*, 2011; Lee *et al.*, 2012; Lee *et al.*, 2013). Inflammation results in reduced performance, but AGPs can again change the composition of the microbiota towards one that is less capable of causing inflammatory response (Huyghebaert *et al.*, 2011; Lee *et al.*, 2013). In essence, AGPs help to restore a productive homeostatic state and this often prevent disturbances from taking place, thus preventing the need for subsequent therapeutic

treatments. The use of antibiotics controls clinical diseases such as NE and cholangiohepatitis (caused by *Clostridium perfringens*) (Ferket, 2004; Lee *et al.*, 2013; Kleyn, 2015).

In South Africa, antibiotics for use in food-producing animals are regulated by the Fertilizers, Farm Feed, Agricultural Remedies and Stock Remedies Act - Act 36, 1947 (Apalata *et al.*, 2011), and administered by the Department of Agriculture, Forestry and Fisheries; and the Medicines and Related Substances Control Act - Act 101, 1965 (Apalata *et al.*, 2011), administered by the National Department of Health (Henton *et al.*, 2011). Antibiotics are registered under Act 36 as stock remedies and can be purchased over the counter (Henton *et al.*, 2011). The use of antibiotics started in the mid-1950s when it was discovered that sub-therapeutic quantities of procaine penicillin and tetracycline (1/10 to1/100) added in animal feed resulted in enhanced performance in poultry, swine and beef cattle (Marshall & Levy, 2011). Of all the available antibiotics used in livestock production in South Africa, 29% are in the form of premixes (Moyane *et al.*, 2013). The most frequently used antibiotics by weight are those used to treat and prevent diseases in poultry and pigs and as growth promoters (Moyane *et al.*, 2013; SAPA, 2016). Literature indicates that the majority of consumed antibiotics in animals are from the macrolide and pleuromutilin classes, followed by the tetracycline, the sulphonamide and lastly the penicillin class (Eagar *et al.*, 2012; Mungroo & Neethirajan, 2014). In 2008, Tylosin was reported as being the most extensively used growth promoter in food-producing animals (Eagar *et al.*, 2012). South Africa has a problem with counterfeit medicines being imported from India and Pakistan that reach pharmacies through illegal means (Essack *et al.*, 2011). Stock remedies are freely available and are used by untrained consumers; therefore no record is kept of their use (Henton *et al.*, 2011). Over-the-counter antibiotics are subjected to quality control inspections. The antibiotics first need to be registered for sale and once approved they can be distributed to veterinary wholesalers, distributors, farmers' co-operatives, feed mix companies or veterinarians by the manufacturer (Henton *et al.*, 2011). Data from the industry on antimicrobial use in livestock production may be underestimating the usage of these counterfeit medicines (Moyane *et al.*, 2013; SAPA, 2016). Farmers believe that using low concentrations of antibiotics in feed and water will help prevent losses in livestock that are disease-driven this is because of increased profit margins despite the lack of well-understood mechanisms (Moyane *et al.*, 2013).

2.6.1 Development of antimicrobial resistance in humans

Antibiotic resistance is seen as a global health issue and regarded as one of the top health challenges today (Marshall & Levy, 2011; Dwoling *et al.*, 2013). Most new antibiotics, which are also used in human health, fall under Act 101 and are regulated by veterinarians (Apalata *et al.*, 2011; Henton *et al.*, 2011). Causes for the resistance include overuse and inappropriate use of antibiotics for nonbacterial infections such as the common cold and general inadequate antibiotic stewardship in the clinical arena (Apalata *et al.*, 2011; Marshall & Levy, 2011; Dwoling *et al.*, 2013). Usage of fluoroquinolones in food producing animals has caused the development of ciprofloxacin-resistant *Salmonella, Campylobacter* and *E. coli*, which have led to human infections difficult to treat (Apalata

et al., 2011; Cervantes, 2015). Certain antimicrobial drug residues have been reported to stay in the beef meat and milk of food animals for extended periods of time (Mgonja *et al.*, 2016). These antibiotic traces have harmful effects on consumers' health, such as liver damage, allergic reactions, yellowing of teeth and gastrointestinal disturbance (Javadi, 2011; Dwoling *et al.*, 2013; Cervantes, 2015). Identical elements were found in bacteria that colonised both animals and humans after doing a molecular analysis of antibiotic resistance genes and antibiotic-resistant mobile elements (Moyane *et al.*, 2013). This has led to the assumptions of raw foods playing a role in the spread of resistant bacteria and resistance genes to humans via the food chain (Moyane *et al.*, 2013). Javadi (2011) stated that the levels of antibiotics in animal tissue are dependent on the type of cooking done on the meat and this can cause variations in the levels of antibiotics. Javadi (2011) did a study on sixty broiler chickens, which were administered with doxycycline in water and feed at 0.1%. The birds were divided into two groups (with and without the antibiotic), they were then slaughtered after 5 days of the drug administration and breast muscles, livers, and gizzards were sampled for analysis. Three cooking methods were compared: boiling, roasting and microwaving; all cooking processes led to a decrease in diameter of inhibition zones in cooked samples. Javadi (2011) came to the conclusion that cooking processes do not guarantee a full break-down of these drugs present in condemned animals, but it did however reduce the quantity and it was concluded that it would be best to discard any juices from the edible tissues as they are cooked. The study highlighted that chicken resulted in lower antibiotic residues after being cooked for 20 minutes at 100°C.

Marshall & Levy (2011) reported that the spread of resistance may result from direct contact or indirectly, through water, food, and the application of animal waste to farm fields. However, the negative impact of antibiotic usage on human health may differ between regions, human population interactions, land usage, contaminated water sources, animal demography, national and international trade and national policies that focus on production, trade, food security and animal health (Moyane *et al.*, 2013). Resistant bacterial infections cause illness to take longer, higher mortality rates and increased costs associated with alternative treatment (Donoghue, 2003; Marshall & Levy, 2011; Cervantes, 2015). Microbiologists and infectious disease experts discovered that farms using AGPs had more resistant bacteria in the intestinal floras of the farm workers and farm animals compared to similar workers and animals on farms without AGPs in their production system (Donoghue, 2003; Marshall & Levy, 2011). Concerns in the aquaculture industry regarding the increased use of antibiotic prophylaxis in shrimp and carnivorous fish such as salmon due to the overcrowding, unhygienic measures also cause pressure in the removal of AGPs (Marshall & Levy, 2011; Cervantes, 2015). Therapeutic treatment of fish occurs in a group through inclusion in fish food, which results in exposure of the entire body of water to the antibiotics. Broad application of antibiotics in fish leads to leaching from unconsumed food and faeces into the water and pond sediments and this could affect wild fish and shellfish (Marshall & Levy, 2011).

The assumption is that each animal fed antibiotics becomes a 'factory' for the production and subsequent distribution of antibiotic-resistant bacteria (Marshall & Levy, 2011). Another major problem is that a single antibiotic selects for resistance to multiple structurally unrelated antibiotics via linkage of genes on plasmids and transposons, which may result in the propagation of multidrug complex ecologic and genetic factors involved in building resistance as some resistance was found in antibiotic free animals (Marshall & Levy, 2011). Avoparcin was banned after finding a bacterial cross-resistance between the use of avoparcin in feed and vancomycin, which is used for humans therapeutically (Engster *et al.,* 2002; Marshall & Levy, 2011). The emergence of vancomycin-resistant enterococci became a serious human pathogen (Donoghue, 2003; Marshall & Levy, 2011). The use of virginiamycin in broilers correlated with the rise in resistant *E. faecium* prevalence, which was later banned in the Danish poultry farms (Marshall & Levy, 2011). Homologous links between bacterial resistance genes in humans and farm animals were reported for food-borne pathogens such as *E.coli* and *Salmonella* (Marshall & Levy, 2011; Cervantes, 2015). Shuffling of products every 6 months is a standard practice done by farmers to try and prevent bacteria from becoming resistant to AGPs (Kleyn, 2013). For South Africa, the reality is that the resistance of organisms in hospitals and communities is not as high as other countries in the world, due to the fact that the use of antibiotics is not rife and communities cannot purchase these drugs without prescriptions (Apalata *et al.*, 2011).

The routine practice of giving antibiotic agents to domestic livestock is regarded as a vital factor in the emergence of antibiotic resistant bacteria in humans and animals (Moyane *et al.*, 2013). There is an increase in antibiotic resistance in both commensal and pathogenic bacteria, which can be attributed to two combined factors (Carlet *et al.,* 2012). The first factor is that micro-organisms become extremely resistant to existing antibiotics, specifically Gram-negative rods such as *Escherichia coli, Salmonella spp, Klebsiella spp, Acinetobacter spp*. These micro-organisms are resistant to almost all currently available antibiotics. The second factor is the availability of new antibiotics (Carlet *et al.*, 2012). There are powerful compounds available for Gram-positive cocci, but nothing has been made available for Gram-negative rods (Carlet *et al.,* 2012). All the classes that were authorised for use include growth promoters such as ionophores, macrolides, quinoxaline, polypeptides, streptogramins, glycolipids, oligosaccharides, phosphonic acids and polymeric compounds, and all are banned from use in the EU (Henton *et al.,* 2011). When used to treat animals, the emergence and propagation of antimicrobial-resistant strains are controlled (Marshall & Levy, 2011; Mungroo & Neethirajan, 2014). This is achieved due to their relatively short-term applications, but if their application is prolonged an ecological imbalance results (Marshall & Levy, 2011). This imbalance favours emergence and propagation of large numbers of resistance genes (Donoghue, 2003; Marshall & Levy, 2011; Mungroo & Neethirajan, 2014). Unfortunately, only 50% of the bacterial species present in the tract have been cultured (Patterson & Burkholder, 2003), which makes it difficult to fully understand the effects of AGPs in the gut. Gut health problems in the poultry industry related to subclinical necrotic enteritis and nonspecific small intestinal overgrowth of certain gut

intestinal bacteria (dysbacteriosis) have increased since the ban of AGPs in the European Union (Ranjitkar *et al.,* 2016). Studies on the impact of nontherapeutic antibiotic use on resistance in land food animals have focused primarily on *Enterococcus, Escherichia,* and *Campylobacter* and less on *Salmonella* and *Clostridium* (Marshall & Levy, 2011).

2.6.2 Consequences of removing antibiotics from animal feed

Bt 2003, the only attributable effect on humans following the ban of antibiotic use in animal feed has been some reduction in vancomycin resistance in enterococci isolated (VRE) from human faecal carriers (Casewell *et al.,* 2003). Increased antibiotic resistance in Salmonella is expected in response to the increased use of therapeutic antibiotics in animal feed consequent to the ban (Casewell *et al.*, 2003; Cervantes, 2015). This is due to the fact that the antibiotics that were banned had a Gram-positive spectrum of activity and both Salmonella and Campylobacter are Gram-negative organisms (Casewell *et al.*, 2003).

A ban on AGPs would impact producers differently due to the differences in location, farm size, contracting arrangements, production practices, species and stage of production (Engster *et al.*, 2002; Casewell *et al.,* 2003; Teillant & Laxminarayan, 2015). The first ban was imposed on tetracycline in the mid-1970s by the European Common Market, which resulted in a rise in tetracycline-resistant *Salmonella spp*. (Marshall & Levy, 2011). The ban of AGPs in Denmark resulted in an increase in the feed conversion ratio by 0.016 kg/kg from November 1995 to May 1999 from 1.78 to 1.796 (Dibner & Richards, 2005; Teillant & Laxminarayan, 2015). An increase of less than 1% of feed conversion ratio was also reported in a study of the effect of withdrawing AGP in two US broiler farms (Engster *et al.,* 2002). In Sweden, 16 years after the ban of growth promoters, the loss in production from pigs has not yet been fully recovered on a national basis (Casewell *et al.*, 2003; Teillant & Laxminarayan, 2015). In Sweden, the removal of AGPs may be partially effective, but it comes with an increased financial burden (Casewell *et al.,* 2003). Potential economic effects with the removal of AGPs from feed include reduced growth rate, reduced feed efficiency, high mortality rate, increased morbidity, longer time to market and lower stocking density, higher input cost with regards to increased feed intake, cost of more biosecurity measures and adjustments in housing to compensate for AGP termination and increased variability of product produced (Engster *et al.*, 2002; Casewell *et al.*, 2003; Teillant & Laxminarayan, 2015). The poor uniformity that results in body size of broilers not supplemented with growth promoters leads to rupture of the gastrointestinal tract at slaughter, faecal spillage, and potential contamination with Campylobacter and Salmonella (Engster *et al.*, 2002; Casewell *et al.*, 2003; Cervantes, 2015). A substantial increase in the use of therapeutic antibiotics for food animals in Europe has increased from 383 tonnes in 1999 to 437 in 2000 since the ban (Casewell *et al.,* 2003). There was an increase of 7 tonnes in pigs, 13 tonnes in poultry and 37 tonnes of therapeutics authorised for more than one species. In the pig industry, the increase was ascribed to the EU ban in 1999 and to the presence of diseases such as porcine

dermatitis, nephritis syndrome and post weaning multisystemic wasting syndrome (Casewell *et al.*, 2003).

2.6.3 Necrotic enteritis in broilers

Necrotic enteritis (NE) is the inflammation of the small intestine leading to the dying of tissue of the intestinal wall, with the villi tips disappearing (Olkowski *et al.*, 2006; Cooper & Songer, 2009; Stanley *et al.*, 2012; Abid *et al.*, 2016). It is characterised by a watery gaseous material in the small intestine together with necrotic lesions and in the presence of coccidiosis the intestine becomes bloody and red (Timbermont *et al.*, 2010; Lee *et al.*, 2013; Abid *et al.*, 2016; Kaldhusdal *et al.*, 2016). It is associated with the Clostridium bacteria and certain predisposing factors including housing density, high energy diets (Antonissen *et al.*, 2015; Tsiouris *et al.*, 2015; Abid *et al.*, 2016; Moore, 2016), poor feed viscosity, coccidiosis, fat oxidation damage in the gut, high pathogen load in the gut and free nitrogen in the lower gut (Riddell & Kong, 1992; Kaldhusdal & Skjerve, 1996; Lee *et al.*, 2013; Abid *et al.*, 2016). The onset of NE is linked with a change in the microbiota present within the GIT (Abid *et al.*, 2016). *Clostridium perfringens* is a Gram positive spore forming anaerobic bacterium that is found commonly in the environment and the GIT of the birds and humans as part of the normal gut microbiota (Cooper & Songer, 2009; Titball, 2009; Timbermont *et al.*, 2010; Lee *et al.*, 2013; Kaldhusdal *et al.*, 2016). It is the most important clostridial pathogen of poultry, causing avian malignant disease, gizzard erosions and gangrenous dermatitis (Cooper & Songer, 2009). There are five different types of strains namely A-E; this classification is based on the production of four major toxins (McDonel, 1908; Cooper & Songer, 2009; Titball, 2009; Timbermont *et al.*, 2010; Lee *et al.*, 2013; Antonissen *et al.*, 2015; Moore, 2016). These exotoxins produced by *C. perfringens* isolates are toxinotype by the presence of four major toxins (α, β, ε and ι toxins) various strains have the potential to produce other minor toxins such as CPE, β2 toxin, perfringolysin O (θ-toxin) and collagenase (κ-toxin) (Abid *et al.*, 2016).

Necrotic enteritis is present in both clinical and non-clinical forms. The clinical form is characterised by extensive necrosis of the small intestine, with increased mortality rate, whereas the sub-clinical form causes lesser mortality with milder intestinal lesions and hepatitis. It is the sub-clinical form of the disease that is regarded as the major problem, leading to extreme production losses (Cooper & Songer, 2009; Timbermont *et al.*, 2010; Al-Baadani *et al.*, 2016; Kaldhusdal *et al.*, 2016). Recently, it was found that the majority of *C. perfringes* isolates from chickens with clinical signs of NE carry the necrotic enteritis B-like toxin (NetB) (Abid *et al.*, 2016; Awad *et al.*, 2017). NetB is a cytotoxic, haemolytic, pore-forming toxin encoded on a large conjugative plasmid (approximately 85 kilobase) within a 42-kilobase pathogenicity locus, for avian cells and uses TJ proteins directly as cell surface receptors to bind the CPE (Olkowski *et al.*, 2008; Abid *et al.*, 2016; Awad *et al.*, 2016; Fernandes da Costa *et al.*, 2016). The structure of the NetB monomer has a β-sandwich, latch, rim and prestem domain that belong to the α-haemolysin family of β-pore-forming toxins (Abid *et al.*, 2016). NetB pore channels prefer cations over anions (Abid *et al.*, 2016;

Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). Besides alpha and Net B toxins, various secretary products of *C. perfringens* and the host factors are also involved in the pathogenesis of NE. These include hydrolytic enzymes and proteolytic enzymens that take part in the destruction of the basal lamina and lateral domains of enterocytes (Abid *et al.*, 2016).

Enteric pathogens target the intercellular tight junctions through the disruption of specific TJ proteins or indirectly by altering the cellular cytoskeleton through changes in the peri-junctional actomyosin ring (Olkowski *et al.*, 2006; Stanley *et al.*, 2012; Awad *et al.*, 2017). The TJ proteins can be disrupted by degradation by proteases derived from bacteria or by biochemical alterations such as phosphorylation or dephosphorylation (Awad *et al.*, 2017). Other mechanisms include direct reorganisation or degradation of specific TJ proteins, reorganisation of the cell cytoskeleton and activation of host signalling events (Awad *et al.*, 2017). Pathogens can stimulate the localised secretion of pro-inflammatory cytokines from immune and intestinal epithelial cells (Olkowski *et al.*, 2008; Stanley *et al.*, 2012; Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). Consequently, these inflammatory and stress responses result in phosphorylation of myosin light chain by myosin light chain kinase, resulting in contraction and opening of the intestinal epithelial tight junctions and an increased intestinal permeability (Olkowski *et al.*, 2006; Awad *et al.*, 2017). The disruption of gut barrier results in a malabsorption of nutrients and translocation of enteric bacteria to different internal organs, leading to disease and poor growth performance (Olkowski *et al.*, 2006; Stanley *et al.*, 2012; Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). It is one of the most common and economically devasting bacterial diseases in modern broiler production in terms of performance, welfare and mortality (Abid *et al.*, 2016). It was reported that NE caused an economic loss of as much as $0.05 per bird in the year 2000 (Jayaraman *et al.*, 2013). The “epidemic” of NE in birds is under control for now because of the implementation of specific management factors (Tsiouris *et al.*, 2015; Tsiouris, 2016) and, again, the therapeutic use of antimicrobials and ionophorous. Currently, the use of coccidiostats of ionophore type is regarded as the main strategies to manage NE when AGPs are removed in the diet because ionophores exert an effect against coccidia and also against several intestinal bacteria including *Clostridium perfringens* (Dahiya *et al.*, 2006; Lee *et al.*, 2013; Kaldhusdal *et al.*, 2016). There are two types of anticoccidials namely ionophores and chemical drugs. Examples of ionophers include monensin, narasin, salinomycin, lasalocid and maduramicin and for chemical drugs, amprolium, nicarbazin, and robenidine (Kleyn, 2013; Lee *et al.*, 2013). Ionophores are polyether compounds and form lipid soluble complexes with cations and facilitate specific ion transport across biological membranes (Kleyn, 2013; Lee *et al.*, 2013). These products are used in the industry to also control coccidiosis in broiler birds. Ionophores are classified into monovalent ionophores, which bind with sodium ion and potassium ion and divalent ionophores, which bind with sodium, potassium, calcium and magnesium ions (Kleyn, 2013; Lee *et al.*, 2013). This causes an interference with the normal transport of cations across the surface membranes of the parasite. Therefore, the intracellular sodium ion will increase and inhibit the functions of the mitochondria of the coccidia cell (Lee *et al.*,

2013). The calcium ions also increase in the cytoplasm due to the modified membrane and results in damaged membrane and cell bursting from the swelling (Kleyn, 2013; Lee *et al.*, 2013). These products have helped in preventing the coccidial damage which leads to NE (Al-Sheikhly & Al-Saieg, 1979; Dahiya *et al.*, 2006). Research reports from laboratory trials and natural field cases highlight the chances that coccidiasis or coccidiosis especially *Eimeria* species, may predispose birds to clostridial enteritis (Al-Sheikhly & Al-Saieg, 1979; Jackson *et al.*, 2003; Lee *et al.*, 2013). Other studies have indicated that coccidial vaccines were able to prevent *C. perfringens*-associated necrotic enteritis (Al-Sheikhly & Al-Saieg, 1979; Jackson *et al.*, 2003; Williams *et al.*, 2003; Lee *et al.*, 2013). Reports have shown that birds receiving a live attenuated anticoccidial vaccine in drinking water containing seven species of *Eimeria* were protected against necrotic enteritis, whereas birds fed ionophores with AGPs suffered from clostridiosis (Lee *et al.*, 2013; Sivaseelan *et al.*, 2013). The link between coccidiosis, anticoccidial vaccines and necrotic enteritis in broiler chickens have been examined (Al-Sheikhly & Al-Saieg, 1979; Lee *et al.*, 2013; Sivaseelan *et al.*, 2013). This led to the observation that immunisation against virulent coccidial challenge decreased the degree of severity of the later clostridial challenge (Al-Sheikhly & Al-Saieg, 1979). This could be due to the fact that any coccidial lesions that might have predisposed birds to necrotic enteritis were prevented because of vaccination (Lee *et al.*, 2013). In contrast to this, the vaccination itself resulted in mild coccidial lesions in some birds, but those lesions were not severe enough to predispose immunised birds to necrotic enteritis (Al-Sheikhly & Al-Saieg, 1979; Williams & Andrews, 2001; Williams *et al.*, 2003; Lee *et al.*, 2013).

To decrease the antibiotic usage, the industry needs to develop alternate strategies to control intestinal health problems (Van Immerseel *et al.*, 2016). The claudin family is used as cellular receptors and it binds to claudin-3 and 4 of MDCK cell monocytes (Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). After binding, *C. perfringes* enterotoxin (CPE) destroys the membrane permeability and leads to calcium influx into the cell, resulting in cell damage (Abid *et al.*, 2016; Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). *Clostridium perfringens* alpha toxin is zinc-dependent, consists of two domains which are associated with phospholipase C activity (N-domain, 1-250 residues) and membrane recognition (C-domain, 251-370 residues) (Abid *et al.*, 2016). A 43-KDa phospholipase C enzyme that breaks down membrane phospholipids in a calcium-dependent manner (Abid *et al.*, 2016) and has a distinctive haemolytic activity (Olkowski *et al.*, 2006; Olkowski *et al.*, 2008; Zekarias *et al.*, 2008; Abid *et al.*, 2016). It has been shown that, only immune responses against the C-domain gave protection against a subsequent challenge (Abid *et al.*, 2016). Hence the C-terminal domain of the alpha toxin was used as a vaccine against C. perfringens infection, in a purified protein or live attenuated bacteria form (Abid *et al.*, 2016). The catalytic effect of the alpha toxin on membranes relies on the Ca^{2+} mediated binding of its carboxy-terminal tail to membrane phospholipids (Zekarias *et al.*, 2008; Fernandes da Costa *et al.*, 2016). The blockage of the C-terminal domain with epitope-specific antibody neutralises both phospholipase and haemolytic activities of the toxin (Zekarias *et al.*, 2008; Fernandes da Costa *et al.*, 2016).

Biosecurity is an important preventative management tool which should be strictly followed by employees to reduce endemic pathogen spread on the farm or between farms (Collett, 2009; Tsiouris *et al.*, 2015; Abid *et al.*, 2016; Tsiouris, 2016). An "all in all out" system should be followed instead of multi-age sites for an ideal biosecurity program. The houses should be managed with regards to sanitation, pest control, environmental quality and litter quality; these factors may be influenced by stocking density levels (Collett, 2009; Tsiouris *et al.*, 2015). These preventive measures should be based on applied microbiology and epidemiology, must be practical, enforceable and cost-effective (Collett, 2009). Physiological stress of the birds can lead to compromised immune systems and increase the birds' susceptibility to pathogenic effects (Collett, 2009; Tsiouris *et al.*, 2015).

2.7 Alternatives to antibiotic growth promoters

South Africa is regarded as competitive in global terms because the country has achieved some of the highest yields per meter of floor space. This is possible due to the use of AGPs in broiler diets in order to improve performance of birds kept at high stocking density and under high bacterial challenges (Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). However, the use of AGPs in South Africa is under political and scientific scrutiny and this has led to the limitation of the use of these products in the SA market. In order to avoid reduction in performance in the birds, one needs to prevent or avoid infection; this is done by decreasing the pathogen load that the animal is exposed to. Pathogen status should be monitored routinely to help manage the causative organisms associated with specific diseases in the poultry industry. Reasons for declining the use of antibiotics include changes in animal demographics, changes in data collecting systems, restrictions of use, bench-marking, increased awareness of the threat of antimicrobial resistance, and/or the setting of targets (Laxminarayan *et al.*, 2016). The impact of phasing out AGPs can be reduced if attention is given to the implementation of alternative disease-preventing strategies and management factors, such as alternative husbandry practices in food animal production (Huyghebaert *et al.*, 2011; Lee *et al.*, 2012). The AGP ban by itself is not a sustainable, long-term solution and there is a need for nutritional strategies and interventions, effective monitoring and disease controlling measures in place. Therefore, the poultry industry is currently in search of alternative management or dietary strategies to prevent and control the incidence and severity of different diseases. However, the majority of the non-antibiotic alternatives studied are not likely to compensate fully for the loss of AGPs, as these alternatives will only help to compensate partially and not replace AGPs (Huyghebaert *et al.*, 2011; Al-Baadani *et al.*, 2016). Studies of feed additives used for preventing necrotic enteritis include direct-fed microbials including competitive exclusion products and probiotics, prebiotics, plant extracts, organic acids, essential oils, feed enzymes, hen egg antibodies, vaccination against *C. perfringens*, anticoccidial vaccination, bacteriophages, diet formulation and ingredient selection (Weber *et al.*, 2012; Khosravinia, 2015; Abid *et al.*, 2016; Al-Baadani *et al.*, 2016; Hanczakowska *et al.*, 2016). The cost of the alternatives is a major problem and extracting a premium for 'organic'

chicken is small in South Africa as the majority prefers cheap poultry meat. The identification of antibiotic-free, alternative disease control strategies has been hindered by the difficulty of experimentally reproducing NE by *C. perfringens* infection alone (Abid *et al.*, 2016; Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017). Key factors in experimental reproduction of NE, and major risk factors for development of natural disease, are inoculation with a virulent strain of *C. perfringens* and creation of an intestinal environment that favours anarchic growth of the organism (Cooper & Songer, 2009). The intestinal environment is prepared by creating minor enterocyte damage by challenging the bird with *Eimeria* spp. or immunising with commercial coccidial vaccine (Cooper & Songer, 2009). Another approach involves manipulation of the diet and reports from NE studies should be compared based on challenge methods and lesion scoring system in mind due to the extreme variation that could result (Cooper & Songer, 2009).

2.7.1 Vaccination

The use of feed additives as a nutritional strategy aims to decrease the stress levels of the animals, maintain cellular integrity, enhance the immune system, improve establishment of beneficial microflora, improve growth of beneficial microbes and block adhesion of pathogens (Kaczmarek *et al.*, 2015; Khosravinia, 2015; Abid *et al.*, 2016; Al-Baadani *et al.*, 2016; Hanczakowska *et al.*, 2016). Studies are done on practical strategies for vaccination in the field, and protein and toxins have been tested as vaccine candidates (Abid *et al.*, 2016). The major question with this strategy is how the birds will get protection from the vaccination in the limited time span of 3 to 4 weeks before the lesions are most likely to develop (Abid *et al.*, 2016). Keeping in mind that practically, vaccination of young broilers is affected by their immature immune systems and mass parental vaccination is possible at day 1 and not beyond this point (Abid *et al.*, 2016). However, the need for antibiotic alternatives has become essential in order to prevent NE from occurring and the consequent economic losses that follow (Abid *et al.*, 2016; Fernandes da Costa *et al.*, 2016; Awad *et al.*, 2017).

2.7.2 Probiotics

Probiotics means “for life” in Greek, and is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Loh *et al.*, 2010; Mookiah *et al.*, 2012; Ebrahimi *et al.*, 2016; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). Probiotics can be mono- or mixed cultures of living micro-organisms that affect the host by improving the properties of the indigenous microbiota which is regarded as beneficial (Mookiah *et al.*, 2012; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). Probiotics, also called direct-fed microbials (DFMs) modify the gastrointestinal microflora by stimulating the bacterial activities advantageous to the host and suppressing those adverse to the host’s health (Dahiya *et al.*, 2006; Loh *et al.*, 2010; Ebrahimi *et al.*, 2016; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). The available probiotics can be classified into (1) ‘colonising’ species, such as *Lactobacillus* and *Enterococcus* spp., and (2) free flowing ‘non-colonising’ species, such as *Bacillus* spp. (spores) and *Saccharomyces cerevisiae* (Loh *et al.*, 2010; Mookiah *et al.*, 2012; Ebrahimi *et al.*, 2016; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). Competitive

exclusion (CE) describes the treatment of day-old chicks with an undefined microbiota derived from adult animals resulting in colonisation resistance against pathogenic microorganisms (Loh *et al.*, 2010; Mookiah *et al.*, 2012; Denli *et al.*, 2003). This is accomplished by different mechanisms such as competition for mucosal binding sites, competition for luminal nutrients or production of inhibitory substances such as volatile fatty acids, low pH and bacteroicins which are bacteriostatic or bacteriocidal for pathogenic bacteria (Loh *et al.*, 2010; Huyghebaert *et al.*, 2011; Ebrahimi *et al.*, 2016; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). The modes of action of probiotics include: (1) maintaining "normal" intestinal microflora by competitive exclusion and antagonism of pathogens, (2) altering metabolism by increasing digestive enzyme activity and reducing bacterial enzyme activity and ammonia production, (3) improving feed intake and digestion, (4) neutralising enterotoxins and (5) stimulating the immune system (Mookiah *et al.*, 2012; Ebrahimi *et al.*, 2016; Palamidi *et al.*, 2016; Gadde *et al.*, 2017). Some studies have indicated a potential benefit of "normal gut flora" on necrotic enteritis in broiler chickens including low mortality and caecal colonisation of *C. perfringens*. Hofacre *et al.* (1998) challenged broiler chickens experimentally with *C. perfringens*. The study concluded that normal gut flora products reduced gross intestinal lesions and improved feed efficiency in their disease model. Thus, results have been reported indicating the ability of probiotics in controlling *C. perfringens*, however, some of the suggested mechanisms of action remain hypothetical and much work needs to be done to confirm the efficacy of this approach.

2.7.3 Prebiotics

Prebiotics are described as non-digestible feed ingredients that positively affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Patternson & Burkholder, 2003; Tuohy *et al.*, 2003; Mookiah *et al.*, 2012). Prebiotics are regarded as ideal when they are not hydrolysed or absorbed in the small intestine and be a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the large intestine (Tuohy *et al.*, 2003; Mookiah *et al.*, 2012). Examples of prebiotics include polysaccharides such as fructooligosaccharides (FOS), inulin, trans-galactooligosaccharides, glucooligosaccharides, glycooligosacchriades, lactulose, lactitol, maltooligosaccharides, xylooligosaccharides, stachyose, raffinose and sucrose thermal oligosaccharides (Tuohy *et al.*, 2003; Mookiah *et al.*, 2012). Oligosaccharides like mannan oligosaccharides (MOS) have also been described as prebiotics. MOS works by blocking pathogens from binding to mannan receptors on the mucosal surface and stimulate the immune system (Spring *et al.*, 2000; Tuohy *et al.*, 2003; Mookiah *et al.*, 2012). Non-digestible oligosaccharides stimulate Lactobacillus and Bifidobacterium growth and development (Tuohy *et al.*, 2003; Mookiah *et al.*, 2012).

2.7.4 Exogenous enzymes

Non-starch polysaccharides (NSPs) are a complex group of components with different chemical compositions, physical properties and physiological activities (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011). Examples of NSPs include hemi-celluloses, pectins and

oligosaccharides as well as arabinoxylans and ß-glucans (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011). Different cereal types such as maize, wheat, barley and rye contain variable NSP levels (soluble and insoluble types) (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011). NSPs have a viscous nature and this is regarded as the main cause for their anti-nutritive effect in poultry (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011). Dietary NSP-enzymes function by decreasing the viscosity of the digesta in the small intestine, so that digesta passage and nutrient digestion rate increase resulting to less substrate and less time for the fermentation organisms to proliferate (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011). The normal and efficient endogenous enzymatic digestions of nutrients in the small intestine are then restored (Sarica *et al.*, 2005; Bozkurt *et al.*, 2008; Huyghebaert *et al.*, 2011).

2.7.5 Plant extracts

Herbs and spices are rich sources of molecules displaying antimicrobial properties (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). They have appetising properties that enhance feed utilisation efficiency by promoting feed intake, secretion of endogenous enzymes and digestive juices, which lead to better digestibility (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). Plant extracts rationalise energy consumption by enhancing the metabolism of energy and reducing the energy requirement for maintenance, which includes immune response (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). Examples used in broilers include garlic, black pepper and hot red pepper (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). Their principal mode of action is the stabilisation of feed hygiene and the beneficial effect on the gut microbiota through controlling pathogens (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). However, their effect is far more complex than simply providing antimicrobial action. Unfortunately, there are no clear guidelines regarding dosage levels and phytochemical compositions of these extracts (Garcia *et al.*, 2007; Ocak *et al.*, 2008; Chisoro, 2016). The fibre in the plant extracts is required to ensure gizzard and GIT function, and may enhance growth efficiency and meat yield. Further research is necessary before plant extracts can be used on a large scale in the livestock industry (Garcia *et al.*, 2007; Chisoro, 2016).

2.7.6 Organic acids

Bacteria can use organic acids as a carbon or energy source and these organic acids can be long-, medium- or short chain fatty acids (Van Immerseel *et al.*, 2006). The long chain fatty acids are made up of twelve or more carbon atoms and can be moved across the cell membrane by carrier mechanisms (Van Immerseel *et al.*, 2006). The medium chain fatty acids range from six to ten carbon atoms and can be transported by carrier proteins or diffuse freely across the cell membrane in undissociated form (Van Immerseel *et al.*, 2006). The short chain fatty acids on the other hand, consist of less than four carbon atoms and also cross the outer cell membrane through diffusion in the undissociated form (Van Immerseel *et al.*, 2006). Organic acids such as acetate and propionate have been successfully used as water sanitisers in poultry facilities (Panda *et al.*, 2009; Adil *et al.*, 2010; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016). The supplementation of organic acids to poultry diets

resulted in suppression of the growth of certain species of bacteria, mainly acid-intolerant species, such as Salmonella, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes* and Campylobacter (Samanta *et al.*, 2010; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016; El-Ghany *et al.*, 2016). Formic, fumaric and citric acid showed a beneficial effect on growth and feed-to-gain ratio in weaned piglets and fattening pigs (Isabel & Santos, 2009; Adil *et al.*, 2010; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016). The mode of action of organic acids is believed to be related to the reduction of pH and the ability to dissociate (Abdel-Fattah *et al.*, 2008; Isabel & Santos, 2009; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016). Andreopoulou *et al.* (2014) found that the efficacy of organic acids depended on certain factors including the Pka of the organic acid, the pH of the surrounding milieu and the form of the organic acid. The Pka is the expression of the acidity of acids and both the Pka and pH values determine the amount of organic acid remaining in the undissociated form (Andreopoulou *et al.*, 2014). It has been assumed that undissociated forms of organic acids penetrate the lipid membrane of the bacterial cell and dissociate within the cell (Garcia *et al.*, 2007; Denli *et al.*, 2013; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016). Therefore, the antimicrobial activity of organic acids is pH dependent as the activity increases with a decrease in the pH (Isabel & Santos, 2009; Andreopoulou *et al.*, 2014; Aristimunha *et al.*, 2016; Ebrahimi *et al.*, 2016).

Butyric acid is a short chain aliphatic carboxylic fatty acid that is found naturally in many plants, fruits, vegetables, beverages, dairy products and in the essential oils of a number of herbs and spices (Eeckhaut *et al.*, 2008; Jiang *et al.*, 2015; Bedford & Gong, 2017). It is also produced in the GIT of mammals, resulting from the fermentation of fibre or starch in the diet by resident bacteria (Jiang *et al.*, 2015; Bedford & Gong, 2017). Butyric acid has higher bacterial activity when the acid is dissociated (Leeson, 2005; Eeckhaut *et al.*, 2008; Panda *et al.*, 2009; Song *et al.*, 2017). The acid is absorbed primarily by colonic epithelial cells called colonocytes, but may also be absorbed into the portal vein and transported to the liver, where it is metabolised to substances that are used for energy or eliminated (Jiang *et al.*, 2015; Song *et al.*, 2017). It is metabolised to produce acetyl coenzyme A (CoA), hydroxybutyrate, acetoacetate and acetone (Jiang *et al.*, 2015; Song *et al.*, 2017). The bacterial cell takes up the undissociated fatty acid and a change in the intracellular pH results soon after dissociation (Panda *et al.*, 2009). This change in intracellular pH causes death of the bacterial cells which is regarded harmful in the gut of the chickens (Eeckhaut *et al.*, 2008; Panda *et al.*, 2009; Song *et al.*, 2017). In the United States, butyric acid has been approved for use in animal feed as a synthetic flavouring substance (Jiang *et al.*, 2015). The form used in diets has been debated due to the release area in the gut of the chicken. Supplementation of pure sodium butyrate does not have a significant influence on the intestinal environment because it is water soluble (Leeson *et al.*, 2005; Panda *et al.*, 2009; Guilloteau *et al.*, 2010; Song *et al.*, 2017). Butyrate derived from fermentation of non-starch polysaccharides is vital for normal growth of epithelial cells with better gastrointestinal health and lower incidence of colon cancer in humans (Brouns *et al*, 2002; Panda *et al.*, 2009; Guilloteau *et al.*, 2010). Butyrate has also been studied in controlling the infections caused by Salmonella which has

proven to be successful (Van Immerseel *et al.*, 2002; Eeckhaut *et al.*, 2008; Andreopoulou *et al.*, 2014), but this is not covered in the scope of this review. Natural production of butyric acid in the GIT is significant only after 10 days of life and studies showed that it was beneficial to supplement it during brooding (Guilloteau *et al.*, 2010; Bedford & Gong, 2017). Even though the levels of short-chain fatty acids are not sufficient in the distal small intestine and caeca of the young chick, there is an increase by approximately 15 days of age (Leeson *et al.*, 2005; Song *et al.*, 2017). Butyrate is important in sodium and water absorption and improving gut defence systems (Panda *et al.*, 2009; Guilloteau *et al.*, 2010; Andreopoulou *et al.*, 2014; Bedford & Gong, 2017).

The mucus layer covers the entire surface of the chicken intestinal tract and is regarded as part of the innate host response, it plays a role in the effectiveness of 1-monocapric acid and sodium salt of the SCFA butyric acid (Hermans *et al.*, 2010). Butyrate has a strong capacity to enhance synthesis of endogenous antimicrobial host defense peptides (HDPs) in humans, rabbits and chickens (Sunkara *et al*., 2011; Pan & Yu, 2014), which are critical components of the animal's innate immunity (Pan & Yu, 2014; Bauwens, 2016). It induces the expression of multiple HDPs in different cell and tissue types including HD11 macrophages, primary monocytes, bone marrow cells, jejunum and caecal explants as well as in the crop, caecum and caecal tonsils of the bird (Sunkara *et al*,. 2011). At physiological concentrations, butyrate is unable to inhibit the bacteria directly, but increases the antibacterial activity of host innate immune cells by stimulating the synthesis of an array of HDPs with a lower impact on the phagocytic and oxidative killing capacity as well as activation status of host cells (Sunkara *et al*,. 2011). The potency of butyrate in stimulating the expression of HDPs is through synergising with the agonists of the cyclic adenosine monophosphate (cAMP) signalling pathway in inducing HDP expression (Zhang & Sunkara, 2014). Zhang and Sunkara (2014) found that mitogen-activated protein kinase signalling pathways were involved in the HDPs- inducing synergy between butyrate and plant extracts containing forskolin. It has shown an increase in the expression of mucin-2, the most abundant intestinal mucin protein present in the mucus (Bauwens, 2016). Research also indicated an increase in the expression levels of several major tight junction proteins including claudin-1 and TJ-protein-1 when coated (Bauwens, 2016; Song *et al.*, 2017). Birds previously supplemented with butyrate can withstand the stress of coccidial challenge much better at 21 days of age (Leeson *et al.*, 2005). Inflammation causes a reduction in the levels of butyrate in the gut and low rates of oxidation of butyrate by the mucosa (Brouns *et al.*, 2002; Song *et al.*, 2017). Brouns *et al.* (2002) suggested that this may be due to a reduced supply of fermentable substrate and a non-balanced intestinal flora, in which sulphate reducing bacteria are present in large quantities.

Research has reported that butyric acid increases the villi height, crypt depth, and plica area and mucosa thickness in the small intestine (mainly in jejunum and proximal ileum) as it is associated with the mucosal immune response and has an anti-inflammatory effect in birds and animals (Kaczmarek *et al.*, 2016). This leads to increased absorptive capacity on the small intestine, improved performance, enhanced enterocytes and intestinal velocity development (Song *et al.*, 2017). The

increase in cell proliferation from supplementing with butyric acid may be the reason for the increase in villi height, villi surface area, absorptive epithelial cell area and intestinal weight reported in some studies (Andreopoulou *et al.*, 2014; Abdelqader & Al-Fatafah, 2016; Song *et al.*, 2017). This improvement contributes to the maintenance of intestinal epithelial integrity by decreasing breakage in the mucosal barrier and improved tightness, which restricts passage of luminal antigens to blood circulation (Abdelqader & Al-Fatafah, 2016; Song *et al.*, 2017). Infusion of butyrate into fistulated rats caused an increase in the proliferation of crypt cells in both the small and large intestines (Sakata, 1987; Panda *et al.*, 2009). This effect could have resulted from changes in the microflora, which is known to be a major modulator of epithelial cell activity (Sakata, 1987; Panda *et al.*, 2009; Song *et al.*, 2017). This is not well documented as not enough information is available in poultry (Panda *et al.*, 2009).

Butyrate can balance microflora with selective control on pathogens and microflora while it enhances the barrier by facilitating tight junction assembly and also acts as a signalling molecule (Song *et al.*, 2017). It has also been shown to limit the invasiveness of Salmonella reducing colonisation in studies involving piglets and poultry (Abdelqader & Al-Fatafah, 2016; Bauwens, 2015). The efficacy of organic acids in controlling microbes such as *Campylobacter* is controlled by the concentration, form of the acid and the degree of any dissociation as it indicates the capability of entering the cell wall of the bacteria (Leeson *et al.*, 2005; Andreopoulou *et al.*, 2014). In contrast, butyrate does not inhibit *C. perfringens* (Timbermont *et al.*, 2010; Song *et al.*, 2017).This simple molecule can have very complex and diverse modes of action. Butyric acid is commonly used in its butyrate (B) form (calcium or sodium salt) (Andreopoulou *et al.*, 2014). The animal produces butyrate from dietary fibre, but large amounts of fermentable fibres may have a negative effect on faecal characteristics such as loose stools and flatulence can be observed. Butyrate can be added to animal diets as different metal salts (Na, K, Mg or Ca salt) (Jiang *et al.*, 2015).

Nutritionists have found that the best way to ensure efficiency of the feed additive is to use a coated product which allows release at the lower part of the tract. Due to rapid absorption and metabolism, free uncoated SCFA showed a marginal effect in disease control (Bauwens, 2016; Bedford & Gong, 2017; Song *et al.*, 2017). Butyrate has an influence on virulence gene expression and it is improved by coating which enables it to reach the major sites of colonisation (ileum, caecum, colon) in the tract of the chicken (Ahsan *et al.*, 2016; Song *et al.*, 2017). Butyric acid is extremely potent and the use of the coating can help reduce the smell, and also avoid excretion of the active ingredient in the faeces (Guilloteau *et al.*, 2010; Bedford & Gong, 2017). Uncoated butyrate is directly available and is immediately absorbed in the crop, almost 60% of the feed source is found intact in the crop (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012) before reaching the large intestine where it is needed (Andreopoulou *et al.*, 2014; Ahsan *et al.*, 2016; Song *et al.*, 2017). Less than 1% is recovered from the upper small intestine hence the efficacy of butyrate will be improved by protection from immediate absorption in the upper tract (Leeson *et al.*, 2005). If not in the crop, it is absorbed

from the duodenum, limiting its ability for systemic absorption and nourishment of colonocytes (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012; Jiang *et al.*, 2015; Dolan *et al.*, 2016). Fat coated butyrate becomes easily absorbed along the entire intestinal tract of the chicken and also improves the butyrate's ability to nourish the colonocytes (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012; Jiang *et al.*, 2015; Dolan *et al.*, 2016 Leeson *et al.* (2005) indicated that butyrate needs to be stabilised and they used glycerides to achieve their goal. This butyrate glyceride has only a mild buttery type odour and not the rancid odour often associated with butyric acid (Leeson *et al.*, 2005).

In the GIT, both butyric acid and sodium butyrate dissociate into n-butyrate and the corresponding cation H^+ for butyric acid and Na^+ for sodium butyrate (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012; Jiang *et al.*, 2015). Sodium butyrate has effects at the molecular, cellular and tissue level, also regarded as an acidifier (Dolan *et al.*, 2016; Sinkandar *et al.*, 2017). It has long been known as an inhibitor of histone deacetylases (HDACs) (Lu *et al.*, 2008; Mazzoni *et al.*, 2016; El-Ghany *et al.*, 2016; Sinkandar *et al.*, 2017). In cells, this affects the expression of a certain group of genes containing butyrate response elements and may also include Sp1/Sp3 binding sites which causes reduced invasion (Andreopoulou *et al.*, 2014). Sodium butyrate also causes suppression in the growth, differentiation and apoptosis in cancer cells, primarily through its effects on HDAC activity. It also suppresses inflammation by decreasing the expression of pro-inflammatory cytokines, including interferon-γ, interleukin (IL)-6, and IL-1β. The growth of beneficial bacteria Lactobacillus and Bifidobacterium and inhibition of the growth of the harmful coliform bacteria in the gastrointestinal tract were seen with butyrate supplementation (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012). In addition to its antineoplastic activity, sodium butyrate induces changes in cellular morphology, alters the expression of cellular genes, and modulates hormone action and hormone receptors as well as growth factor receptors (Dolan *et al.*, 2016; Sinkandar *et al.*, 2017). Sodium butyrate supplementation causes an inhibition of high fat diet-induced mammary tumorigenesis (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012). Butyric acid is found to be volatile and corrosive as a free acid and the formation of the corresponding salt stabilises it; therefore, the sodium salt of butyric acid (sodium butyrate) is the preferred form for addition to feed (Jiang *et al.*, 2015; Dolan *et al.*, 2016; Sinkandar *et al.*, 2017). Sodium butyrate has hydrophilic and lipophilic properties (Jerzsele *et al.*, 2012; Dolan *et al.*, 2016; Mazzoni *et al.*, 2016; El-Ghany *et al.*, 2016). Sodium butyrate is a good attractant, which can significantly increase feed intake and regulate intestinal microbiological balance. Sodium butyrate is perfectly water soluble with no sedimentation, unlike calcium butyrate with its poor water-solubility (Van Immerseel *et al.*, 2005; Jerzsele *et al.*, 2012). It has a higher bio-availability and is a preferred source for all in-feed applications, specifically for milk replacers and liquid applications (Lu *et al.*, 2008; Dolan *et al.*, 2016; Mazzoni *et al.*, 2016; El-Ghany *et al.*, 2016). The percentage of dust is much less for sodium butyrate. This is due to the uniform particle size distribution and fine particles. Calcium butyrate has also a lower anti-bacterial effect, as its molecular weight is two times higher, which results in less diffusion capacity than sodium butyrate. Calcium

butyrate has a much stronger 'typical' odour (free butyric acid) than sodium butyrate. That is because calcium butyrate is not spray-dried, and sodium butyrate is. Furthermore, calcium butyrate has lower levels of active ingredients, and often contains a high level of non-nutritional anti-caking products.

2.7.7 Monolaurin

Glycerides are made up of a glycerol molecule plus a fatty acid. The glycerol molecule has 3 positions where the fatty acid can bind to make it a triglyceride. By esterifying a fatty acid at the alpha position of the glycerol molecule, a 1-monoglyceride is formed and depending on the type of fatty acid attached this molecule acquires antibacterial and antiviral activity (Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010; Mostafa *et al.*, 2013).

The geometric configurations are important because the *trans* isomers are less active than the *cis* isomers. Addition of a second doublebond further increased the bacteriostatic effect of the cis isomer (Kabara *et al.*, 1972; Tsuji *et al.*, 2001; Van Immerseel *et al.*, 2004; Zhang *et al.*, 2009). Changing the COOH group to CONMe2 also increased the activity. Reduction of the amide group to give an amine (laurylamine HCl) yielded a compound which was active against both Gram-positive and Gram-negative organisms (Kabara *et al.*, 1972; Tsuji *et al.*, 2001; Van Immerseel *et al.*, 2004; Zhang *et al.*, 2009). In contrast it was found that free fatty acid derivatives such as aldehydes, acetate, ethyl ester, amide, a substituted amide are less active than the corresponding acids (Wyss *et al.*, 1945; Tsuji *et al.*, 2001; Van Immerseel *et al.*, 2004; Zhang *et al.*, 2009). Only the cis forms of unsaturated acids were found to be bacteriostatic in a study done by Kabara *et al.* (1972). The free carboxyl group is necessary for bactericidal activity, because ester formation generally decreased the bactericidal activity of the fatty acids (Van Immerseel *et al.*, 2004; Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010). The oxidation of the terminal end of the alkyl chain to form a dicarboxylic acid destroys the activity of lauric acid (Kabara *et al.*, 1972; Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010).

Coconut oil and certain coconut products contain an estimated 50 percent of lauric acid and capric acid (Lieberman *et al.*, 2006; Tsuji *et al.*, 2001; Van Immerseel *et al.*, 2004; Zhang *et al.*, 2009). Lauric acid is the main antiviral and antibacterial substance found in human breast milk (Lieberman *et al.*, 2006; Zhang *et al.*, 2009). Research suggests that monolaurin has virucidal and bactericidal effects by solubilising the lipids and phospholipids in the envelope of the pathogen causing the disintegration of its envelope (Lieberman *et al.*, 2006; Zhang *et al.*, 2009).

Some literature indicates that the antimicrobial effects of monolaurin are related to the interference caused by monolaurin of the signal transduction in cell replication (Lieberman *et al.*, 2006; Zhang *et al.*, 2009). Monolaurin has antimicrobial effects against a wide range of pathogenic bacteria such as *Staphylococcus aureus* (Lieberman *et al.*, 2006) and *Streptococcus agalactiae*. Among these, lauric acid has high antimicrobial activity against *Clostridium perfringens*, *Salmonella typhymurium*, and *Campylobacter jejuni* (Antongiovanni *et al.*, 2006; Zhang *et al.*, 2009; Timbermont *et al.*, 2010; Van Gerwe *et al.*, 2010; Khosravinia, 2015). MCFAs have a shorter chain length

compared to long chain fatty acids and this gives them the ability to penetrate cell membranes easily. Many experiments *in vitro* have also confirmed the antimicrobial properties of MCFA against enteric pathogens *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* and *Salmonella typhymurium*. Aromabiotic is a commercial product that contains a mixture of MCFA that demonstrates antimicrobial, physiological, and immunological properties (Antongiovanni *et al.*, 2006; Solis de los Santos *et al.*, 2008; Timbermont *et al.*, 2010; Van Gerwe *et al.*, 2010; Khosravinia, 2015).

It has been found that monolaurin has antiviral, antibacterial and antifungal properties (Lieberman *et al.*, 2006; Solis de los Santos *et al.*, 2008; Van Gerwe *et al.*, 2010). Monolauric is more biologically active than lauric acid in killing viruses and bacteria (Lieberman *et al.*, 2006; Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010). Lauric acid has greater antiviral activity than caprylic acid (C-8), capric acid (C-10) or myristic acid (C-14) (Lieberman *et al.*, 2006; Solis de los Santos *et al.*, 2008; Van Gerwe *et al.*, 2010; Donoghue *et al.*, 2015). Monoglycerides have anti-pathogenic properties that are independent of pH, making them active throughout the whole digestive tract. This gives them an advantage against traditional organic acids, as they are known to dissociate at high pH. Monolaurin has been shown to destroy the lipid-coated viruses such as influenza and binding to the lipid-protein envelope of the virus, which prevents its attachment and entry into the host cells. Both lauric acid and 1-monolaurin have antibacterial properties against gram-positive bacteria, and antiviral properties against fat-coated viruses (Lieberman *et al.*, 2006; Solis de los Santos *et al.*, 2008; Batovska *et al.*, 2009; Van Gerwe *et al.*, 2010). Various studies show that 1-monolaurin disrupts the plasma membrane lipid bilayer of gram-positive bacteria and the envelope of the fats that envelop the virus. In this way, gram-positive bacteria and fat-coated viruses cannot adhere to and penetrate into a host cell so that infection and reproduction are not possible (Van Immerseel *et al.*, 2004; Antongiovanni *et al.*, 2006; Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010). Also, parts of viruses without a fat coating are recognised more easily by the animal's immune system, so the animal's body is able to control the infection.

Lauric acid esterified with the monohydric alcohols, cholesterol and methanol showed no inhibitory properties. Free fatty acids have been shown to have antifungal and bactericidal properties as well as antitumor activity, which suggest that these compounds may affect some fundamental processes of cellular growth (Kabara *et al.*, 1972). Free fatty acids need monoglycerides to be absorbed as micelles with bile salts (Antongiovanni *et al.*, 2006; Solis de los Santos *et al.*, 2008; Van Gerwe *et al.*, 2010). Mono-esters of lauric acid and myristic acid are characterised by the maximum absorption rate, the longer the chain the lower the absorption (Antongiovanni *et al.*, 2006; Zhang *et al.*, 2009). Monolaurin has been approved by the FDA as a nontoxic direct feed additive. It adversely affects bacteria, yeast, fungi, protozoa and enveloped viruses. The mono-, di-, and triglyceryl derivatives were also studied and showed that the monoglyceride (1-mono-laurin) was more active than the free acid (Kabara *et al.*, 1972; Solis de los Santos *et al.*, 2008; Zhang *et al.*, 2009; Van Gerwe *et al.*, 2010). Fatty acids function as anionic surface agents, and the anionic surfactants are less potent

at physiological pH values (Kabara *et al.*, 1972; Dufour *et al.*, 2007; Solis de los Santos *et al.*, 2008; Van Gerwe *et al.*, 2010). 1-monoglycerides are taken up into the blood stream and in mammals pass into the mother's milk. Monolaurin benefits the animal's skin, eyes, heart and immune system by strengthening the overall immune status. Monolaurin helps to improve the general condition of the skin and the feathers. They are a good source of energy in animal nutrition due to less strain on the digestive system and ease of digestibility (Zhang *et al.*, 2009; Timbermont *et al.*, 2010; Van Gerwe *et al.*, 2010; Khosravinia, 2015). Medium chain 1-monoglycerides are reported to be active in the entire GIT and the blood stream and pathogens cannot develop resistance against them, hence they are considered in replacing AGPs (Van Immerseel *et al.*, 2004; Antongiovanni *et al.*, 2006; Timbermont *et al.*, 2010; Van Gerwe *et al.*, 2010; Khosravinia, 2015).

The alpha-monolaurin is a fat-like molecule that is tolerant to heat up to 160° C and is produced by the esterification of lauric acid together with glycerol. Additionally, this molecule is independent to pH fluctuations and will not dissociate in the GIT. The active ingredient of this additive is not transported via the hepatic portal vein to the liver, but rather enters the blood stream via the lymphatic system and bloodstream (Antongiovanni *et al.*, 2006; Solis de los Santos *et al.*, 2008; Van Gerwe *et al.*, 2010; Khosravinia, 2015). Considering such antibacterial properties, MCFA are among the candidates for new non antibiotic feed additives which are helpful in providing healthy gastrointestinal conditions in broilers (Timbermont *et al.*, 2010; Khosravinia, 2015; Zeitz *et al.*, 2015).

2.8 Conclusion

The animal food-producing industry is under pressure to find alternative strategies to using antibiotic growth promoters when farming. Consumers are concerned for their health as the use of antibiotics in animal feed has been linked to antibiotic resistant bacteria. This has shown to make treatment of human diseases more difficult due to the spread of resistant bacteria from animals to humans. The removal of anitbiotics in animal feed has resulted in an increase in the incident of bacterial infections such as necrotic enteritis. The sub-clinical form of this disease is a major problem which leads to extreme production losses due to reduction in efficiency of the birds. The gut health is compromised as the *Clostridium perfringens* bacteria causes inflammation of the small intestine leading to the dying of tissue of the intestinal wall, with the villi tips disappearing.

Studies have been done on different products in hope of replacing antibiotic use in animal feed. The use of butyric acid as an alternative has shown positive results on the performance of the broilers, but their efficacy is dependent on the site of release and the coating of the butyric acid. This product has beneficial effects on the gut health of the bird and improves the morphology of the gut in terms of villi length. The monolaurin is more antimicrobial, antifungal and bactericidal which suggest that this compound may affect some fundamental processes of cellular growth of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Clostridium perfringens*, *Salmonella typhymurium* and *Campylobacter jejuni*. Due to their shorter chain length, this product has the ability to penetrate cell

membranes and interfere with the signal transduction in cell replication. The use of both butyric acid and monolaurin together could result in improved broiler performance, reduced pathogen load, improved gut health and reduced mortality rate.

Chapter 3

Materials and Methods

3.1 Birds and housing

This trial was approved by the Faculty of Natural and Agricultural Sciences ethics committee with the reference number EC 170419-108. The trial was conducted at the trial facility of Sovereign Foods (Wincanton Farm, Uitenhage). The trial house was an environmentally controlled broiler house fitted with a coal boiler (HEATCO) attached to a heat sock as a heat source. The house contained 96 pens in total, divided into two rows consisting of forty-eight pens each over the length of the house. The pens were 1.1 meters long and 1 meter wide. Pre-assembled pens were built with rubberised-coated galvanised pipes. The 96 pens were covered with wood shavings approximately 40 mm deep supplied by Timer Shavings. The pens were separated from one another with a netting wire frame approximately 1 meter high, allowing birds to interact socially within each pen and between neighbouring pens. The house was divided into 12 blocks, with 8 pens per block and one replication of each treatment per block. All pens and housing conditions were inspected three times daily for general health of the birds and temperature and ventilation were set according to the guidelines described in the Ross Management Manual. Minimum and maximum temperatures were recorded on a daily basis and are given in Appendix A. Temperature and humidity sensors were used to monitor any changes throughout the trial using the SKOV system. The house temperature was initially adjusted at 35ºC prior to placement to ensure that the temperature at the bird level and floor temperature were acceptable. The temperature was gradually lowered from 35ºC to reach approximately 27ºC by day 10 of age and was kept constant until 35 days of age.

Birds were given *ad libitum* access to feed and water throughout the trial. Each pen was fitted with one tube feeder and 4 to 5 nipple drinkers on a nipple drinker line. The heights of the feeder and drinker lines were adjusted according to bird growth. The feed was placed inside the trial house two days prior to placement day. Feed was provided on scratch pans for the first four days so that chicks would have easy access to the feed. Feeders were shaken and shavings and droppings removed from feed every morning and afternoon, to ensure that the feed was clean and freely available and not dirty for the chicks. During a phase change, the feed from the previous phase was discarded and new feed bags were placed inside corresponding to the pen and treatment numbers.

A standard lighting programme was used according to the following schedule: from day 1 to 7, birds were exposed to 23 hours of light; from day 8 to 21, birds were given 18 hours of light and from day 22 to 31, birds received 20 hours of light. At age 32 to 33 days the birds were exposed to 22 hours of light and at 34 days the birds were given 24 hours of light.

A total of 2304 day-old male Ross 308 broiler chicks with an average body weight of 42.29 grams were randomly distributed among 96 identical pens with concrete floors. The parent flock of the day-old chicks was 38 weeks old. Twenty four birds were placed in each pen, at a stocking density of 21.8 birds/m^2. Chicks were feather sexed at the hatchery on the day of placement. A commercial vaccination programme was used during this study. The birds were vaccinated at day 0 at the hatchery against Newcastle disease (NCD) and infectious bronchitis (IB, IB-Mass, IB-QX, IB-4/91; IBD). On day 7, birds were revaccinated against NCD and on day 12 and 17 the birds were vaccinated for NCD and IBD. On days of vaccination, the lights were switched off to allow the vaccinator to spray the birds evenly and accurately with less movement of birds in the house. The drinking lines were also treated on days 26, 27, 33 and 34 with a disinfecting product (Pathopure, Techniblend, Innovative Technology, Nywerheid Singel, Malmesbury) to sterilise the water and to prevent bacterial infections. All the birds that died during the trial from unknown causes were subjected to a necropsy to ascertain the cause of death. Mortalities were weighed, recorded and removed from the pens and incinerated after full post mortem.

3.2 Feed rations and analysis

Three phases were fed according to age. The starter was fed from 0 to 14 days of age in a crumble form, the grower from 14 to 27 days as pellets and the finisher, also as pellets, from 27 to 35 days of age. A least cost feed formulation program (Format International, U.K) was used to formulate a maize-soybean based diet for the trial. The basal diet was mixed as a single batch to reduce diet variation after which the respective feed additive(s) were added to create the different treatments. The ingredients are shown in Table 3.1.

Table 3.1. Ingredient composition (%) and calculated nutrient content (g/kg) of the basal diets used in the trial

Ingredients (%)	**Starter**		**Grower**		**Finisher**	
Maize (Yellow)	55.1		61.0		65.2	
Soybean oilcake meal(46.5%)	34.6		29.7		26.1	
Sunflower oilcake meal (36%)	4		4		4	
Soya oil	2.2		2.1		1.9	
Limestone	1.63		1.33		1.21	
Mono-di-calcium phosphate	1.09		0.55		0.36	
Salt (Fine)	0.13		0.12		0.12	
Lysine (HCl 78%)	0.28		0.30		0.30	
Methionine (DL 98%)	0.29		0.27		0.24	
Threonine (98%)	0.05		0.06		0.06	
Sodium bicarbonate	0.28		0.31		0.32	
Axtra Phy 100g/t SK	0.01		0.01		0.01	
Choline CL (60%)	0.2		0.2		0.2	
Robenidine HCL (6.6%)	0.05		0.05		0.05	
Broiler premix	0.15		0.15		0.10	
Nutrient Composition (g/kg)	Calculated	Analysed	Calculated	Analysed	Calculated	Analysed
Dry matter	883.4	887.7	882.8	883.7	882.4	886.4
AME (MJ/Kg)[1]	11.30		11.65		11.85	
Moisture	116.6	112.3	117.2	116.3	117.6	113.6
Crude protein	225	213	207	202	194	187
Fat	51.7	53.5	51.5	49.3	50.1	49.5
Crude fibre	35.5	49.3	35.4	39.8	35.3	40.5
Ash	60.9	51.5	50.4	42.6	45.6	41.8
Lysine SPL[2]	11.6		10.6		10.2	
Methionine SPL	5.7		5.4		5.1	
Total sulphur amino	8.8		8.2		7.6	
acids SPL	7.4		6.9		6.4	
Threonine SPL	2.2		2.0		1.8	
Tryptophan SPL	8.2		7.5		6.9	
Isoleucine SPL	13.4		12.1		11.2	
Arginine SPL	9.0		8.2		7.7	
Valine SPL	17.2		15.7		14.6	
Glycine and serine SPL						
Calcium	10.5	8.85	8.4	7.1	7.6	6.4
Total phosphorus	6.7	6.0	5.3	4.62	4.7	4.4
Sodium	1.6	1.24	1.6	1.28	1.6	1.26
Potassium	11	8.36	9.9	7.8	9.1	7.6

[1]AME- Apparent metabolisable energy
[2]SPL –Digestible amino acids

The antibiotic growth promoter (AGP) used in the dietary treatments was zinc bacitracin (Zinc Bacitracin 15%, antibiotic performance promoter, Virbac, South Africa) at 500 g/ton feed. The sodium butyrate added in the treatment diets was an organic acid feed additive product (Novyrate® C,

Innovad NV/SA, Essen, Belgium) and the monolaurin added was a medium-chain monoglyceride feed additive product (FRA® AC12, Framelco, Allied Nutrition). The inclusion levels were all at the recommended levels i.e. 100 g/ton for starter, 75 g/ton for grower, and 25 g/ton for finisher feed for sodium butyrate and 100 g/ton for monolaurin. All the phases contained the chemical coccidiostat, Robenidine HCL 6.6% (Zoetis, USA) at 500 g/ton feed. Phytase was also used in the diets (AXTRA Phy 100 g/ton SK, Danisco Animal Nutrition, UK) with inclusion levels at 100 g/ton feed. The test products were combined in treatment 5 and treatment 8 to investigate possible synergistic effects. The treatments were as follows:

Treatment 1: Positive control (contained the AGP, Zinc Bacitracin)

Treatment 2: Negative control (contained no AGP)

Treatment 3: Positive control + Novyrate® C

Treatment 4: Positive control + FRA® AC12

Treatment 5: Positive control + Novyrate® C+ FRA® AC12

Treatment 6: Negative control + Novyrate® C

Treatment 7: Negative control + FRA® AC12

Treatment 8: Negative control + Novyrate® C+ FRA® AC12

The raw materials included in the feed were all sampled at the feed mill prior to formulation and mixing. Chemical composition were determined using an NIR scanner (Perten Instruments, DAF200, Sweden) to obtain the moisture, crude protein, fat, crude fibre, ash, calcium, and phosphorus contents of each raw material. The diets were blended at Pennville (PTY) Ltd feedmill in Pretoria, South Africa. The feed was delivered to Sovereign Foods after manufacturing. Samples were analysed by Chem Nutri Analytical Services and Cumberland Valley Analytical services-USA for crude protein, moisture, ash, crude fibre, ether extract and Ca, P, Na and K content. Dry matter of feed and ash were analysed according to AOAC's official method of analysis (AOAC, 2000, Official method of analysis 942.05). Crude fibre was determined following the AOAC's method of analysis (AOAC, 2000, Official method of Analysis 962.09) using the Fibre-Tech apparatus, as was crude fat, using the Ether Extract method (AOAC, 2000, Official method of Analysis 920.39). The Leco FP-428 (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396) was used to analyse the nitrogen content of the feed according to the AOAC's official method of analysis (AOAC,

2000, Official method of Analysis 988.05), with the Dumas method used to calculate total crude protein content. The calcium and phosphorus concentrations in feed were determined using the AOAC's official method of analysis, calcium (AOAC, 2000, Official method of Analysis 935.13), and phosphorus (AOAC, 2000, Official method of Analysis 965.17).

3.3 Performance measurements

All activities, observations and mortalities were recorded daily. Performance measurements were recorded weekly, from day of placement (day 0) to slaughter at day 35.The body weights were recorded as the average bird weight per pen. The feed conversion ratio (FCR), feed intake (FI) and body weight gain (BWG) were calculated from the recorded data using the formulae below:

Feed intake/bird (kg) = [(Feed given (kg) – Feed refused (kg))/ (24 birds-number of mortalities)]

Individual bird weight (kg) = Pen weight (kg)/ (24 birds-number of mortalities)

FCR = (Feed intake of pen over a period of time) / (Body weight gain of pen over a period of time)

$FCR_{corrected}$ = (Feed intake of pen over a period of time) / [Body weight gain of pen (live + mortality) over a period of time]

3.4 Bird sampling, carcass characteristics and histopathology

On days 20 and 33, two birds with body weight close to the weight average of the pen group were slaughtered. The birds were marked for ease of identification and they were culled using cervical dislocation. The selected birds were recorded as mortalities.

Sections of the small intestine were cut into 2 cm pieces and stored in 10% neutral buffered formalin for 3 weeks. The samples were cut into 1 cm pieces and put in a cassette. They were processed overnight and waxed the next morning. The samples from the two birds of the same pen shared a cassette, i.e. six samples were in one cassette. Four to five micron sections were dewaxed in xylene for 5 minutes and rinsed in 100%, 96%, and 70% for 1 minute, respectively. The sections were then placed in distilled water and stained in haematoxylin for 10 minutes. This was followed by rinsing in tap water and differentiating in acid alcohol in 1 dip. After rinsing with tap water for 10 minutes it was rinsed in 70% alcohol for 3 minutes followed by a counterstain in Eosin for 2½ minutes. This was done to produce a contrasting background or make a clearer distinction between the different kinds of tissues. The sections were then dehydrated for 3 min in 96% and 100% alcohol, respectively, cleared in

xylol, after which the samples were mounted in Entellan. These H & E stained intestinal section were used to measure the crypt depth and villus height. Villus height was measured as the length between the villus-crypt axis and the tip of the villus. The other slides were stained using Alcian Blue / Periodic Acid Schiff (PAS) to determine the number of neutral mucin goblet cells on the villi. The goblet cells were counted per 100 µm of villi. The staining method of Brancroft (2003) was adapted for this experiment. Sections were dewaxed in xylene for 5 minutes and rinsed in 100%, 96% and 70% for 1 minute respectively. Samples were processed in duplicate and were put in distilled water for diastase treatment and treated with undiluted periodic acid for 10 minutes. This was then followed by washing several times with distilled water and covered with Schiff's solution for 30 minutes. The slides were dehydrated in 96% alcohol and 100% alcohol after staining the nuclei with Lilly Mayer's haematoxylin for 1 minute with 10 minutes of rinsing with tap water in between. To capture images of the villi from the slides, the Zeiss Axiovert 200 microscope with AxioVision Rel. 4.8.2 software was used.

On day 35, two additional birds per pen were weighed and marked for carcass sampling. The birds were selected according to average pen weight. The birds were portioned at Sovereign Foods abattoir and all the portions were weighed and recorded. This included carcass, thighs, drumsticks, wings, breast, fillets and the viscera. The birds were grouped per treatment and a colour system was used to separate the treatments.

3.5 Experimental design

The experimental design for this trial had two levels (0 - No AGP and 1 - AGP). There were 12 pens (replications) for each treatment in the house. There were 12 blocks, 8 pens per block and each treatment appeared once per block. All the birds used were male birds and there was only one fixed factor in the trial, which was the specific diet given to each treatment group. The experiment was conducted in an ethical manner and care was taken to limit stress as far as possible.

3.6 Statistical analysis

The Generalised Line Treatment Model (GLM) function was used in preference to the balanced ANOVA. This was to ensure that the *post hoc* multiple comparison tests could find significant differences in performance between treatments. The *post hoc* multiple comparison tests used was the Fisher's Least Significant Difference (LSD), which was used to identify which pairs of means were statistically different. It is the same as the Duncan's MRT, but with t-values instead of Q values. The confidence level was set at 95%. The variables analysed were body weight, weekly body gains, weekly feed intake, cumulative intake, feed conversion ratio, cumulative feed conversion ratio, carcass weight, dressing percentage, and weights of meat portions. The gut morphology

measurements included villi height, crypt depth, and goblet cells. Gut morphology measurements and performance traits such as FCR and BW were analysed statistically as a randomised block design with the GLM model (Statistical Analysis Systems, 2018) for the average effects over time. The Repeated Measures Analysis of Variance with the GLM model was used for repeated week or period measures. Means and standard error were calculated and significance of difference ($P < 0.05$) between means was determined by Fischers test (Samuals, 1989).

The linear model used is described by the following equation:

$$Yij = \mu + T_i + L_j + TL_{ij} + B_k + e_{ijk}$$

Where Y = variable studied during the period

μ = overall mean of the population

T = effect of the ith treatment

H = effect of the jth level

TL = effect of the ij^{th} interaction between treatment and level

B = effect of the kth block

e = error associated with each Y

CHAPTER 4

Results

4.1 Body weight of broilers from 0 to 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the body weight (BW) of broilers is summarised in Tables 4.1.1 to 4.1.6.

Table 4.1.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 0 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	42.27 (±0.09)	42.34 (±0.09)	42.31 (±0.06)
2 (+ Butyric acid[#])	42.39 (±0.09)	42.25 (±0.09)	42.32 (±0.06)
3 (+ Monoglyceride[*])	42.28 (±0.09)	42.26 (±0.09)	42.27 (±0.06)
4 (+ Butyric acid + monoglyceride)	42.26 (±0.09)	42.28 (±0.09)	42.27 (±0.06)
Mean	42.30 (±0.04)	42.28 (±0.04)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Body weight was not significantly different between treatments ($P > 0.05$) at day 0 (placement day).

Table 4.1.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 7 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	186.43[b] (±2.31)	191.52 (±2.31)	188.98[ab] (±1.63)
2 (+ Butyric acid[#])	193.17[a] (±2.31)	193.69 (±2.31)	193.43[a] (±1.63)
3 (+ Monoglyceride[*])	187.96[ab] (±2.31)	189.12 (±2.31)	188.54[b] (±1.63)
4 (+ Butyric acid + monoglyceride)	190.77[ab] (±2.31)	192.16 (±2.31)	191.47[ab] (±1.63)
Mean	189.58 (±1.15)	191.62 (±1.15)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Body weight was not significantly different between the groups supplemented with AGP and the groups without AGP ($P > 0.05$) at 7 days of age. The group of birds supplemented with butyric acid without AGP was heavier in body weight than the negative control group at 7 days of age ($P < 0.05$). There was also a significant increase in body weight for birds supplemented with butyric acid regardless of AGP inclusion compared to the monoglyceride groups at 7 days of age ($P < 0.05$).

Table 4.1.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 14 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	489.75[b1] (±3.91)	501.15[2] (±3.91)	495.45[b] (±2.77)
2 (+ Butyric acid[#])	503.79[a] (±3.91)	506.45 (±3.91)	505.12[a] (±2.77)
3 (+ Monoglyceride[*])	496.69[ab] (±3.91)	503.54 (±3.91)	500.11[ab] (±2.77)
4 (+ Butyric acid + monoglyceride)	498.39[ab] (±3.91)	496.87 (±3.91)	497.63[ab] (±2.77)
Mean	497.16 (±1.96)	502.00 (±1.96)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The supplementation of butyric acid resulted in a significant increase in body weight compared to the negative control group ($P < 0.05$). The positive control group outperformed the negative control group in terms of 14 day body weights ($P < 0.05$). The average body weight of the group of birds supplemented with butyric acid regardless of AGP inclusion resulted in a higher weight compared to the control groups ($P < 0.05$). However, there was no significant difference between the group of birds supplemented with AGP and group of birds without AGP ($P > 0.05$).

Table 4.1.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 21 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1089.58 (±9.71)	1092.69 (±9.71)	1091.13 (±6.87)
2 (+ Butyric acid[#])	1090.87 (±9.71)	1106.07 (±9.71)	1098.47 (±6.87)
3 (+ Monoglyceride[*])	1090.74 (±9.71)	1100.15 (±9.71)	1095.45 (±6.87)
4 (+ Butyric acid + monoglyceride)	1095.00 (±9.71)	1086.64 (±9.71)	1090.82 (±6.87)
Mean	1091.55 (±4.86)	1096.39 (±4.86)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Body weights were not significantly different between treatments ($P > 0.05$) at 21 days of age.

Table 4.1.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 28 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1731.80 (±13.55)	1750.60 (±13.55)	1741.20[b] (±9.58)
2 (+ Butyric acid[#])	1742.71 (±13.55)	1774.25 (±13.55)	1758.48[ab] (±9.58)
3 (+ Monoglyceride[*])	1764.95 (±13.55)	1776.23 (±13.55)	1770.59[a] (±9.58)
4 (+ Butyric acid + monoglyceride)	1736.45 (±13.55)	1748.33 (±13.55)	1742.39[b] (±9.58)
Mean	1743.98 (±6.77)	1762.35 (±6.77)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

On day 28, the group supplemented with monoglyceride regardless of AGP inclusion showed a significant increase in body weight compared to the control groups and the group supplemented with both butyric acid plus monoglyceride regardless of AGP inclusion ($P < 0.05$), but this was not significantly different to the group supplemented with butyric acid regardless of AGP inclusion ($P > 0.05$).

Table 4.1.6. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the body weight (g) of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	2436.13 (22.54)	2467.31 (22.54)	2451.72 (±15.94)
2 (+ Butyric acid[#])	2465.89 (±22.54)	2491.28 (±22.54)	2478.58 (±15.94)
3 (+ Monoglyceride[*])	2469.47 (±22.54)	2498.76 (±22.54)	2484.12 (±15.94)
4 (+ Butyric acid + monoglyceride)	2440.57 (±22.54)	2447.79 (±22.54)	2444.18 (±15.94)
Mean	2453.01 (±11.27)	2476.28 (±11.27)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Body weights were not significantly different between treatments ($P > 0.05$) at 35 days of age.

4.2 Cumulative feed intake of broilers from 0 to 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the cumulative feed intake of broilers is summarised in Tables 4.2.1 to 4.2.5.

Table 4.2.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative feed intake (kg/b) of broilers at 0-7 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	152.73 (±1.96)	154.59 (±1.96)	153.66 (±1.38)
2 (+ Butyric acid[#])	156.41 (±1.96)	157.87 (±1.96)	157.14 (±1.38)
3 (+ Monoglyceride[*])	155.21 (±1.96)	153.72 (±1.96)	154.46 (±1.38)
4 (+ Butyric acid + monoglyceride)	158.11 (±1.96)	155.86 (±1.96)	156.98 (±1.38)
Mean	155.61 (±0.98)	155.51 (±0.98)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference in cumulative feed intake between treatments at 7 days of age ($P > 0.05$).

Table 4.2.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative feed intake (kg/b) of broilers at 0-14 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	534.26[b] (±4.53)	546.09 (±4.53)	544.68[b] (±3.20)
2 (+ Butyric acid[#])	558.55[a] (±4.53)	555.51 (±4.53)	557.03[a] (±3.20)
3 (+ Monoglyceride[*])	552.88[ab] (±4.53)	546.82 (±4.53)	549.85[ab] (±3.20)
4 (+ Butyric acid + monoglyceride)	546.44[ab] (±4.53)	549.96 (±4.53)	548.20[ab] (±3.20)
Mean	550.28 (±2.26)	549.59 (±2.26)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The negative control group had a lower feed intake compared to the group supplemented with butyric acid without AGP ($P < 0.05$) at 14 days of age (Table 4.2.2). The group of birds supplemented with butyric acid regardless of AGP inclusion also resulted in a higher feed intake compared to that of the control groups ($P < 0.05$).

Table 4.2.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative feed intake (kg/b) of broilers at 0-21 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1401.51 (±17.61)	1374.99 (±17.61)	1388.25 (±12.46)
2 (+ Butyric acid[#])	1402.67 (±17.61)	1388.01 (±17.61)	1395.34 (±12.46)
3 (+ Monoglyceride[*])	1389.21 (±17.61)	1390.04 (±17.61)	1389.62 (±12.46)
4 (+ Butyric acid + monoglyceride)	1387.67 (±17.61)	1362.33 (±17.61)	1375.00 (±12.46)
Mean	1395.27 (±8.81)	1378.84 (±8.81)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference between treatments at 21 days of age.

Table 4.2.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative feed intake (kg/b) of broilers at 0-28 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	2368.56 (±20.23)	2353.37 (±20.23)	2360.97 (±14.31)
2 (+ Butyric acid[#])	2386.10 (±20.23)	2357.91 (±20.23)	2372.01 (±14.31)
3 (+ Monoglyceride[*])	2355.31 (±20.23)	2357.51 (±20.23)	2356.41 (±14.31)
4 (+ Butyric acid + monoglyceride)	2334.18 (±20.23)	2354.98 (±20.23)	2344.58 (±14.31)
Mean	2361.04 (±10.12)	2355.95 (±10.12)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference in terms of feed intake between treatments at 28 days of age.

Table 4.2.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative feed intake (kg/b) of broilers at 0-35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	3729.33[b] (±34.36)	3762.56 (±34.36)	3745.95[ab] (±24.30)
2 (+ Butyric acid[#])	3829.50[a] (±34.36)	3782.28 (±34.36)	3805.89[a] (±24.30)
3 (+ Monoglyceride[*])	3727.68[b] (±34.36)	3733.81 (±34.36)	3730.74[b] (±24.30)
4 (+ Butyric acid + monoglyceride)	3705.03[b] (±34.36)	3792.82 (±34.36)	3748.92[ab] (±24.30)
Mean	3747.89 (±17.18)	3767.87 (±17.18)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 35 days of age, the group of birds supplemented with butyric acid without AGP resulted in a higher cumulative feed intake compared to the negative control group, the monoglyceride group and group of birds supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$). All the birds supplemented with butyric acid regardless of supplementing it

with AGP, resulted in a higher cumulative feed intake compared to the monoglyceride group of birds regardless of adding AGP, at 35 days of age ($P < 0.05$).

4.3 Weekly feed intake of broilers from 0 to 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the weekly feed intake of broilers is summarised in Tables 4.3.1 to 4.3.6.

Table 4.3.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly feed intake (kg/b) of broilers at 0-7 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	152.73 (±1.96)	154.59 (±1.96)	153.66 (±1.38)
2 (+ Butyric acid[#])	156.41 (±1.96)	157.87 (±1.96)	157.14 (±1.38)
3 (+ Monoglyceride[*])	155.21 (±1.96)	153.72 (±1.96)	154.46 (±1.38)
4 (+ Butyric acid + monoglyceride)	158.11 (±1.96)	155.86 (±1.96)	156.98 (±1.38)
Mean	155.61 (±0.98)	155.51 (±0.98)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference between feed intake of birds that were supplemented with butyric acid or monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) during the first week of the trial ($P > 0.05$).

Table 4.3.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly feed intake (kg/b) of broilers at 7-14 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	390.54[b] (±3.75)	391.51 (±3.75)	391.02[b] (±2.65)
2 (+ Butyric acid[#])	402.14[a] (±3.75)	397.64 (±3.75)	399.89[a] (±2.65)
3 (+ Monoglyceride[*])	397.66[ab] (±3.75)	393.10 (±3.75)	395.38[ab] (±2.65)
4 (+ Butyric acid + monoglyceride)	388.34[b] (±3.75)	394.10 (±3.75)	391.22[b] (±2.65)
Mean	394.67 (±1.87)	394.09 (±1.87)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 7-14 days, the group supplemented with butyric acid without AGP showed a higher FI compared to the negative control group and the group of birds supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$).

Table 4.3.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly feed intake (kg/b) of broilers at 14-21 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	858.25 (±15.24)	828.90 (±15.24)	843.57 (±10.78)
2 (+ Butyric acid[#])	844.12 (±15.24)	832.51 (±15.24)	838.31 (±10.78)
3 (+ Monoglyceride[*])	836.34 (±15.24)	843.22 (±15.24)	839.78 (±10.78)
4 (+ Butyric acid + monoglyceride)	841.23 (±15.24)	812.37 (±15.24)	826.80 (±10.78)
Mean	844.98 (±7.62)	829.25 (±7.62)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference on the weekly feed intake between treatments at 14-21 days of age ($P > 0.05$).

Table 4.3.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly feed intake (kg/b) of broilers at 21-28 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	967.05 (±14.99)	978.38 (±14.99)	972.72 (±10.60)
2 (+ Butyric acid[#])	983.43 (±14.99)	969.90 (±14.99)	976.67 (±10.60)
3 (+ Monoglyceride[*])	966.10 (±14.99)	967.47 (±14.99)	966.79 (±10.60)
4 (+ Butyric acid + monoglyceride)	946.50[1] (±14.99)	992.65[2] (±14.99)	969.58 (±10.60)
Mean	965.77 (±7.50)	977.10 (±7.50)	

[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference between the birds supplemented with AGP and birds without AGP on the weekly feed intake at 21-28 days of age (P > 0.05). However, the group of birds supplemented with both butyric acid plus monoglyceride without AGP showed a significantly lower feed intake at 21-28 days of age compared to the group of birds supplemented with both the butyric acid plus the monoglyceride with AGP (P < 0.05).

Table 4.3.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly feed intake (kg/b) of broilers at 28-35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1360.77[b] (±25.76)	1409.19 (±25.76)	1384.98[ab] (±18.21)
2 (+ Butyric acid[#])	1443.41[a] (±25.76)	1422.01 (±25.76)	1432.71[a] (±18.21)
3 (+ Monoglyceride[*])	1372.37[ab] (±25.76)	1376.30 (±25.76)	1374.33[b] (±18.21)
4 (+ Butyric acid + monoglyceride)	1370.85[ab1] (±25.76)	1438.91[2] (±25.76)	1404.88[ab] (±18.21)
Mean	1386.85 (±12.88)	1411.60 (±12.88)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group of birds supplemented with both butyric acid plus monoglyceride without AGP resulted in a lower FI compared to the group of birds supplemented with both butyric acid

plus monoglyceride with AGP at 28-35 days ($P < 0.05$). The group of birds supplemented with butyric acid without AGP resulted in a higher feed intake compared to the negative control group ($P < 0.05$). However, the group of birds supplemented with monoglyceride regardless of AGP inclusion showed a lower feed intake compared to the group supplemented with butyric acid regardless of AGP inclusion ($P < 0.05$).

4.4 Cumulative FCR of broilers from 0 to 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the cumulative feed conversion ratio of broilers is summarised in Tables 4.4.1 to 4.4.5.

Table 4.4.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative FCR of broilers at 0-7 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.06 (±0.01)	1.04 (±0.01)	1.05 (±0.01)
2 (+ Butyric acid[#])	1.04 (±0.01)	1.05 (±0.01)	1.04 (±0.01)
3 (+ Monoglyceride[*])	1.07 (±0.01)	1.05 (±0.01)	1.06 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.06 (±0.01)	1.04 (±0.01)	1.05 (±0.01)
Mean	1.06 (±0.01)	1.04 (±0.01)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference found between treatments in terms of the cumulative FCR at 7 days of age ($P > 0.05$).

Table 4.4.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative FCR of broilers at 0-14 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.21 (±0.01)	1.19[ab] (±0.01)	1.20 (±0.01)
2 (+ Butyric acid[#])	1.21 (±0.01)	1.20[ab] (±0.01)	1.20 (±0.01)
3 (+ Monoglyceride[*])	1.21[1] (±0.01)	1.19[b2] (±0.01)	1.20 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.20 (±0.01)	1.21[a] (±0.01)	1.20 (±0.01)
Mean	1.21[1] (±0.004)	1.20[2] (±0.004)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data revealed a significant difference between the groups supplemented with AGP and the groups without AGP (P < 0.05) at 14 days of age. The group supplemented with monoglyceride without AGP resulted in a higher FCR compared to the monoglyceride group with AGP (P < 0.05) at 14 days, but was not significantly different to the positive control group. The group of birds supplemented with both butyric acid plus monoglyceride with AGP showed a higher FCR compared to the monoglyceride group with AGP (P < 0.05) at 14 days of age, but were both not significant to the positive control group and the butyric acid group with AGP.

Table 4.4.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative FCR of broilers at 0-21 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.29 (±0.02)	1.27 (±0.02)	1.28 (±0.01)
2 (+ Butyric acid[#])	1.29 (±0.02)	1.25 (±0.02)	1.27 (±0.01)
3 (+ Monoglyceride[*])	1.27 (±0.02)	1.26 (±0.02)	1.27 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.25 (±0.02)	1.30 (±0.02)	1.27 (±0.01)
Mean	1.28 (±0.01)	1.27 (±0.01)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference between the cumulative FCR of treatments for birds at 21 days of age ($P > 0.05$).

Table 4.4.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative FCR of broilers at 0-28 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.36 (±0.02)	1.34[ab] (±0.02)	1.35 (±0.01)
2 (+ Butyric acid[#])	1.37 (±0.02)	1.33[ab] (±0.02)	1.35 (±0.01)
3 (+ Monoglyceride[*])	1.33 (±0.02)	1.32[b] (±0.02)	1.32 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.34 (±0.02)	1.36[a] (±0.02)	1.35 (±0.01)
Mean	1.35 (±0.01)	1.34 (±0.01)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group of birds supplemented with both butyric acid plus monoglyceride with AGP showed a higher FCR compared to the monoglyceride group with AGP ($P < 0.05$) 28 days, but were both not significant to the positive control group and the butyric acid group with AGP ($P > 0.05$).

Table 4.4.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the cumulative FCR of broilers at 0-35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.48 (±0.01)	1.47[ab] (±0.01)	1.48[ab] (±0.01)
2 (+ Butyric acid[#])	1.51 (±0.01)	1.47[b] (±0.01)	1.49[a] (±0.01)
3 (+ Monoglyceride[*])	1.47 (±0.01)	1.44[b] (±0.01)	1.46[b] (±0.01)
4 (+ Butyric acid + monoglyceride)	1.47[1] (±0.01)	1.51[a2] (±0.01)	1.49[a] (±0.01)
Mean	1.48 (±0.01)	1.48 (±0.01)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group of birds supplemented with butyric acid and the group supplemented with monoglyceirde with AGP showed a significantly lower FCR at 35 days of age compared to the group of birds supplemented with both butyric acid plus monoglyceride with AGP ($P < 0.05$). None of these treatments, however, differed from the positive control group ($P > 0.05$). The group of birds supplemented with both butyric acid plus monoglyceride with AGP resulted in a higher FCR compared to the same group without AGP ($P < 0.05$) at 35 days of age (Table 4.4.5). The group supplemented with butyric acid regardless of the presence of AGP and the group of birds supplemented with both butyric acid plus monoglyceride regardless of AGP inclusion had the same FCR of 1.49, which was significantly higher than the group that was supplemented with monoglyceride regardless of including AGP ($P < 0.05$).

4.5 Weekly FCR of broilers from 0 to 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the weekly feed conversion ratio of broilers is summarised in Tables 4.5.1 to 4.5.6.

Table 4.5.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly FCR of broilers at 0-7 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.06 (±0.01)	1.04 (±0.01)	1.05 (±0.01)
2 (+ Butyric acid[#])	1.04 (±0.01)	1.04 (±0.01)	1.04 (±0.01)
3 (+ Monoglyceride[*])	1.07 (±0.01)	1.05 (±0.01)	1.06 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.06 (±0.01)	1.04 (±0.01)	1.05 (±0.01)
Mean	1.06 (±0.01)	1.04 (±0.01)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The weekly FCR was not significant between treatments at 7 days of age ($P > 0.05$).

Table 4.5.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly FCR of broilers at 7-14 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.29 (±0.01)	1.26[ab] (±0.01)	1.28 (±0.01)
2 (+ Butyric acid[#])	1.29 (±0.01)	1.27[ab] (±0.01)	1.28 (±0.01)
3 (+ Monoglyceride[*])	1.28 (±0.01)	1.25[b] (±0.01)	1.27 (±0.01)
4 (+ Butyric acid + monoglyceride)	1.26 (±0.01)	1.29[a] (±0.01)	1.28 (±0.01)
Mean	1.28 (±0.01)	1.27 (±0.01)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group supplemented with butyric acid plus monoglyceride with AGP showed a higher FCR compared to the group supplemented with monoglyceride with AGP ($P < 0.05$), but did not show any significant difference against the positive control group ($P > 0.05$) at 14 days of age.

Table 4.5.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly FCR of broilers at 14-21 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.31 (±0.03)	1.36 (±0.03)	1.34 (±0.02)
2 (+ Butyric acid[#])	1.33 (±0.03)	1.30 (±0.03)	1.31 (±0.02)
3 (+ Monoglyceride[*])	1.32 (±0.03)	1.32 (±0.03)	1.32 (±0.02)
4 (+ Butyric acid + monoglyceride)	1.30 (±0.03)	1.32 (±0.03)	1.31 (±0.02)
Mean	1.31 (±0.02)	1.32 (±0.02)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The weekly FCR was not significantly different between treatments at 21 days of age ($P > 0.05$).

Table 4.5.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly FCR of broilers at 21-28 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.49 (±0.03)	1.46 (±0.03)	1.48[ab] (±0.02)
2 (+ Butyric acid[#])	1.48 (±0.03)	1.45 (±0.03)	1.47[ab] (±0.02)
3 (+ Monoglyceride[*])	1.44 (±0.03)	1.42 (±0.03)	1.43[b] (±0.02)
4 (+ Butyric acid + monoglyceride)	1.51 (±0.03)	1.47 (±0.03)	1.49[a] (±0.02)
Mean	1.48 (±0.02)	1.45 (±0.02)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 28 days of age, the group supplemented with monoglyceride regardless of AGP inclusion, resulted with a lower FCR compared to the group of birds supplemented with both butyric acid plus monoglyceride with and without AGP (P < 0.05). However, this was not significant to the butyric acid group and the control groups (P > 0.05).

Table 4.5.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weekly FCR of broilers at 28-35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1.80 (±0.04)	1.84[ab] (±0.04)	1.82 (±0.03)
2 (+ Butyric acid[#])	1.87 (±0.04)	1.86[ab] (±0.04)	1.87 (±0.03)
3 (+ Monoglyceride[*])	1.88 (±0.04)	1.79[b] (±0.04)	1.83 (±0.03)
4 (+ Butyric acid + monoglyceride)	1.82[1] (±0.04)	1.93[a2] (±0.04)	1.88 (±0.03)
Mean	1.84 (±0.02)	1.85 (±0.02)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group of birds supplemented with both butyric acid plus monoglyceride with AGP was found to have a higher FCR compared to the same group without AGP (P < 0.05) at 35 days of age. The group supplemented with monoglyceride with AGP resulted in a lower weekly

FCR compared to the group of birds supplemented with both butyric acid plus monoglyceride with AGP at 35 days of age ($P < 0.05$).

4.6 Carcass traits of broilers at 35 days of age

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the carcass traits of broilers is summarised in Tables 4.6.1 to 4.6.6.

Table 4.6.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the viscera weight relative to carcass weight percentage of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	11.27 (±0.27)	11.00 (±0.27)	11.13 (±0.19)
2 (+ Butyric acid[#])	11.48 (±0.27)	11.12 (±0.27)	11.30 (±0.19)
3 (+ Monoglyceride[*])	11.59 (±0.27)	10.99 (±0.27)	11.29 (±0.19)
4 (+ Butyric acid + monoglyceride)	11.72 (±0.27)	11.35 (±0.27)	11.54 (±0.19)
Mean	11.51[1] (±0.14)	11.12[2] (±0.14)	

[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data showed no significant difference between the treatments regarding the weight of the viscera in relation to the carcass weight at 35 days of age ($P > 0.05$), but the average viscera weight (expressed as a percentage of carcass weight) of birds supplemented with AGP was lower compared to that of the birds without AGP ($P < 0.05$).

Table 4.6.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weight of wings expressed as a percentage of carcass weight of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	8.94[b] (±0.15)	8.61[b] (±0.15)	8.78[b] (±0.10)
2 (+ Butyric acid[#])	9.22[ab1] (±0.15)	8.60[b2] (±0.15)	8.91[b] (±0.10)
3 (+ Monoglyceride[*])	9.06[ab] (±0.15)	8.95[ab] (±0.15)	9.00[b] (±0.10)
4 (+ Butyric acid + monoglyceride)	9.39[a] (±0.15)	9.23[a] (±0.15)	9.31[a] (±0.10)
Mean	9.15[1] (±0.07)	8.85[2] (±0.07)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Both the positive control and negative control groups had a lower weight of wings expressed as percentage of carcass weight compared to the group of birds supplemented with both butyric acid plus monoglyceride with and without AGP, respectively (P < 0.05). The group supplemented with butyric acid without AGP had a significantly heavier weight of wings expressed as percentage of carcass weight compared to butyric acid with AGP (P < 0.05) at 35 days of age (Table 4.6.2). The group average of birds supplemented with AGP and without AGP resulted in a significantly different weight of wings relative to carcass weight i.e. 8.85% and 9.15%, respectively (P < 0.05).

Table 4.6.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weight of thighs expressed as a percentage of carcass weight of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	26.37 (±0.33)	26.43 (±0.33)	26.40 (±0.24)
2 (+ Butyric acid[#])	25.81 (±0.33)	26.31 (±0.33)	26.06 (±0.24)
3 (+ Monoglyceride[*])	25.93 (±0.33)	26.55 (±0.33)	26.24 (±0.24)
4 (+ Butyric acid + monoglyceride)	26.37 (±0.33)	26.68 (±0.33)	26.53 (±0.24)
Mean	26.12 (±0.17)	26.49 (±0.17)	

[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference in the weight of thighs relative to carcass weight between treatments ($P < 0.05$), this is shown in Table 4.6.3.

Table 4.6.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weight of drumsticks expressed as a percentage of carcass weight of the carcass of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	11.26 (±0.19)	11.57 (±0.19)	11.41 (±0.14)
2 (+ Butyric acid$^{\#}$)	11.11^{1} (±0.19)	11.75^{2} (±0.19)	11.43 (±0.14)
3 (+ Monoglyceride*)	11.47 (±0.19)	11.25 (±0.19)	11.36 (±0.14)
4 (+ Butyric acid + monoglyceride)	11.29 (±0.19)	11.27 (±0.19)	11.28 (±0.14)
Mean	11.28 (±0.10)	11.46 (±0.10)	

^{1,2} Row means with the same superscript do not differ significantly ($P < 0.05$)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA$_{®}$ AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The weight of drumsticks relative to carcass weight for the group supplemented with butyric acid with AGP was found to be heavier compared to the group supplemented with butyric acid without AGP ($P < 0.05$), however the rest of the treatments were not significantly different from each other (Table 4.6.4).

Table 4.6.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weight of breast with bone expressed as percentage of the carcass weight of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	40.82 (±0.42)	39.84^{b} (±0.42)	40.33^{ab} (±0.30)
2 (+ Butyric acid$^{\#}$)	41.17 (±0.42)	41.02^{ab} (±0.42)	41.09^{a} (±0.30)
3 (+ Monoglyceride*)	40.29 (±0.42)	41.05^{a} (±0.42)	40.67^{ab} (±0.30)
4 (+ Butyric acid + monoglyceride)	40.15 (±0.42)	39.90^{b} (±0.42)	40.02^{b} (±0.30)
Mean	40.61 (±0.21)	40.45 (±0.21)	

^{a,b} Column means with the same superscript do not differ significantly ($P < 0.05$)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA$_{®}$ AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

Table 4.6.5 shows a significantly heavier breast weight relative to carcass weight for the group of birds supplemented with monoglyceride and AGP as compared to the positive control group and the group of birds supplemented with both butyric acid plus monoglyceride with AGP ($P < 0.05$). The butyric acid group regardless of AGP inclusion showed a heavier breast weight expressed as a percentage of carcass weight compared to the group supplemented with both butyric acid plus monoglyceride ($P < 0.05$) at 35 days on age (Table 4.6.5).

Table 4.6.6. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the weight of breast fillet expressed as a percentage of carcass weight of broilers at 35 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	22.14 (±0.33)	21.09 (±0.33)	21.62 (±0.24)
2 (+ Butyric acid[#])	22.51 (±0.33)	21.57 (±0.33)	22.04 (±0.24)
3 (+ Monoglyceride[*])	21.78 (±0.33)	21.85 (±0.33)	21.82 (±0.24)
4 (+ Butyric acid + monoglyceride)	21.70 (±0.33)	21.43 (±0.33)	21.57 (±0.24)
Mean	22.03[1] (±0.17)	21.49[2] (±0.17)	

[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The breast fillet data showed a significant difference between the groups supplemented with AGP (21.49%) compared to the groups without AGP (22.03%) at 35 days of age ($P < 0.05$).

4.7 Morphology and histology of the gastrointestinal tract

The data indicated significant differences between the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) regarding villi height in the duodenum, jejunum and ileum on 20 and 33 days of age. This is shown in Tables 4.7.1 to 4.7.6.

Table 4.7.1. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in the duodenum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	2829.66[b] (±75.24)	2708.57[b] (±75.24)	2769.12[b] (±53.20)
2 (+ Butyric acid[#])	3188.21[a] (±75.24)	2976.97[a] (±75.24)	3082.59[a] (±53.20)
3 (+ Monoglyceride[*])	3219.30[a1] (±75.24)	2938.28[a2] (±75.24)	3078.79[a] (±53.20)
4 (+ Butyric acid + monoglyceride)	3170.11[a1] (±75.24)	2893.01[a2] (±75.24)	3031.56[a] (±53.20)
Mean	3101.82[1] (±37.62)	2879.21[2] (±37.62)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was a significant increase in the villi height in the duodenum for birds that were supplemented with butyric acid, monoglyceride and the group of birds supplemented with both butyric acid plus monoglyceride without zinc bacitracin compared to the negative control group at 20 days of age ($P < 0.05$). The positive control group showed a decrease in the villi length in the duodenum compared to the groups supplemented with butyric acid, monoglyceride and the group of birds supplemented with both butyric acid plus monoglyceride with AGP at 20 days of age ($P < 0.05$).

Table 4.7.2. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in the duodenum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	2989.78[a1] (±86.23)	2595.58[b2] (±86.23)	2792.68[c] (±60.97)
2 (+ Butyric acid[#])	3187.82[a1] (±86.23)	2906.90[a2] (±86.23)	3047.36[b] (±60.97)
3 (+ Monoglyceride[*])	3462.50[b1] (±86.23)	3086.72[a2] (±86.23)	3274.61[a] (±60.97)
4 (+ Butyric acid + monoglyceride)	3486.65[b1] (±86.23)	3074.47[a2] (±86.23)	3280.56[a] (±60.97)
Mean	3281.69[1] (±43.11)	2915.92[2] (±43.11)	

[a-c] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 33 days of age, the duodenal villi height of the negative control group was not significantly different (P > 0.05) compared to the butyric acid group without AGP, but showed significantly shorter villi height compared to the group supplemented with monoglyceride and the group of birds supplemented with both butyric acid plus monoglyceride without AGP (P < 0.05). All the treatments without AGP had longer duodenal villi height than the groups supplemented with AGP (P < 0.05) at 33 days of age (Table 4.7.2).

Table 4.7.3. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in the jejunum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1770.21[1] (±74.03)	1472.10[c2] (±74.03)	1621.16[c] (±52.35)
2 (+ Butyric acid[#])	1808.52[1] (±74.03)	1642.83[bc2] (±74.03)	1752.67[bc] (±52.35)
3 (+ Monoglyceride[*])	1937.13[1] (±74.03)	1687.65[ab2] (±74.03)	1812.39[ab] (±52.35)
4 (+ Butyric acid + monoglyceride)	1964.48 (±74.03)	1888.85[a] (±74.03)	1926.66[a] (±52.35)
Mean	1870.09[1] (±37.01)	1672.85[2] (±37.01)	

[a-c] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The jejunal villi height of the birds supplemented with AGP was shorter than birds without AGP at 20 days of age averaging at 1672.85 µm and 1870.09 µm, respectively ($P < 0.05$). The group supplemented with both butyric acid plus monoglyceride with AGP resulted with a longer villi length compared to the positive control group and the butyric acid group with AGP ($P < 0.05$) at 20 days of age. The group of birds supplemented with butyric acid without AGP had a higher villi length value compared to the group supplemented with butyric acid with AGP ($P < 0.05$). There was a significant increase in the villi length of the birds supplemented with monoglyceride without AGP compared to the group supplemented with monoglyceride with AGP added ($P < 0.05$). Regardless of AGP inclusion to the treatments, the group supplemented with both butyric acid plus monoglyceride resulted with a longer villi length compared to the control groups and the group supplemented with butyric acid with and without AGP ($P < 0.05$). The data also revealed that the group of birds supplemented with monoglyceride with and without AGP had an increased jejunal villi length compared to the control groups ($P < 0.05$).

Table 4.7.4. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in the jejunum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1591.21[b] (±83.88)	1806.88 (±83.88)	1699.05[b] (±59.32)
2 (+ Butyric acid[#])	1822.23[ab] (±83.88)	1799.30 (±83.88)	1810.76[ab] (±59.32)
3 (+ Monoglyceride[*])	1983.43[a1] (±83.88)	1700.20[2] (±83.88)	1841.81[ab] (±59.32)
4 (+ Butyric acid + monoglyceride)	1966.81[a] (±83.88)	1812.18 (±83.88)	1889.50[a] (±59.32)
Mean	1840.92 (±41.94)	1779.64 (±41.94)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference with regards to the jejunal villi length for birds supplemented with AGP and birds without AGP (P > 0.05) at 33 days of age. However, the group supplemented with monoglyceride without AGP showed a significant increase in villi length in the jejunum compared to the monoglyceride supplemented group with AGP (P < 0.05). The group of birds supplemented with both butyric acid plus monoglyceride without AGP showed an increase in length in the jejunum villi of 1966.81 µm than the negative control group with an average length of 1591.21 µm (P < 0.05) at 33 days of age (Table 4.7.4)

Table 4.7.5. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in the ileum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	1064.83 (±40.49)	983.93[b] (±40.49)	1024.38[b] (±28.63)
2 (+ Butyric acid[#])	1164.02 (±40.49)	1146.42[a] (±40.49)	1155.22[a] (±28.63)
3 (+ Monoglyceride[*])	1091.94 (±40.49)	1087.94[ab] (±40.49)	1089.94[ab] (±28.63)
4 (+ Butyric acid + monoglyceride)	1172.10 (±40.49)	1090.91[ab] (±40.49)	1131.50[a] (±28.63)
Mean	1123.22 (±20.25)	1077.30 (±20.25)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 20 days of age the length in the ileum villus showed no significant difference between the groups supplemented with and groups without AGP (P > 0.05). The group of birds supplemented with butyric acid with AGP resulted with a longer villus length compared to the positive control group (P < 0.05). Both the groups supplemented with butyric acid and the group supplemented with both butyric acid plus monoglyceride regardless of AGP inclusion showed longer villus length compared to both control groups (P < 0.05).

Table 4.7.6. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the villi height (µm) in ileum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	13375.34^{1} (±63.72)	1157.70^{b2} (±63.72)	1266.52 (±45.06)
2 (+ Butyric acid$^{\#}$)	1367.42 (±63.72)	1330.12^{ab} (±63.72)	1348.77 (±45.06)
3 (+ Monoglyceride*)	1320.83 (±63.72)	1260.44^{ab} (±63.72)	1290.63 (±45.06)
4 (+ Butyric acid + monoglyceride)	1197.11^{1} (±63.72)	1380.94^{a2} (±63.72)	1289.03 (±45.06)
Mean	1315.17 (±31.86)	1282.30 (±31.86)	

^{a,b} Column means with the same superscript do not differ significantly (P < 0.05)
^{1,2} Row means with the same superscript do not differ significantly (P < 0.05)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA® AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 33 days of age the length in the ileum villus showed no significant difference between the groups supplemented with and groups without AGP (P > 0.05), but the group of birds supplemented with both butyric acid plus monoglyceride with AGP had a longer villi length compared to the same group without AGP (P < 0.05) at 33 days of age (Table 4.7.6). The group of birds supplemented with both butyric acid plus monoglyceride with AGP had higher villi height in the ileum of 1380.94 µm compared to the positive control group with an average of 1157.70 µm at 33 days of age (P < 0.05).

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the crypt depth in the duodenum, jejunum and ileum of broilers is summarised in Tables 4.7.7 to 4.7.12.

Table 4.7.7. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the duodenum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	407.78[a] (±15.29)	382.36 (±15.29)	395.07 (±10.81)
2 (+ Butyric acid[#])	389.59[ab] (±15.29)	408.30 (±15.29)	398.94 (±10.81)
3 (+ Monoglyceride[*])	370.00[ab1] (±15.29)	419.44[2] (±15.29)	394.72 (±10.81)
4 (+ Butyric acid + monoglyceride)	364.23[b] (±15.29)	402.25 (±15.29)	383.24 (±10.81)
Mean	382.90 (±7.64)	403.09 (±7.64)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data indicated no significant difference in the crypt depth in the duodenum between birds supplemented with and birds without AGP at 20 days of age (P > 0.05). However, the depth in the duodenum crypt of the group supplemented with monoglyceride with AGP was increased at 20 days of age compared to the monoglyceride group without AGP (P < 0.05). The group supplemented with both butyric acid and monoglyceride without AGP showed a decreased depth of the crypt compared to the same group with AGP (P < 0.05).

Table 4.7.8. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the duodenum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	364.16 (±48.57)	425.80 (±48.57)	394.98 (±34.35)
2 (+ Butyric acid[#])	356.92 (±48.57)	382.52 (±48.57)	369.72 (±34.35)
3 (+ Monoglyceride[*])	352.21 (±48.57)	314.11 (±48.57)	333.16 (±34.35)
4 (+ Butyric acid + monoglyceride)	288.33[1] (±48.57)	434.25[2] (±48.57)	361.29 (±34.35)
Mean	340.40 (±24.29)	389.17 (±24.29)	

[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data indicated no significant difference in the crypt depth in the duodenum between birds supplemented with and birds without AGP at 33 days of age ($P > 0.05$). However, the group of birds supplemented with both butyric acid plus monoglyceride without AGP resulted in a decreased crypt depth compared to the group of birds supplemented with the same products with AGP ($P < 0.05$).

Table 4.7.9. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the jejunum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	361.38^{a1} (±13.22)	306.37^{b2} (±13.22)	333.88 (±9.35)
2 (+ Butyric acid[#])	300.71^{b1} (±13.22)	355.50^{a2} (±13.22)	328.10 (±9.35)
3 (+ Monoglyceride[*])	335.64^{ab} (±13.22)	362.92^{a} (±13.22)	349.28 (±9.35)
4 (+ Butyric acid + monoglyceride)	310.76^{b} (±13.22)	344.23^{a} (±13.22)	327.50 (±9.35)
Mean	327.12 (±6.61)	342.25 (±6.61)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The negative control group resulted in an increased crypt depth compared to the group of birds supplemented with butyric acid and the group supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$). The group supplemented with butyric acid without AGP showed a decreased jejunal crypt depth compared to the group supplemented with butyric acid with AGP ($P < 0.05$). The negative control group resulted in an increased crypt depth (361.38 µm) compared to the positive control group (306.37 µm) at 20 days of age (Table 4.7.9). The positive control group indicated a decrease in the crypt depth in the jejunum compared to the group of birds supplemented with butyric acid, group of birds supplemented with monoglyceride and the group of birds supplemented with both butyric acid plus monoglyceride with AGP ($P < 0.05$) at 20 days of age.

Table 4.7.10. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the jejunum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	376.84[a] (±33.15)	327.45[ab] (±33.15)	352.14 (±23.44)
2 (+ Butyric acid[#])	308.37[ab] (±33.15)	296.56[ab] (±33.15)	302.47 (±23.44)
3 (+ Monoglyceride[*])	341.36[ab1] (±33.15)	239.68[b2] (±33.15)	290.52 (±23.44)
4 (+ Butyric acid + monoglyceride)	278.79[b] (±33.15)	351.37[a] (±33.15)	315.08 (±23.44)
Mean	326.34 (±16.57)	303.77 (±16.57)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 33 days of age, the group supplemented with both butyric acid plus monoglyceride without AGP had a decrease in crypt depth compared to the negative control group (P < 0.05). The group supplemented with monoglyceride without AGP showed a greater crypt depth at 33 days of age compared to the monoglyceride group with AGP (P < 0.05). The data also indicated an increased crypt depth for the birds supplemented with both butyric acid plus monoglyceride with AGP compared to the group of birds supplemented with monoglyceride with AGP (P < 0.05).

Table 4.7.11. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the ileum of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	387.64[a1] (±14.98)	321.34[2] (±14.98)	354.49[a] (±10.59)
2 (+ Butyric acid[#])	332.41[b] (±14.98)	330.35 (±14.98)	331.38[ab] (±10.59)
3 (+ Monoglyceride[*])	272.25[c1] (±14.98)	337.57[2] (±14.98)	304.91[b] (±10.59)
4 (+ Butyric acid + monoglyceride)	306.87[bc] (±14.98)	297.06 (±14.98)	301.96[b] (±10.59)
Mean	324.79 (±7.49)	321.58 (±7.49)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The ileum crypt depth of birds supplemented with monoglyceride without AGP was lower ($P < 0.05$) compared to the monoglyceride group with AGP at 20 days of age (Table 4.7.11). The negative control group showed a significant increase in the crypt depth at 20 days of age compared to the butyric acid group, monoglyceride group and the group supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$). The crypt depth in the ileum of the positive control group was lower ($P < 0.05$) than the negative control group at 20 days of age (Table 4.7.11).

The monoglyceride group without AGP also showed a decreased ileum crypt depth compared to monoglyceride group with AGP ($P < 0.05$). However, no significant difference was found between the average depth of the group with AGP and the group without AGP ($P < 0.05$). The groups supplemented with monoglyceride with and without AGP and the group of birds supplemented with both butyric acid plus monoglyceride with and without AGP showed a significant reduction on the crypt depth compared to both the negative and positive control groups at 20 days of age ($P < 0.05$).

Table 4.7.12. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the crypt depth (µm) in the ileum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	316.49^{a} (±19.49)	275.44 (±19.49)	295.96^{a} (±13.79)
2 (+ Butyric acid$^{\#}$)	276.11^{ab} (±19.49)	278.11 (±19.49)	277.11^{ab} (±13.79)
3 (+ Monoglyceride*)	279.09^{ab} (±19.49)	232.29 (±19.49)	255.69^{b} (±13.79)
4 (+ Butyric acid + monoglyceride)	229.70^{b} (±19.49)	271.04 (±19.49)	250.37^{b} (±13.79)
Mean	275.35 (±9.75)	264.22 (±9.75)	

^{a,b} Column means with the same superscript do not differ significantly ($P < 0.05$)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA® AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

No significant difference was found between the average depth of the group with AGP and the group without AGP ($P < 0.05$). The groups supplemented with monoglyceride with and without AGP and the group supplemented with both butyric acid plus monoglyceride with and without AGP showed a significant decrease in the crypt depth compared to both the negative and positive control groups at 33 days of age ($P < 0.05$). The negative control group

had a significantly greater crypt depth of 316.49 µm compared to the group of birds supplemented with both butyric acid plus monoglyceride without AGP, which averaged to 229.70 µm at 33 days of age (Table 4.7.12).

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the villi length to crypt depth ratio in the duodenum, jejunum and ileum of broilers is summarised in Tables 4.7.13 to 4.7.18.

Table 4.7.13. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the duodenal villi length to crypt depth ratio of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	6.98^{b} (±0.35)	7.21 (±0.35)	7.10^{b} (±0.25)
2 (+ Butyric acid$^{\#}$)	8.37^{a} (±0.35)	7.41 (±0.35)	7.89^{a} (±0.25)
3 (+ Monoglyceride*)	8.72^{a1} (±0.35)	7.17^{2} (±0.35)	7.94^{a} (±0.25)
4 (+ Butyric acid + monoglyceride)	8.77^{a1} (±0.35)	7.32^{2} (±0.35)	8.04^{a} (±0.25)
Mean	8.21^{1} (±0.17)	7.28^{2} (±0.17)	

^{a,b} Column means with the same superscript do not differ significantly ($P < 0.05$)
^{1,2} Row means with the same superscript do not differ significantly ($P < 0.05$)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA® AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data indicated a significant difference in the villi height to crypt depth ratio in the duodenum between the groups with and the groups without AGP ($P < 0.05$). The negative control group showed a lower ratio compared to the butyric acid group, monoglyceride group and the group supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$), at 20 days of age (Table 4.7.13).

The ratio was significantly lower for the monoglyceride group and the group of birds supplemented with both butyric acid plus monoglyceride with AGP than the monoglyceride group and the group supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$), respectively.

Table 4.7.14. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the duodenal villi length to crypt ratio of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	8.79[b1] (±0.62)	6.47[b2] (±0.62)	7.63[b] (±0.44)
2 (+ Butyric acid[#])	9.22[b] (±0.62)	7.93[b] (±0.62)	8.58[b] (±0.44)
3 (+ Monoglyceride[*])	9.97[b] (±0.62)	10.21[a] (±0.62)	10.09[a] (±0.44)
4 (+ Butyric acid + monoglyceride)	12.48[a1] (±0.62)	9.15[a2] (±0.62)	10.82[a] (±0.44)
Mean	10.12[1] (±0.31)	8.44[2] (±0.31)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

At 33 days of age, the ratio was lower (8.44) for the groups supplemented with AGP than the groups without AGP (10.12). Furthermore, the positive control group resulted in a significant decrease in the ratio of 6.47 compared to the negative control group with a ratio of 8.79 ($P < 0.05$). The highest ratio of 12.48 was observed in the group supplemented with butyric acid plus monoglyceride without AGP compared to the negative control group, butyric acid group and the monoglyceride group without AGP with values of 8.79, 9.22 and 9.97 respectively ($P < 0.05$). The butyric acid plus monoglyceride group with AGP also showed a significantly lower ratio compared to the butyric acid plus monoglyceride group without AGP ($P < 0.05$). The groups supplemented with butyric acid and the control groups showed a lower ratio compared to the monoglyceride group and the butyric acid plus monoglyceride groups ($P < 0.05$) at 33 days of age (Table 4.7.14).

Table 4.7.15. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the jejunal villi length to crypt ratio of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	4.98^{b} (±0.30)	4.95^{ab} (±0.30)	4.96^{b} (±0.21)
2 (+ Butyric acid$^{\#}$)	6.17^{a1} (±0.30)	4.62^{b2} (±0.30)	5.39^{ab} (±0.21)
3 (+ Monoglyceride*)	5.89^{a1} (±0.30)	4.68^{b2} (±0.30)	5.29^{b} (±0.21)
4 (+ Butyric acid + monoglyceride)	6.37^{a} (±0.30)	5.56^{a} (±0.30)	5.97^{a} (±0.21)
Mean	5.85^{1} (±0.15)	4.95^{2} (±0.15)	

^{a,b} Column means with the same superscript do not differ significantly (P < 0.05)
^{1,2} Row means with the same superscript do not differ significantly (P < 0.05)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA® AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

All the birds supplemented with AGP resulted in a lower ratio of 4.95 compared to the groups without AGP with an average ratio of 5.85 at 20 days of age (P < 0.05). Both the butyric acid and monoglyceride groups with AGP showed a significantly lower ratio compared to the butyric acid and monoglyceride groups without AGP, respectively (P < 0.05). The group of birds supplemented with butyric acid plus monoglyceride regardless of supplementing AGP or not showed a higher ratio (5.97) compared to the control groups (4.96) and the monoglyceride groups (5.29) at 20 days of age (P < 0.05).

Table 4.7.16. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the jejunal villi length to crypt ratio of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP$^{\alpha}$ inclusion	With AGP inclusion	Mean
1 (No additives)	4.98^{b} (±0.43)	5.11^{b} (±0.43)	5.04^{b} (±0.30)
2 (+ Butyric acid$^{\#}$)	6.16^{ab} (±0.43)	6.29^{ab} (±0.43)	6.22^{a} (±0.30)
3 (+ Monoglyceride*)	5.87^{b1} (±0.43)	7.23^{a2} (±0.43)	6.55^{a} (±0.30)
4 (+ Butyric acid + monoglyceride)	7.18^{a} (±0.43)	6.36^{a} (±0.43)	6.77^{a} (±0.30)
Mean	6.05 (±0.21)	6.25 (±0.21)	

^{a,b} Column means with the same superscript do not differ significantly (P < 0.05)
^{1,2} Row means with the same superscript do not differ significantly (P < 0.05)
$^{\#}$Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
*Monoglyceride: FRA® AC12 (1 kg/t)
$^{\alpha}$Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

There was no significant difference between the groups supplemented with AGP and the groups without AGP at 33 days of age regarding the jejunum ratio of the villi height to crypt depth ($P < 0.05$). The data indicated a lower ratio for the group supplemented with monoglyceride without AGP compared to the group supplemented with monoglyceride with AGP ($P < 0.05$). The positive control group also resulted with lower villi to crypt depth ratio (5.11) compared to the groups supplemented with monoglyceride with AGP (7.23) and the group supplemented with both butyric acid plus monoglyceride with AGP (6.36) at 33 days of age ($P < 0.05$). Both the negative control group and the monoglyceride group without AGP had a significantly lower ratio at 33 days compared to the group of birds supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$).

Table 4.7.17. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the ileum villi length to crypt depth ratio of broilers at 20 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	2.80[a] (±0.20)	3.21 (±0.20)	3.01[b] (±0.14)
2 (+ Butyric acid[#])	3.62[b] (±0.20)	3.50 (±0.20)	3.56[a] (±0.14)
3 (+ Monoglyceride[*])	4.04[b1] (±0.20)	3.27[2] (±0.20)	3.66[a] (±0.14)
4 (+ Butyric acid + monoglyceride)	3.91[b] (±0.20)	3.73 (±0.20)	3.82[a] (±0.14)
Mean	3.59 (±0.10)	3.43 (±0.10)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The villi height to crypt depth ratio at 20 days days of age of the ileum indicated no significant difference between the groups supplemented with and groups without AGP ($P > 0.05$). The negative control group had a lower ratio (2.80) compared to the group supplemented with butyric acid (3.62), the monoglyceride group (4.04) and the group supplemented with both butyric acid plus monoglyceride (3.91) all without AGP ($P < 0.05$). The group supplemented with monoglyceride with AGP showed a lower ratio compared to monoglyceride without AGP ($P < 0.05$) at 20 days of age.

Table 4.7.18. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), in the ileum villi to crypt ratio of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	4.65 (±0.38)	4.44[b] (±0.38)	4.54[b] (±0.27)
2 (+ Butyric acid[#])	5.24 (±0.38)	5.20[ab] (±0.38)	5.22[ab] (±0.27)
3 (+ Monoglyceride[*])	4.87 (±0.38)	5.78[a] (±0.38)	5.33[a] (±0.27)
4 (+ Butyric acid + monoglyceride)	5.35 (±0.38)	5.10[ab] (±0.38)	5.23[ab] (±0.27)
Mean	5.03 (±0.19)	5.13 (±0.19)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The villi height to crypt depth ratio at 33 days of age in the ileum indicated no significant difference between the groups supplemented with and groups without AGP ($P > 0.05$). The positive control group had a significantly lower villi to crypt depth ratio compared to the group supplemented with monoglyceride with AGP at 33 days of age ($P < 0.05$).

The influence of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP) on the number of goblet cells per 100 µm of villi in the duodenum, jejunum and ileum of broilers is summarised in Tables 4.7.19 to 4.7.21.

Table 4.7.19. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the number of goblet cells in the duodenum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	10.72[a] (±0.534)	10.22 (±0.534)	10.47 (±0.377)
2 (+ Butyric acid[#])	11.31[a] (±0.534)	10.27 (±0.534)	10.79 (±0.377)
3 (+ Monoglyceride[*])	12.05[a1](±0.534)	9.80[2](±0.534)	10.92 (±0.377)
4 (+ Butyric acid + monoglyceride)	9.16[b1](±0.534)	10.77[2] (±0.534)	9.96 (±0.377)
Mean	10.81 (±0.267)	10.26 (±0.267)	

[a,b] Column means with the same superscript do not differ significantly ($P < 0.05$)
[1,2] Row means with the same superscript do not differ significantly ($P < 0.05$)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The group of birds supplemented with both butyric acid plus monoglyceride without AGP showed a lower count of goblet cells per 100µm compared to the negative control group, the group supplemented with butyric acid and the group supplemented with monoglyceride without AGP ($P < 0.05$). The data also indicated a significant difference between the group of birds supplemented with monoglyceride without AGP and monoglyceride with AGP ($P < 0.05$). The group supplemented with both butyric acid plus monoglyceride without AGP resulted in a lower goblet cell count compared to the group supplemented with both butyric acid plus monoglyceride with AGP ($P < 0.05$). No significant difference was observed between the group of birds supplemented AGP and the group of birds without AGP ($P > 0.05$).

Table 4.7.20. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the number of goblet cells per 100 µm of villi in the jejunum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	10.37[b](±0.633)	9.89(±0.633)	10.13[b](±0.448)
2 (+ Butyric acid[#])	12.76[a](±0.633)	11.03(±0.633)	11.89[a](±0.448)
3 (+ Monoglyceride[*])	11.58[ab](±0.633)	10.82(±0.633)	11.20[ab](±0.448)
4 (+ Butyric acid + monoglyceride)	10.59[b](±0.633)	10.74(±0.633)	10.66[ab](±0.448)
Mean	11.32(±0. 317)	10.62(±0. 317)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The negative control group and the group of birds supplemented with both butyric acid plus monoglyceride without AGP resulted in a lower count of goblet cells per 100 µm in the jejunum compared to the group of birds supplemented with butyric acid without AGP (P < 0.05). However, the group supplemented with monoglyceride without AGP was not significantly different (P > 0.05). There was no significant difference between the AGP supplemented group and the group of birds without AGP (P > 0.05). The group of birds supplemented with butyric acid regardless of AGP inclusion showed a higher number of goblet cells per 100 µm compared to the control groups (P < 0.05).

Table 4.7.21. The effect of the feed additives, butyric acid and monoglyceride, with and without the inclusion of antibiotic growth promoters (AGP), on the number of goblet cells per 100 µm of villi in the ileum of broilers at 33 days of age (± standard error of the mean)

Treatment	Without AGP[α] inclusion	With AGP inclusion	Mean
1 (No additives)	11.45[b](±0.533)	10.32(±0.533)	10.89[b](±0.377)
2 (+ Butyric acid[#])	12.97[a1](±0.533)	11.42[2](±0.533)	12.20[a](±0.377)
3 (+ Monoglyceride[*])	11.71[ab](±0.533)	10.80(±0.533)	11.25[ab](±0.377)
4 (+ Butyric acid + monoglyceride)	10.86[b](±0.533)	11.41(±0.533)	11.14[ab](±0.377)
Mean	11.75[1](±0.267)	10.99[2](±0.267)	

[a,b] Column means with the same superscript do not differ significantly (P < 0.05)
[1,2] Row means with the same superscript do not differ significantly (P < 0.05)
[#]Butyric acid: Novyrate® C (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t)
[*]Monoglyceride: FRA® AC12 (1 kg/t)
[α]Antibiotic growth promoter: Zinc Bacitracin 15% (0.5 kg/t)

The data highlights a significant difference between the group supplemented with butyric acid without AGP (12.97) and the negative control group (11.45) and the group supplemented with both butyric acid plus monoglyceride without AGP ($P < 0.05$). The group of birds supplemented with AGP showed a lower count of goblet cells compared to the group without AGP ($P < 0.05$). There was also a significant difference between the group supplemented with butyric acid without AGP and the group of birds supplemented with butyric acid with AGP ($P < 0.05$). The data also reflects a significant difference in the group average of birds supplemented with butyric acid regardless of adding AGP and the control groups ($P < 0.05$).

4.8 Mortality

There was no significant difference in mortality rate between any of the experimental groups.

Chapter 5

Discussion

Butyric acid is an organic acid that has been studied for years. Literature describes it as an easily available source of energy for gut epithelial cells, stimulator of their multiplication and differentiation which improves feed efficiency (Qaisrani *et al.*, 2015). The supplementation of butyric acid is believed to reduce hindgut protein fermentation, improve gut health and promote microbes that are beneficial for the host, which all will translate to a positive influence on growth performance (Qaisrani *et al.*, 2015). Lauric acid has the greatest antibacterial activity among all the medium chain aliphatic fatty acids and the effect becomes magnified when the acid is esterified to glycerol (Batovska *et al.*, 2009). Monolaurin is active against gram-positive and some cases gram-negative pathogens when combined with substances such as citrate (cation chelator), giving it a broader spectrum (Batovska *et al.*, 2009). From a practical point of view, the main characteristic of a good alternative is that it must improve performance at least as well as AGPs (Huyghebaert *et al.*, 2010).

5.1 Effect of feed additives on broiler performance

In the present study, there was no significant difference between the broilers that received zinc bacitracin and those that did not receive zinc bacitracin in terms of body weight throughout the trial, except on day 14. Leeson *et al.* (2005) reported heavier body weights in birds fed bacitracin compared to all the other treatments, but the difference was not significant. This could be due to the fact that broilers will not respond to a performance promoting additive when in good health and reared under optimal bio-security conditions (García *et al.*, 2007; Nkukwana *et al.*, 2015). In the present study, at 7 and 14 days of age, the group supplemented with butyric acid without AGP resulted in heavier body weights compared to the negative control group. El-Ghany *et al.* (2016) reported an increase in the weight gain of birds supplemented with sodium butyrate compared to their control group in both the grower and finisher phases. The body weight was not significantly different in the present study on 21, 28 and 35 days of age between birds supplemented with butyric acid and the control groups. Similarly to a study done by Leeson *et al.* (2005) and Jerzsele *et al.* (2012), no significant difference on body weights with supplementing butyric acid in the starter, grower and finisher periods was found. The effect of butyric acid on the body weight could be absent due to the inclusion levels in the present study, as Panda *et al.* (2009) indicated that the inclusion level of 0.2% was not sufficient to maintain performance. However, some studies have reported benefits with supplementing at 0.2% inclusion (Leeson *et al.*, 2005; Antongiovanni et *al.*, 2007).

There was no significant difference on body weight with supplementing monoglycerides with and without zinc bacitracin throughout the present study. This was similar to the findings by Zeitz *et*

al. (2015), in a study done on male broilers supplemented with dietary fats rich in lauric acid and myristic acid. However, the study done by Khosravinia (2015) resulted in an improved early weight gain with supplementing a mixture of medium chain fatty acids (C6 to C12) compared to the control group. Khosravinia (2015) suggested that this was due to the reduction of intestinal infection pressure and improved intestinal morphology translating to a better digestive and absorptive capacity. The combination of the two products did not influence the body weight of the birds throughout the current study either.

The cumulative and weekly feed intake in the present study for birds supplemented with zinc bacitracin was also not significantly different compared to the broilers that did not receive zinc bacitracin. The group of birds supplemented with butyric acid resulted in a higher cumulative and weekly feed intake compared to the control groups at 14 and 35 days of age. El-Ghany *et al.* (2016) only reported this increase in feed intake during the first, third and fourth weeks of the cycle. Contrary to the present study, Kaczmarek *et al.* (2016) found that feed intake decreased during the starter period when the birds were given a 0.4 g/kg dose. There was no significant difference between birds supplemented with butyric acid and the control groups at 7, 21 and 28 days of age regarding cumulative and weekly feed intake. This was in agreement with the findings reported by Adil *et al.* (2010) in a study to determine the effect of dietary supplementation of organic acids on broiler performance. There was no significant difference on feed intake with supplementing monoglycerides with and without zinc bacitracin throughout the present study. At 28 and 35 days, the weekly feed intake was lower for the group of birds supplemented with butyric acid plus monoglyceride without zinc bacitracin compared to the group with zinc bacitracin. The cumulative feed intake was not affected by the supplementation of butyric acid plus monoglycerides.

The cumulative FCR at 14 days of age for birds supplemented with zinc bacitracin was significantly lower compared to the group of birds that were not supplemented with zinc bacitracin. The beneficial effect of growth promoter substances on performance is related to efficient use of nutrients, which results in an improved FCR (García *et al.*, 2007). In a study done by Engberg *et al.* (2000), a reduction in the *C. perfringens* counts was reported in the caeca of broiler birds fed zinc bacitracin compared to the control group, which led to the conclusion that the use of dietary antibiotics can suppress outbreaks of necrotic enteritis. Contrary to the present study, Leeson *et al.* (2005), Adil *et al.* (2010), Kaczmarek *et al.* (2016) and Song *et al.* (2017) all reported an improvement in the FCR regardless of the different inclusion levels of butyric acid used in the studies. This was attributed to the improved digestion and absorption of nutrients, as a result of increased pancreatic enzyme secretion and effects on gut mucosa together with their antimicrobial activity (Leeson *et al.*, 2005; Qaisrani *et al.*, 2015; Kaczmarek *et al.*, 2016; Song *et al.*, 2017). The FCR was lower for the group of birds supplemented with monoglycerides with zinc bacitracin compared to the group without zinc bacitracin at 14 days of age. This could be due to the fact that free fatty acids may

cause effects in the digestive tract or in the intermediary metabolism, which results in improved usage of feed (Zeitz *et al.*, 2015). However, this effect is not clear because the zinc bacitracin was also present. The digestion of fat in the digestive tract involves hydrolysis of triglycerides and emulsification of monoglycerides and free fatty acids by bile acids and this forms micelles before absorption occurs (Zeitz *et al.*, 2015). With free fatty acids, this process is not necessary, meaning the short and medium chain fatty acids can be absorbed directly eliminating the need for emulsification and micelle formation (Zeitz *et al.*, 2015). Zeitz *et al.* (2015) reported a lower FCR for birds supplemented with free lauric and myristic acid during the 27 to 35 day period and concluded that the bile acid formation and lipid hydrolysis in the broilers during this period was not limiting and these birds required less lipases for hydrolysis, hence the improvement in feed conversion efficiency resulted. The cumulative and weekly FCR showed a decrease with the combination of the products without zinc bacitracin compared to the group with zinc bacitracin at 35 days of age. This decrease in FCR could be due to the high villi height to crypt depth ratio reported on these birds which indicates better nutrient absorption and utilisation. It can also be attributed to the use of medium chain fatty acids and their direct absorption without the need for emulsification (Zeitz *et al.*, 2015) and the enhancement of healthy tissue turnover and maintenance in the gut from butyric acid allowing for a more efficient use of nutrients in the gut (García *et al.*, 2007).

5.2. **Effect of feed additives on carcass traits**

A significant decrease was revealed in the present study between the groups supplemented with zinc bacitracin and without zinc bacitracin with regards to the viscera weight. Miles *et al.* (2006) also reported a decreased intestinal tract weight for birds supplemented with antibiotics as compared to the control group. However, there was no significant difference recorded in the rest of the treatments. Literature indicates that antibiotics may reduce gut weight, which involves thinner intestinal villi and total gut wall and this has a sparing effect on nutrient usage which improves performance (García *et al.*, 2007). Panda *et al.* (2009) reported an improvement in dressing percentage and reduction in the abdominal fat content of birds supplemented with butyrate at 0.4 % inclusion rate. However, it is not clear what role butyric acid has on the content of abdominal fat of broilers (Panda *et al.*, 2009). Leeson *et al.* (2005) and Panda *et al.* (2009) reported no significant effect on the breast meat yield, both absolute and percentage yield when supplementing with butyric acid. This was not in agreement with the study done by Antongiovanni *et al.* (2007) where an increase in the weight of breasts, thighs and abdominal fat was recorded. There was no significant difference in the present study between treatments in terms of thighs and breast meat weights. However, in the present study the group of birds supplemented with monoglycerides with zinc bacitracin had heavier breast meat percentage yield compared to the positive control group at 35 days of age, this was similar to the findings of Zeitz *et al.* (2015). This may be attributed to the fact that medium chain fatty acids cause a higher percentage of fat to be oxidised and less retained, whereas the usage of breast meat yield may be

enhanced (Zeitz *et al.*, 2015). The group of birds that did not receive zinc bacitracin, that was supplemented with butyric acid without zinc bacitracin and that received both feed additives with and without zinc bacitracin all resulted in heavier wings, but the supplementation with monoglycerides had no significant influence on the weight of the wings. The group of birds supplemented with butyric acid with zinc bacitracin resulted in heavier drumsticks; however no influence was recorded between the other treatments. Birds that did not receive zinc bacitracin showed a heavier breast fillet weight, whereas nothing significant was reported between the other treatments.

5.3. Effect of feed additives on gut morphology of the broiler birds

Intestinal villus height, crypt depth, the ratio of the villi height and crypt depth, the balance of the microbiota and the intestinal bacterial translocation are vital parameters for gut health, recovery and function (Song *et al.*, 2017). The epithelium functions as a natural barrier against pathogenic bacteria and toxic substances (Pelicano *et al.*, 2005). When the epithelium is penetrated by pathogens or chemical substances that are harmful a disturbance results which causes a shift in the microflora and alters the permeability of this natural barrier (Pelicano *et al.*, 2005). Consequently, the gut will undergo changes such as a decrease in villi length, increase in the cell turnover and decrease in the digestive and absorptive activities (Pelicano *et al.*, 2005). Literature correlates long villi with improved gut health, which would translate to better response to growth performance and nutrient absorption and utilisation efficiency (Nkukwana *et al.*, 2015). The crypt depth is referred to as the villus factory, and a large crypt depth indicates fast cell turnover to allow renewal of the villus in response to normal sloughing or inflammation from pathogens or toxins which means there is a high demand for new tissue (Xu *et al.*, 2003; Awad *et al*., 2009). The maintenance of the gut has a higher demand for nutrients such as energy and protein compared to other organs and any additional tissue turnover will increase nutrient requirements for maintenance causing a reduction in the efficiency of the animal (Xu *et al.*, 2003). Literature indicates that addition of organic acids such as butyric acid effectively reduce the pH in the intestine, which inhibits pathogenic microorganisms that are sensitive to low pH such as *Clostridiidium* sp, *Salmonella* sp and *Escherichia coli* and allowing the villi to grow (Pelicano *et al.*, 2005). Butyric acid had no significant antimicrobial effect against *C. perfringens* as indicated by Timbermont *et al.* (2010), but the synergistic effect of butyric acid and medium chain fatty acids resulted in the best protection for birds predisposed to necrotic enteritis. Timbermont *et al.* (2010) suggested that this could be because of the elimination of both the *Eimeria* lesions and *C. perfringens* bacteria. The butyric acid improved the efficiency of the gut in terms of nutrient absorption and more nutrients were available for growth.

In the present study it was reported that the villi height in the duodenum of birds supplemented with zinc bacitracin was shorter compared to birds that were not supplemented with the antibiotic at 20 and 33 days of age. Nkukwana *et al.* (2015) reported narrow or thinner villous width with

supplementing zinc bacitracin but could not explain why it reduced the villi properties compared to the other treatments in that study. Panda *et al.* (2009) also reported an increase in the villi length in the duodenum of the group of birds supplemented with butyric acid compared to the control groups. In the present study at 33 days, the group supplemented with butyric acid without zinc bacitracin had longer villi compared to the group supplemented with butyric acid with zinc bacitracin. There was a significant increase in the duodenum villi height for birds supplemented with monoglycerides with and without zinc bacitracin compared to the positive and negative control groups, respectively. The group of birds supplemented with monoglycerides without zinc bacitracin showed higher villi height compared to the group of birds supplemented with monoglyceride with zinc bacitracin at both 20 and 33 days of age. The combination of the products revealed an increase in the villi height in the duodenum compared to the control groups at 20 and 33 days of age. The groups supplemented with butyric acid plus monoglycerides without zinc bacitracin had a significantly longer villi length compared to the group with zinc bacitracin at 20 and 33 days of age.

At 20 days, villi in the jejunum of birds that were not given the zinc bacitracin were longer than those of the group that was supplemented with zinc bacitracin. However, at 33 days of age there was no significant difference noted in the villi length in the jejunum between the two control groups. In contrary to the study done by Antongiovanni *et al.* (2007), the present study reported an increase in the villi height in the jejunum of birds supplemented with butyric acid without zinc bacitracin compared to the group supplemented with zinc bacitracin at 20 days of age. However, there was no significant difference reported at 33 days of age. This could be the reason why the birds did not show any significant difference in body weight, feed intake and feed conversion ratio at 21, 28 and 33 days of age and could be suggested that the birds may respond to butyric acid at different inclusion levels with age. The jejunum is responsible for digestion and absorption of all the major nutrients such as starch, and an increase in length would mean improved digestive capacity (Kadhim *et al.*, 2011; Svihus, 2014). The supplementation of monoglycerides without zinc bacitracin revealed an increased villi height in the jejunum compared to the group with zinc bacitracin and the negative control group at 33 days of age. The villi length in the jejunum showed an increase for the group supplemented with butyric acid plus monoglycerides with zinc bacitracin compared to the positive control group at 20 days. At 33 days of age, the negative control group resulted in a shorter villi length in the jejunum compared to the group supplemented with butyric acid plus monoglycerides without zinc bacitracin.

The villi length in the ileum did not differ between the two control groups on both 20 and 33 days of age. In the second experimental study done by Kaczmarek *et al.* (2016), an increase was reported in the villi height in the ileum from the group of birds supplemented with butyric acid with zinc bacitracin compared to the positive control group, but no difference at 33 days of age. This was in accordance with the first experiment done by Kaczmarek *et al.* (2016), which also resulted in an increased villi height in the ileum of the birds at 35 days of age supplemented at 0.3 g/kg. No

significant difference was reported in the ileum regarding villi height for birds supplemented with monoglycerides in the present study at 20 and 33 days of age. In the ileum, the data revealed a shorter villi length for the group supplemented with butyric acid plus monoglycerides with zinc bacitracin compared to the group without zinc bacitracin at 33 days of age. This increase villi length with monoglycerides could be attributed to the antibacterial effect of the acids in the crop and stomach of the birds which prevented harmful bacteria from reaching and colonising the caeca and allowing better villi development as more nutrients are available (Hermans *et al.*, 2012). Due to the lack of response at 33 days in the gut, it could be concluded that the body weight was not influenced because the gut was also not influenced by the supplementation of monoglycerides. The increase in villi length could be attributed to the fact that supplementing with butyric acid causes a decrease in bacterial colonisation in the intestine resulting in less damage of the epithelial cells from toxic components produced by pathogenic microbiota (Adil *et al.*, 2010; Qaisrani *et al.*, 2015).

No significant difference was reported in the duodenal crypt depth for birds with and without zinc bacitracin in the feed at 20 and 33 days of age. Contrary to the study done by Leeson *et al.* (2005), supplementing the birds in the present study with bacitracin resulted in a reduction in the villi crypt depth in the duodenum compared to the control group. Contrary to Panda *et al.* (2009), the present study found no significant difference in the crypt depth in the duodenum of birds supplemented with butyric acid and the control group similar to Adil *et al.* (2010) findings. Supplementation of monoglycerides with zinc bacitracin resulted in an increased crypt depth of the duodenum at 20 days of age compared to the group without zinc bacitracin. No significant difference was reported with supplementing monoglycerides compared to the control groups at 33 days of age. The crypt depth in the duodenum of birds in the negative control group revealed an increase compared to the group of birds supplemented with butyric acid plus monoglycerides without zinc bacitracin only at 20 days of age. An increase in the crypt depth of the duodenum was reported for birds supplemented with butyric acid plus monoglycerides with zinc bacitracin compared to the group without zinc bacitracin at 33 days of age.

The positive control group showed a reduced jejunal crypt depth at 20 days of age compared to the negative control group. In the present study at 33 days of age, the crypt depth in the jejunum showed no significant difference between the two control groups. However, the crypt depth in the jejunum revealed a decrease in the group of birds supplemented with butyric acid without zinc bacitracin compared to the negative control group, but an increase with the group supplemented with butyric acid with zinc bacitracin compared to the positive control group only at 20 days of age. A greater crypt depth was also reported in the jejunum for birds supplemented with 2 g/kg of butyric acid glycerides compared to the control group, indicating higher proliferative cellular activity or cell turnover (Antongiovanni *et al.*, 2007). The group supplemented with monoglycerides with zinc bacitracin showed an increase in the crypt depth of the jejunum compared to the positive control

group at 20 days of age. The data revealed a higher jejunal crypt depth in the group of birds supplemented with monoglycerides without zinc bacitracin compared to the group with zinc bacitracin at 33 days of age, but nothing significant compared to the control groups. The supplementation of the two products with zinc bacitracin resulted in a decreased crypt depth in the jejunum compared to the negative control group at 20 days. The positive control group showed a decrease in the crypt depth of the jejunum at 20 days compared to the group supplemented with butyric acid plus monoglycerides with zinc bacitracin. Whereas, the negative control group resulted in an increased crypt depth in the jejunum compared to the group supplemented with butyric acid plus monoglycerides without zinc bacitracin at 33 days of age.

Miles *et al.* (2006) also reported a reduced crypt depth in the ileum for birds supplemented with antibiotics compared to the negative control group, but only in the fourth week instead of the third week as in the present experiment. In the present study at 33 days of age, the crypt depth in the ileum showed no significant difference between the two control groups. The ileum showed a lower crypt depth for birds supplemented with butyric acid without zinc bacitracin compared to the negative control group at 20 days. Adil *et al.* (2010) and Kaczmarek *et al.* (2016) reported no significant difference in the crypt depth of the ileum, which was also the case with the present study at 33 days of age. The group of birds supplemented with monoglycerides without zinc bacitracin resulted in a decreased crypt depth in the ileum compared to the negative control group and the groups with monoglycerides with zinc bacitracin at 20 days of age, but at 33 days difference was not significant. In the ileum, the supplementation of the two products without zinc bacitracin resulted in a decrease in the crypt depth at 20 and 33 days of age compared to the negative control group. Adil *et al.* (2010) and Kaczmarek *et al.* (2016) reported no significant difference in the crypt depth of the ileum, which was also the case with the present study at 33 days of age. This decrease in crypt depth through the gut indicates a slower tissue development and less inflammation (Pelicano *et al.*, 2005; Miles *et al.*, 2006). It can be suggested that due to the low demand for tissue renewal more nutrients are available for growth instead of gut maintenance. Pelicano *et al.* (2005) suggested that the crypt depth will increase to compensate for a loss in the villi height in the gut. These morphological changes that result in the gut are associated with an increase in the pancreatic and digestive enzymes activity, which is similar to zinc bacitracin's mode of action (Engberg *et al.*, 2000; Adil *et al.*, 2010; Nkukwana *et al.*, 2015). The lack of pancreatic enzyme hydrolysis in the intestine is suggested to decrease the digestibility of the dietary components and suppress growth (Kadhim *et al.*, 2011).

The ratio between the villi height and the crypt depth is regarded as an important parameter for intestinal health (Kaczmarek *et al.*, 2016). A higher ratio indicates a long villus in which the epithelium is sufficiently matured and functionally active, in combination with a shallow crypt with constant cell renewal (Kaczmarek *et al.*, 2016). The villi height to crypt depth ratio of the duodenum was higher for the group supplemented with butyric acid without zinc bacitracin compared to the

negative control group at 20 days of age and nothing significant was reported at 33 days of age. This high ratio translates to maximum absorption and digestion capacity and it is essential to animal development (Pelicano *et al.*, 2005). A significantly higher ratio was reported at 20 days of age for the group of birds supplemented with butyric acid without zinc bacitracin compared to the negative control group and the group supplemented with butyric acid with zinc bacitracin. The villi height to crypt depth ratio in the duodenum of birds supplemented with monoglycerides without zinc bacitracin was reported to be higher compared to the negative control group and the group with zinc bacitracin at 20 days of age. There was no significant difference with supplementing monoglycerides at 33 days compared to the control groups in the duodenum. The group of birds supplemented with butyric acid plus monoglycerides without zinc bacitracin resulted into a high villi height to crypt depth ratio compared to the negative control group and the group supplemented with butyric acid plus monoglycerides with zinc bacitracin at 20 and 33 days in the duodenum of the chicken.

A lower ratio was reported in the jejunum for the positive control group compared to the negative control group at 20 days of age. However, no significant difference between these groups was reported regarding the villi height to crypt depth ratio in the jejunum at 33 days and in the ileum at 20 and 33 days of age. The villi height to crypt depth ratio in the jejunum of birds supplemented with monoglycerides without zinc bacitracin was reported to be higher compared to the negative control group and the group with zinc bacitracin at 20 days of age. However, Zeitz *et al.* (2015) reported decreased villi to crypt ratio in the jejunum of the birds supplemented with the lauric and myristic free fatty acid. However, the jejunum showed a higher ratio for the group supplemented with monoglycerides with zinc bacitracin compared to the group without zinc bacitracin at 33 days of age. The villi height to crypt depth ratio was reported higher for birds supplemented with butyric acid plus monoglycerides without zinc bacitracin compared to the negative control group at 20 and 33 days in the jejunum. The positive control group resulted with a lower ratio compared to the group supplemented with butyric acid with zinc bacitracin in the jejunum at 33 days of age.

The villi height to crypt depth ratio in the ileum was higher for the group supplemented with butyric acid without zinc bacitracin compared to the negative control group at 20 days of age. The ratio in this study was influenced at 20 days of age and this could be the reason why a response in body weight was only observed at 7 and 14 days of age. The villi height to crypt depth ratio in the ileum of birds supplemented with monoglycerides without zinc bacitracin was reported to be higher compared to the negative control group and the group with zinc bacitracin at 20 days of age. There was no significant difference with supplementing monoglycerides at 33 days compared to the control groups in the ileum. The data revealed a higher ratio in the ileum for birds supplemented with butyric acid plus monoglycerides without zinc bacitracin at 20 days of age compared to the negative control group, but nothing significant at 33 days of age.

The mucus-gel layer is regarded as the first line of defense that prevents foreign bacteria and pathogens from invading the intestinal mucosa (Forder *et al.*, 2007; Pan & Yu, 2014). The evaluation of mucin and the cells that secrete mucin also play a role in the protection of the intestinal epithelium from infections, maintenance of the gut integrity, the immune status and absorption of cations such as Ca^{2+} (Uni *et al.*, 2003; Pan & Yu, 2014; Song *et al.*, 2017). Mucin synthesis and secretion is suggested to be influenced by agents that uncouple glycosylation and protein synthesis, diet types and different intestinal microbial populations (Uni *et al.*, 2003). The goblet cells secrete polymeric mucin glycoproteins which compete with bacteria for adherence via heterogenous oligosaccharide chains and preventing toxins from having contact with the epithelial cells (Forder *et al.*, 2007). The mucins are vital sources of carbon, nitrogen and energy for some commensal and pathogenic bacteria (Pan & Yu, 2014). Literature indicates that specific bacteria attach to the mucin layer and degrade the mucin with specific enzymes (Pan & Yu, 2014). Birds affected with NE may result in a reduction in the mucin-2 gene expression which means less protection of the birds against infections (Song *et al.*, 2017). It was then suggested that *C. perfringens* may be mucolytic and increased host mucus production is linked to coccidiosis or viscous intestinal environment (Jia *et al.*, 2009). However, some literature also indicates that high intestinal viscosity reduces nutrient absorption by the host animal, fast feed passage rate and enhanced mucus production (Jia *et al.*, 2009). Forder *et al.* (2007) suggested that the presence of the neutral mucin in the jejunum and ileum was the result of increased intestinal maturity to facilitate the breakdown of complex carbohydrates and serve as a protective mechanism against invasion by pathogenic bacteria. The biosynthesis of mucin is altered by changes in the rate of migration of epithelial cells moving from the proliferating crypt zone and by perturbations in the rates of differentiation of precursor cells into mature goblet cells (Uni *et al.*, 2003). The mucus layer found in the small intestine is vital in protecting the small intestinal epithelial cells and transporting substances between the lumen and the brush border membrane (Uni *et al.*, 2003). Forder *et al.* (2007) reported a drastic change in the mucin composition in conventionally reared birds at 4 days of age which coincided with an increase in immune system development. Where an up-regulation of mRNA expression of proteins involved in immune function such as antimicrobial peptides and pro-inflammatory cytokines was increased in the gut-associated lymphoid tissue (Forder *et al.*, 2007). Butyrate has a strong capacity to enhance synthesis of endogenous antimicrobial host defense peptides (HDPs) in humans, rabbits and chickens (Sunkara *et al.*, 2011; Pan & Yu, 2014), which are critical components of the animal's innate immunity (Pan & Yu, 2014; Bauwens, 2016). They are small cationic peptides which function to kill various intestinal pathogens by disrupting cell membrane permeability leading to cell lysis (Pan & Yu, 2014).

No significant difference was observed in the number of goblet cells in the duodenum at 33 days of age for both control groups. The supplementation of butyric acid without zinc bacitracin showed an increase in the number of goblet cells per 100µm in the duodenum compared to the

negative control group at 33 days of age. The group of birds supplemented with monoglycerides without zinc bacitracin resulted in an increase in the number of goblet cells compared to the group of birds with zinc bacitracin at 33 days in the duodenum. This suggests that supplementing monoglycerides has the capability to improve the first line of defence for birds. Uni *et al.* (2003) indicated that after hatch the proportion of goblet cells is similar and increases in number with age throughout the small intestine. The physiological importance of the different mucin subtypes is not well documented, but it is suggested that the acidic mucin protects against bacterial translocation whereas the sulphated mucin is less degradable by bacterial glycosidases (Uni *et al.*, 2003; Forder *et al.*, 2007). Santin *et al.* (2001) suggested that a reduction in the number of goblet cells may indicate that the gut is not exposed to stressing conditions. Lauric acid with its high antimicrobial activity against *C. perfringens* together with the effects of butyric acid such as anti-inflammatory effects, reinforcing the colonic defence barrier by increasing production of mucins and host antimicrobial peptides, increasing the expression of tight junction proteins, lowering extracellular pH and providing energy to the epithelial cells may be the reasons for all the gut morphological changes in this study (Timbermont *et al.*, 2010; Pan & Yu, 2014). A decrease in the number of goblet cells was reported in birds supplemented with butyric acid plus monoglycerides without zinc bacitracin compared to the negative control group at 33 days of age in the duodenum.

No significant difference was observed in the number of goblet cells in the jejunum at 33 days of age for both control groups. The supplementation of butyric acid without zinc bacitracin showed an increase in the number of goblet cells per 100µm in the jejunum compared to the negative control group at 33 days of age. The group of birds supplemented with monoglycerides without zinc bacitracin resulted in no significant difference in the number of goblet cells compared to the group of birds with zinc bacitracin at 33 days in the jejunum. No significant difference was reported in the number of goblet cells in birds supplemented with butyric acid plus monoglycerides without zinc bacitracin compared to the negative control group at 33 days of age in the jejunum.

The ileum on the other hand, resulted in an increased number of goblet cells at 33 days of age for the negative control group compared to the positive control group which could mean that there was a need for protection in the intestine. The supplementation of butyric acid without zinc bacitracin showed an increase in the number of goblet cells per 100µm in the ileum compared to the negative control group at 33 days of age. The ileum had an increase in the number of goblet cells in the group supplemented with butyric acid without zinc bacitracin compared to the group with zinc bacitracin at 33 days of age. This increase in goblet cells in the ileum was expected with the supplementation of butyric acid because butyric acid has been reported to increase the production of mucins and host antimicrobial peptides (Timbermont *et al.*, 2010; Pan & Yu, 2014). The group of birds supplemented with monoglycerides without zinc bacitracin resulted in no significant difference in the number of goblet cells in the jejunum. The number of goblet cells in birds supplemented with butyric acid plus

monoglycerides without zinc bacitracin compared to the negative control group at 33 days of age was not significantly different in the ileum. Uni *et al.* (2003) concluded that the mucus layer develops in the late embryonic and immediate post-hatch period, and its development is influenced by the time of access to feed. Delayed access to first feed was associated with a decrease in the number of enterocytes and an increase in the density of goblet cells in the jejunum and ileum (Uni *et al.*, 2003). The increased mucosal development in the gut is suggested to be caused by the bacterial-diet interactions and the need for a greater absorptive area to accommodate the by-products from microbial fermentation (Forder *et al.*, 2007).

Chapter 6

Conclusion

The birds responded to the supplementation of butyric acid (1 kg/t) in terms of BW at 7 and 14 days of age compared to the negative control. This could indicate that butyric acid has a more significant effect during early life when the microbiome is still immature. The butyric acid (Starter- 1 kg/t, Grower- 0.75 kg/t, Finisher- 0.25 kg/t) resulted in an increased FI at 14 and 35 days compared to the negative control group. A significant increase in the wing percentage weight was observed with supplementation of butyric acid without AGP compared to the group with AGP. However, the drumstick weight percentage was higher for the birds supplemented with butyric acid with AGP compared to the group without AGP. The gut showed a significantly higher response with supplementing butyric acid in terms of the villi height, crypt depth and goblet cells in the duodenum, jejunum and the ileum of the birds. The monoglyceride (1 kg/t) had a significantly higher BW regardless of the addition of AGP at 28 days of age compared to the control groups.

The birds responded to the monoglyceride with AGP in terms of breast meat with bone weight percentage compared to the positive control group. The birds responded with an increase in the villi height in the duodenum, jejunum and the ileum with supplementing monoglycerides without AGP compared to the control groups and the groups with AGP. The crypt depth only decreased in the ileum of the birds supplemented with monoglyceride without AGP compared to the negative control group at 20 days of age. The combination of the two products without AGP caused a reduction in the FI at 28 and 35 days compared to the combination with AGP.

The birds responded to the combination of the feed additives without AGP by significantly lowering the FCR at 35 days. The combination had a significantly higher wing weight percentage compared to both the positive and negative control groups. An increase in the villi height was observed with supplementation of both products and a decrease in the crypt depth and goblet cells in the group of birds without AGP compared to the negative control. This study revealed that both butyric acid and monoglyceride have an effect on the gut morphology of the birds and these changes have beneficial impacts on the performance of the birds. More research is required to understand the effect these products have on the microflora of the birds which relates to the histological changes reported in this study. The beneficial implications of the products influencing the number of goblet cells is still unclear, however this study has indicated a slight correlation between goblet cells and gut changes which need further investigating.

Chapter 7

Critical Review and Recommendations

This study did not use birds challeneged with a pathogenic bacterial load due to the fact that the birds were reared in a commercial house. The inclusion levels used in this study were all recommended levels from the product suppliers. The lack of response in terms of BW, FI and FCR could be due to the absence of a challenge in the birds as it has been shown that birds will only respond to growth promoting feed additives when challenged. Therefore, the findings of this study are only based on birds reared in a clean and controlled environment. There could be some benefit in looking into increasing the inclusion levels of butyric acid in birds as they grow instead of reducing the inclusion levels with age. The combination of the products should be studied further as there may be a synergistic effect shown in this study. More research is needed with challenged birds to understand the protective mechanisms these products may provide for the birds when challenged.

Chapter 8

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Appendix A. Temperature programme of the Broiler house.

Day	Front Temperature		Rear Temperature	
	Lower Temp	Upper Tem	Lower Temp	Upper Temp
0	28.3°c	28.1°c	28.1°c	34.4°c
1	33.6°c	33.0°c	33.0°c	36.3°c
2	33.1°c	32.3°c	32.3°c	35.7°c
3	31.8°c	31.7°c	31.7°c	36.4°c
4	31.6°c	31.3°c	31.3°c	34.3°c
5	30.9°c	30.7°c	30.7°c	33.2°c
6	30.5°c	30.7°c	30.7°c	32.3°c
7	29.7°c	29.1°c	29.1°c	32.2°c
8	29.0°c	28.6°c	28.6°c	31.9°c
9	28.8°c	28.3°c	28.3°c	31.8°c
10	28.2°c	28.5°c	28.5°c	36.0°c
11	28.0°c	27.6°c	27.6°c	30.3°c
12	27.3°c	26.8°c	26.8°c	29.2°c
13	26.8°c	26.6°c	26.6°c	29.8°c
14	26.8°c	26.7°c	26.7°c	33.9°c
15	26.4°c	26.3°c	26.3°c	30.7°c
16	25.6°c	25.5°c	25.5°c	31.9°c
17	25.1°c	25.4°c	25.4°c	27.5°c
18	25.1°c	25.3°c	25.3°c	29.0°c
19	24.8°c	25.0°c	25.0°c	30.4°c
20	25.1°c	25.9°c	25.9°c	28.7°c
21	24.2°c	24.4°c	24.4°c	31.5°c
22	24.1°c	24.3°c	24.3°c	29.9°c
23	24.0°c	24.2°c	24.2°c	30.7
24	23.8°c	23.9°c	23.9°c	27.9°c
25	23.5°c	23.6°c	23.6°c	27.4°c
26	24.4°c	24.1°c	24.1°c	29.5°c
27	23.1°c	23.2°c	23.2°c	30.5°c
28	21.9°c	22.3°c	22.3°c	28.1°c
29	22.1°c	22.2°c	22.2°c	30.1°c
30	23.9°c	23.7°c	23.7°c	31.7°c
31	21.2°c	21.3°c	21.3°c	27.8
32	22.6°c	22.6°c	22.6°c	29.9°c
33	24.2°c	23.9°c	23.9°c	29.5°c
34	23.8°c	25.4°c	25.4°c	29.9°c
35	24.8°c	24.9°c	24.9°c	29.4°c