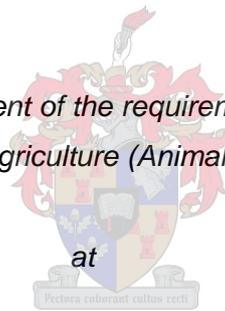


ALTERNATIVES TO REPLACE ANTIBIOTICS IN
BROILER DIETS: EFFECTS ON PROTEIN
UTILIZATION AND PRODUCTION PERFORMANCE

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated), and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Different substances were evaluated and compared to an antibiotic, in terms of their effect on nitrogen - and amino acid digestibilities. Two digestibility trials and one performance trial were conducted.

Trials one and two apparent nitrogen (AND)- and amino acid (AAD) digestibilities were determined from digesta collected at the terminal ileum (ileal digestibility method). In Trial 3 the substances were evaluated in terms of their potential to improve production performance. Broilers were fed a maize-soya based diet throughout the three trials.

In the first trial, garlic and a commercial prebiotic (Bio-Mos®), were tested and compared in terms of AND and AAD, to an antibiotic (doxycyclin, Doxyvete-SOS). A starter and finisher diet were fed as either mash or pellets. The garlic was included at 8g/kg, 13g/kg and 18g/kg to the starter and finisher diets. Bio-Mos® was added at 1g/kg, 2g/kg and 3g/kg to the starter diet, and 0.5g/kg, 1g/kg and 1.5g/kg to the finisher diet. The doxycyclin was added at 0.3 g/kg. None of the treatments had any beneficial effects in terms of AND. Feeding a pellet seem to have some negative effects in terms of AND. In general most of the treatments did not show any improvement in AAD at any determination period (day 21, 28 or 35). At day 21 and day 35, the mash diet supplemented with 18g/kg garlic had a negative effect on AAD, when compared to the negative and positive control. It doesn't seem that feeding either a mash or a pellet had an influence on the effects exerted by the different treatments.

In the second trial the influence of Bio-Mos®, a blend of organic acids, probiotics and electrolytes (Acid-Pak 4-way®) and a medium-chain triglyceride (MCT) were evaluated and compared in terms of AAD and AND, to the effect of an antibiotic, doxycyclin. The starter and finisher diets were fed as a mash. Bio-Mos® was included at 1g/kg, 2g/kg, and 3g/kg in the starter diet, and at 0.5g/kg, 1g/kg, 1.5g/kg in the finisher diet, respectively. Acid-Pak 4-way® was included at 0.4g/kg, 1g/kg and 1.6g/kg for both the starter and finisher diets. Medium-chain triglycerides (MCT) were allocated at 3g/kg, 3.6g/kg, 4.2g/kg for the starter diet, and 2.1g/kg, 2.7g/kg and 3.4g/kg for the finisher diet. An antibiotic, doxycyclin, was included at 0.3 g/kg. With AND, no treatment had any significant effect for the entire experimental period. At day 21, the treatment supplemented with MCT (3.4g/kg) had the most significant beneficial effect on AAD, when compared to the negative- and positive controls, as it increased AAD for the majority of the amino acids. The treatment with Acid-Pak 4-way® (1g/kg) had the most significant negative effect on AAD when compared to the positive control. At day 28, the treatments with Bio-Mos® (0.5g/kg and 1.5g/kg) and Acid-Pak 4-way® (0.4g/kg) had the most significant beneficial effect on AAD when compared to the positive control. It increased AAD for more than half of the 17 amino acids evaluated. The treatment supplemented with MCT (2.7g/kg) has shown the most significant negative effect on AAD, when compared to the positive control.

In the third trial the effect of Bio-Mos®, Acid-Pak 4-way® and MCT on production performance was evaluated, and compared to the effects of the presence or absence of doxycyclin. Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured. The

starter and finisher diets were fed as a mash. Bio-Mos®, MCT and Acid-Pak 4-way® were included at 3.0g/kg, 4.2g/kg and 1.6g/kg, respectively in the starter and finisher diets. Birds were weighed (per pen) on arrival and on days 7, 14, 21, 28, 35. Feed intake (FI) per pen was measured at days 7, 14, 21, 28 and 35, and mortality was recorded daily. In terms of BWG, Acid-Pak 4-way® had a higher BWG, when compared to the negative control, Bio-Mos® and MCT.

It can be concluded that Bio-Mos®, Acid-Pak 4-way®, as well as MCT can be a possible alternatives to antibiotic supplementation. These three treatments did not necessary prove to be more effective than antibiotics, but are definitely competitive alternatives.

OPSOMMING

Verskillende behandelings is geëvalueer en vergelyk met 'n antimikrobiële produk, in terme van hul uitwerking op stikstof- en aminosuur verteerbaarheid. Twee verteringsstudies en produksieprestasiestudie is uitgevoer.

In die eerste twee studies is die skynbare stikstof (AND)- en aminosuur (AAD) verteringskoëffisiënte bepaal deur gebruik te maak van digesta wat by die terminale ileum ingesamel is (ileale verteringsmetode). In die derde studie is die produksieprestasiestudie van braaikuikens op 'n gebalanseerde metaboliseerbare energie (AME) rantsoen, soos beïnvloed deur die verskillende behandelings, geëvalueer.

In die eerste studie is knoffel en 'n kommersiële prebiotikum (Bio-Mos®) geëvalueer en met 'n antibiotikum (doksisisiklien, Doxyveto-SOS) in terme van AND en AAD vergelyk. Beginner- en afrondingsrantsoene is as 'n meel of pille gevoer. Die knoffel is teen 8g/kg, 13g/kg en 18g/kg in die rantsoen ingesluit. Bio-Mos® is teen 1g/kg, 2g/kg en 3g/kg in die beginner rantsoen en teen 0.5g/kg, 1g/kg en 1.5g/kg in die afrondingsrantsoen, ingesluit. Die antibiotikum is teen 0.3g/kg in beide rantsoene ingesluit. Geen van die behandelings het enige positiewe invloed op AND gehad nie. Deur 'n verpilte rantsoen te voer het sekere negatiewe invloed op AND gehad. Oor die algemeen het geen behandelings enige positiewe invloed op AAD gehad nie. Op dag 21 en 35 het die insluiting van knoffel teen 18g/kg in 'n meel rantsoen 'n negatiewe invloed op AAD gehad, wanneer dit met die negatiewe- en positiewe kontroles vergelyk is. Dit blyk nie dat om 'n pil of meel te voer enige invloed op die invloede van die verskillende behandelings gehad het nie.

In die tweede studie is Bio-Mos®, 'n organiese suur (Acid-Pak 4-way®) en 'n medium-ketting trigliseried (MCT) geëvalueer en met 'n antibiotikum, doksisisiklien (Doxyveto-SOS) in terme van AND en AAD, vergelyk. Beginner- en afrondingsrantsoene is gevoer as 'n meel. Bio-Mos® is teen 1g/kg, 2g/kg, and 3g/kg in die beginner rantsoen en teen 0.5g/kg, 1g/kg, 1.5g/kg in die afrondingsrantsoen, ingesluit. Acid-Pak 4-way® is teen 0.4g/kg, 1g/kg en 1.6g/kg vir die beginner –en afrondingsrantsoene ingesluit. Die MCT is teen 3g/kg, 3.6g/kg, 4.2g/kg in die beginner rantsoen en teen 2.1g/kg, 2.7g/kg en 3.4g/kg in die afrondingsrantsoen ingesluit. Die antibiotikum is ingesluit teen 0.3g/kg. Geen behandelings het enige betekenisvolle invloed in terme van AND gehad nie. Op dag 21 het MCT (3.4g/kg), in vergelyking met die negatiewe- en positiewe kontrole, die grootste positiewe invloed op AAD gehad. Acid-Pak 4-way® (1g/kg) het, in vergelyking met die positiewe kontrole, 'n positiewe invloed gehad op AAD. Op dag 28, het Bio-Mos® (0.5g/kg en 1.5g/kg) en Acid-Pak 4-way® (0.4g/kg) die grootste positiewe invloed op AAD gehad. Die behandeling met MCT (2.7g/kg) het die mees negatiewe invloed op AAD gehad.

In die derde studie is die insluiting van Bio-Mos®, Acid-Pak 4-way® en MCT getoets om die invloed op braaikuiken produksieprestasiestudie te evalueer, en te vergelyk met die invloed van die insluiting of afwesigheid van 'n antibiotikum. Liggaamsmassa (BW), liggaamsmassa toename (BWG), voerinnome

(FI) en voeromsetverhouding (FCR) is gemeet. Beginner- en afrondings rantsoene is gevoer as 'n meel. Bio-Mos®, MCT en Acid-Pak 4-way® is onderskeidelik teen 3.0g/kg, 4,2g/kg en 1.6g/kg in die rantsoen ingesluit. Die kuikens is met aankoms (per hok) geweeg, asook op dae 7, 14, 21, 28, 35. Voeriname per hok is gemeet op dae 7, 14, 21, 28 en 35. Mortaliteit is daaglik aangeteken. Die insluiting van Acid-Pak 4-way® het in vergelyking met die negatiewe kontrole, Bio-Mos® en MCT insluiting 'n hoër BWG tot gevolg gehad.

Die gevolgtrekking wat gemaak kan word is dat Bio-Mos®, Acid-Pak 4-way® en MCT gebruik kan word as 'n moontlike alternatief vir antibiotika insluiting. Hierdie drie behandelings was nie noodwendig meer effektief as die antibiotika nie, maar het wel bewys dat dit kompeterende alternatiewe is.

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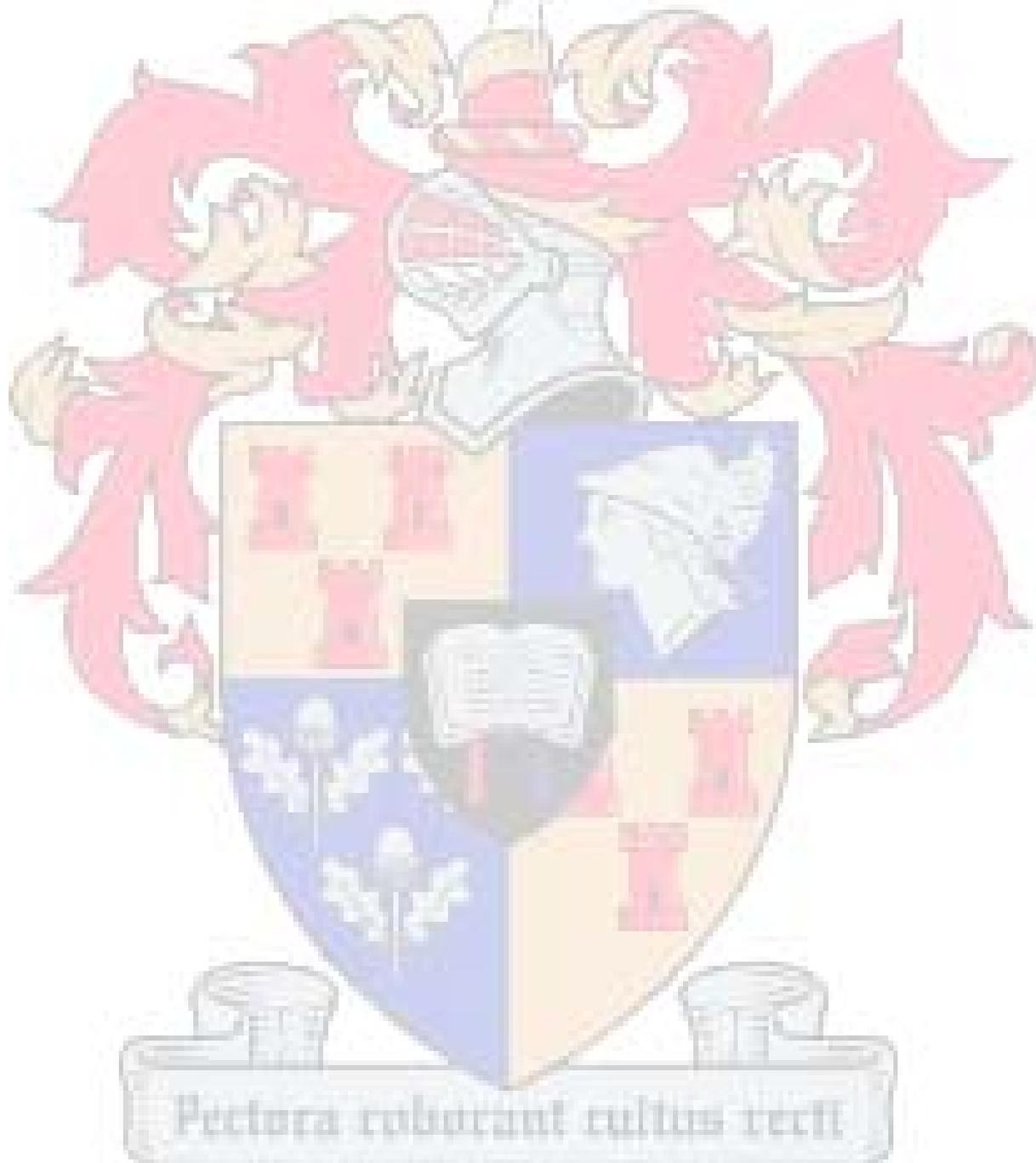
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Chapter 1

General introduction

The main aim of nutrition is to optimise production efficiency under intensive farming conditions. This is usually achieved when flock health is optimised as well (Whitehead, 2002). As a result, broiler diets are supplemented with a number of additives aimed at improving performance, feed intake and thereby optimizing feed utilization (Whitehead, 2002; Wenk, 2003). The growing pressure from consumers on welfare organizations and non-governmental organizations to produce safe and healthy food has become an area of great concern. Human and animal health, as well as the protection of the environment should be considered in modern farming systems.

Enteric diseases are a great concern in the poultry industry due to the loss of productivity and increase in mortality. Therefore, the role of antibiotics in preventing these enteric diseases has long been investigated. An antibiotic can be defined as a chemically complex antimicrobial substance derived from microbial fermentation or synthetic structural derivatives thereof, and that is antagonistic to microbial growth in very low concentration (ACVM Group, 2000). Antimicrobial growth promoters (AGPs) are antibiotics that are included at low levels in rations. At inclusion levels that are lower than therapeutic levels, antibiotics can enhance animal growth and feed efficiency (Maritz, 2005).

Antimicrobials are mainly used in animal production for the treatment and healing of disease. However, it can also be used as growth promoters. The use of antibiotics as growth promoters has made intensive farming possible by means of improved production efficiency through the improvement of feed conversion of animals (Hernandez *et al.*, 2004). The argument about the use of antibiotic growth promoters (AGPs) is part of a worldwide debate about practices of intensive animal farming (Maritz, 2005). There are some concerns regarding the use of antimicrobials as growth enhancers and as a result, alternative feed formulation and management strategies that exclude antimicrobial growth promoters must be developed and evaluated under intensive farming conditions (Collet & Dawson, 2001). Therefore there has been a search for alternative substances to replace antibiotics. Possible alternatives include probiotics, prebiotics, feed enzymes and organic acids.

The main objective of this thesis was to evaluate a number of alternative substances to replace antibiotics as a feed additive in poultry rations and to determine the potential positive or negative effects it may have on production performance and protein digestibility in broilers.



Chapter 2

Literature Review

2.1 Introduction

A large percentage of the feed ingredients consumed by a chicken are in a form that requires chemical and other reactions before it can be utilized by the bird. The alimentary canal is a long tube through which the food passes while these reactions take place. Therefore, digestion can be described as those changes that occur in the alimentary canal to make it possible for feed to be absorbed through the intestinal wall and enter the bloodstream (North & Bell, 1990).

The small intestine is the part of the intestinal tract where most of the digestion and absorption of nutrients take place (Dibner & Richards, 2004). It consists of three parts, i.e. the duodenum, jejunum and ileum. The pancreas is imbedded in the duodenal loop, and secretes the enzymes that are essential in the digestion of lipids (i.e. lipases), starch (i.e. amylases) and proteins (i.e. proteases) in the small intestine. Another function is to neutralize acids that are found in the mixture passed on from the stomach. Most of the absorption takes place in the jejunum. The third section, the ileum, is where enzymes are produced, and contains mainly indigestible material. Bile secreted by the liver, assists with digestion by breaking up large particles, especially fat. The lower part of the intestinal tract consists of the colon and the ceca. These two parts contain mostly the indigestible portions of the feed, i.e. fibre (cellulose). No digestion or absorption of nutrients takes place here (Dibner & Richards, 2004; Guo *et al.*, 2004a, North & Bell, 1990).

Intestinal microflora of animals play an important role in the health status, and especially in the digestion and absorption of feed ingested by the host. It takes part in the metabolism of dietary nutrients such as carbohydrates, protein, lipids and minerals and also in the synthesis of vitamins (Jin *et al.*, 1997). Whilst pathogenic bacteria are always present in the gut, the balance of non-pathogenic and pathogenic bacteria will strongly influence the disease status of the bird. Intestinal bacteria can be divided into species that exert either harmful (pathogenic) or beneficial effects. The intestinal tract contains many micro-organisms like bacteria or viruses. Some of these organisms are harmless and aid in digestion. However, others cause tremendous problems e.g. *Salmonella enteritidis* and are difficult to eliminate (Guo *et al.*, 2004b). Other organisms do not actually cause disease, but impair the functioning of the digestive enzymes. Therefore, a common approach to maintain health of the host animal is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens. The composition and activity of intestinal microbiota can be altered by diet composition and dietary manipulations such as the use of feed additives and antibiotics (Guo *et al.*, 2004b).

Chickens are stressed by various factors such as transportation to the growing site, overcrowding, vaccination, chilling and/or overheating. These factors tend to create an imbalance in the intestinal microflora and a lowering of body defence mechanisms. Under such circumstances, antimicrobial feed additives are often used to suppress or eliminate harmful organisms in the intestine, and to improve

growth and feed efficiency (Jin *et al.*, 1997). Enteric diseases are a problem that continuously challenges the poultry industry through the loss of productivity, increased mortality, and associated contamination of poultry products (Patterson & Burkholder, 2003). It is therefore that so-called bacterial suppressants are added to the feed to depress the proliferation of those bacteria which are harmful to digestion, and thus lowers feed conversion. Moore *et al.* (1946) reported on the first research that indicated the positive effects of antibiotics, such as sulfasuxidine, streptothricin, and streptomycin on chicken growth.

2.2 Antibiotics

Antibiotics have commonly been used in the poultry feed industry in various ways, to improve growth and feed utilisation efficiency and also as a tool in the efficient production of animal products such as milk, meat, eggs and feathers. Therapeutic use involves administering antimicrobials at the highest regulated inclusion levels for a limited period to individual animals showing signs of disease. Prophylactic use involves antimicrobial inclusion for a limited period to large or small groups of healthy animals deemed to be at risk of disease caused by pathogens susceptible to drugs (Cromwell, 1999; ACVM Goup, 2000).

The benefits of AGP's in animal production can be identified as environmental, performance enhancing and the control of disease. AGP's modify the intestinal flora to improve digestion, metabolism and absorption of a variety of essential nutrients. They eliminate Gram-positive bacteria that are associated with the production of undesirable or toxic metabolites and therefore, poorer health and performance of the animal. There are additional direct effects on gut morphology such as increased villus height and surface area and increased mucin secretion. The result is an optimal environment for the intestinal mucosa, which allows efficient nutrient absorption. Consequently nutrient utilization, feed conversion ratio and weight gain are improved. The effect of antibiotics is prominent in young growing animals especially under poor climatic and management conditions (ACVM Group, 2000; Van Immerseel *et al.*, 2002; Wenk, 2003).

The effect of withdrawal of dietary antibiotics on growth and feed conversion efficiency of different farm animals is represented in Table 2.1.

Table 2.1 Effect of withdrawal of antibiotics as performance promoters on growth performance and feed conversion efficiency in different species of farm animals (Wenk, 2003).

	Reduction in daily body mass gain (%)	Increase in feed per gain (%)
Veal calf production	7-8	4-5
Beef production	4	2
Weaned piglets	8	5
Growing pigs	5	3
Fattening pigs	2	1
Pig production	5	2
Growing chickens	3	2

According to Wenk (2003), the withdrawal of antibiotics had the greatest effect on reduction in daily body mass gain in veal calf production and weaned piglets, but seems to have less effect on fattening pigs and growing chickens. It also had the greatest effect on feed intake in veal calf production and weaned piglets.

The controversy surrounding the use of in-feed antimicrobials arises from their use as growth promoters, where antimicrobials are administered to large numbers of healthy animals for long periods at low concentrations, in order to increase the rate and efficiency of growth. The continued use of these AGP's at sub-therapeutic levels has caused concern amongst consumers to the extent that they have forced legislators in Europe to take action regarding the use of AGP's in animal feeds (ACVM Group, 2000; Sun *et al.*, 2005). Antibiotic feed additives were linked to the emergence of multiple drug resistant bacteria. The increase in the level of resistance in bacteria against many of the commonly used antibiotics in human medicine therefore raised major concerns, which resulted in the search for alternatives (Collett & Dawson, 2001; Van Immerseel *et al.*, 2002). Improved bio security, vaccination and genetic selection are some strategies that can be followed to reduce the use of antibiotics (Sun *et al.*, 2005).

A successful alternative to AGP's should comply with certain characteristics. It should be able to mimic the mode of action or effect of the antimicrobial, and therefore have a significant beneficial impact on animal production and health which can be reflected in improved digestion, nutrient metabolism and absorption, as well as a decrease in incidence of diseases. It should also be generally regarded as safe (GRAS) to both the animal and human, be easy to apply and store and be cost-effective. Dry powder products, for instance, are easier to handle than liquid products. Low inclusion rates, heat stability and long shelf-life are all qualities that will make the product more attractive (Collett & Dawson, 2001).

2.3 Probiotics and Prebiotics

Probiotic and prebiotic foods have been consumed by humans for centuries, either as natural components of food, or as fermented foods. The poultry industry has become conscious of the use of probiotics and prebiotics as a potential alternative for antibiotics. Table 2.2 shows the characteristics of probiotics and prebiotics. The proposed mechanisms by which probiotics and prebiotics exert their effects, include competition for substrates, production of toxic compounds that inhibit pathogens, and competition for attachment sites (Patterson & Burkholder, 2003; Angel *et al.*, 2005). Table 2.3 shows the general beneficial effects of probiotics and prebiotics.

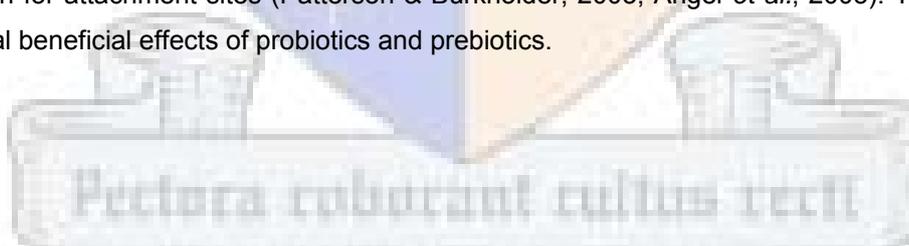


Table 2.2 Characteristics of ideal probiotics and prebiotics (Patterson & Burkholder, 2003).

Probiotics	Prebiotics
Be of host origin	Be neither hydrolysed or absorbed by mammalian enzymes or tissues.
Non-pathogenic	Selectively enrich for one or a limited number of beneficial bacteria.
Withstand processing and storage	Beneficially alter the intestinal microbiota and their activities.
Resist gastric acid and bile	Beneficially alter luminal or systemic aspects of the host defence system.
Adhere to epithelium or mucus	
Persist in the intestinal tract	
Produce inhibitory compounds	
Modulate immune response	
Alter microbial activities	

Table 2.3 Beneficial effects of probiotics and prebiotics (Patterson & Burkholder, 2003).

Probiotics	Prebiotics
Modify intestinal microbiota	Increase production of volatile fatty acids (VFA)
Stimulate immune system	Increase biomass and stool bulking
Reduce inflammatory reactions	Increase B vitamin synthesis
Prevent pathogen colonization	Improve mineral absorption
Enhance animal performance	Prevent cancer
Decrease carcass contamination	Lower serum cholesterol
Decrease ammonia and urea excretion	Lower skatole-, indole-, phenol production, etc.

2.3.1 Probiotics

An alternative method for modifying the gut microflora involves the feeding of probiotics (Whitehead, 2002). Probiotics, or otherwise known as direct-fed microbials (DFM's), can be defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989; Collett & Dawson, 2001; Angel *et al.*, 2005). The statement about probiotics being "live" cells differentiates them from chemical modifiers of the intestinal environment (Partridge, 1991).

The intestine has a mucosa which works as a selective barrier allowing the passage of useful substances and preventing the entering of undesirable agents into the bloodstream. Therefore the health of this mucosa is essential for efficient feed conversion, maintenance and growth, and thus to the well-being of the animal. Healthy birds are generally considered as having a well functioning intestinal tract, and an important characteristic of a healthy, well-functioning intestinal tract is the balance of its microbial population. When animals are stressed through factors such as overcrowding, environmental fluctuations, handling and transport, the population balance of the intestinal microflora gets disturbed. This tends to favour the development of pathogens. The aim of probiotics is to

maintain the population balance in favour of beneficial bacteria. It has been found that continuous probiotic supplementation aid in maintaining that balance (Jin *et al.*, 1997; Cencic *et al.*, 2006).

2.3.1.1 Modes of action

Probiotics that have been specifically investigated for use in livestock include *Bacillus*, *Enterococcus*, *Lactobacillus*, *Saccharomyces* and yeast cultures (Patterson & Burkholder, 2003). The benefits of probiotics are based on two main functions, i.e. stimulating the growth of beneficial microflora in the gastrointestinal tract and suppression of the growth of pathogenic bacteria by means of competitive exclusion (Wenk, 2003). It has been proposed that probiotics compete for substrates, produce toxic compounds inhibitory to pathogens, and competitively exclude potentially pathogenic bacteria by adhering to attachment sites. Mixed bacterial cultures reduce intestinal wall colonisation of pathogens and, therefore, reduce the quantity of toxins produced (Patterson & Burkholder, 2003).

Studies have shown that a low pH in the upper small intestine of monogastric animals helps to suppress the growth of pathogens such as *Escherichia coli* or *Salmonella*. Therefore probiotics used in poultry should stimulate the formation of lactic acid bacteria (LAB) (Wenk, 2003). The antagonistic activity of LAB towards pathogens can be attributed to the production of bacterial substances. Among those produced by lactobacilli are bacteriocins, organic acids and hydrogen peroxide. Bacteriocins are defined as compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action. Antagonism by lactic acid bacteria has also been associated with major end products of their metabolism. The best known are organic acids such as lactic and acetic acids and hydrogen peroxide. Acetic and lactic acids inhibit the growth of many bacteria including pathogenic Gram-negative organisms (Jin *et al.*, 1997).

Another approach is a technique called competitive exclusion. It was found that the resistance, of newly hatched chickens, against *Salmonella* infection could be increased by dosing them with a suspension of gut contents derived from healthy adult chickens (Jin *et al.*, 1997; Van Immerseel *et al.*, 2002).

The gastrointestinal tract of the chick is immunologically prone to rapid colonization by beneficial and pathogenic bacteria during the first 3 to 4 weeks post-hatch (Sun *et al.*, 2005). To avoid transmission of any unsuspected pathogenic bacteria present in the intestinal suspension to the host animals, the components of the protective intestinal flora have to be known.

2.3.1.2 Studies

Researchers have reported that supplementing broiler and layer diets with probiotics leads to improved production performance. Numerous studies with broilers have indicated that supplementation with probiotic preparations improve live weight gain and feed conversion rate, and markedly reduce mortality. It has also been reported that the supplementation of probiotics increases the egg production and feed conversion of layers (Jin *et al.*, 1997). Literature suggests that the effects

may vary between preparations as well as with different environmental and management conditions (Priyankarage *et al.*, 2003b).

Variation in the effects of probiotics on chickens, can possibly be ascribed to differences between the strains and forms of bacteria used, as well as to differences in the level of inclusion in animal diets. The lack of consistency in results contributes to the uncertainty of the positive effects of probiotics in chickens. Given the correct strain of bacteria, optimal concentration in the diets and relatively stress-free conditions, probiotic supplementation should have beneficial effects on production performance in broilers (Jin *et al.*, 1997; Olnood *et al.*, 2007).

Broilers

Several studies found that broilers gained more weight when fed diets supplemented with commercial lactobacilli, compared to birds fed diets without the supplementation of commercial lactobacilli. The birds that were fed lactobacilli in the feed had significantly lower feed to gain ratio i.e. amount of feed needed to gain weight. When adherent *Lactobacillus* cultures, isolated from the GIT of chickens, were used there was an improvement in body weight and feed to gain ratios. It was found that the highest growth rate was obtained when broilers were fed a concentration of 0.1% *Lactobacillus* cultures (Jin *et al.*, 1997).

Yeo and Kim (1997) reported that feeding a diet containing a probiotic (*Lactobacillus casei*) significantly increased the average daily weight gain during the first three weeks of the study period. This increase in weight gain was partly accounted for by increased feed intake. It also indicated a significant decrease ($P < 0.05$) in urease activity in the small intestine of young chicks and can therefore be beneficial for improving animal health and growth.

Another study tested three different commercial probiotic mixtures and compared it to a commercial antibiotic growth promoter. It was found that the weight improvement of the birds fed on the probiotic-containing diets was comparable with birds fed on the antibiotic-containing diets. Significant ($P < 0.05$) positive effects were accomplished only during the starter period for both probiotics and antibiotics (Priyankarage *et al.*, 2003b).

In a study where a live probiotic product containing *Enterococcus faecium* were compared against a negative control (i.e. containing no antibiotic treatment), some positive effects were found. Weight gain and feed conversion ratio were significantly ($P < 0.05$) improved. The same probiotic was also tested against a diet containing antibiotic treatment, but no significant differences were found in terms of performance. These results demonstrate that probiotics can act as a viable alternative to antibiotic feed additives (Leary & Partridge, 2008).

There are also studies that produced no positive results in terms of production performance. A study with host-specific and non-host specific *Lactobacillus*, as well as a commercial product containing 40×10^9 cfu/ml *Lactobacillus* showed no improvement in chicken growth. Another study found no significant

($P \geq 0.05$) difference in body weight of chickens fed with diets containing *L. acidophilus* and *S. faecium* from 8-60 days of age (Jin *et al.*, 1997). Another study with a commercial probiotic (Protexin) showed no significant effect on the performance of the birds (Priyankarage *et al.*, 2003a). In another trial conducted with Cobb broiler chickens, four *Lactobacillus* strains were compared to an antibiotic. The addition of probiotic *Lactobacillus* spp. to the feed did not improve body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) (Olnood *et al.*, 2007).

Layers

Probiotics have also been tested in laying birds. In layers probiotic supplementation also indicated an increase in egg production and feed conversion (Jin *et al.*, 1997). A series of studies were done to investigate the effects of *Lactobacillus* on layer performance, where either 1100mg/kg or 2200mg/kg *Lactobacillus* were fed. An increase in egg size, egg mass, and egg weight, as well as an improvement in body weight gain was observed. Egg production, however, was not influenced by either of the two treatments. Diets supplemented with *Lactobacillus* also increased feed consumption and body weight gain in pullets from 7-19 weeks. During the laying phase (20-59 weeks), layers fed a diet with *Lactobacillus* produced larger eggs than those fed a similar diet without *Lactobacillus* (Nahashon *et al.*, 1996). Tortuero and Fernandez (1995) reported that supplementation with a mixed culture of *L. acidophilus* and *L. casei* improved hen-day egg production, feed conversion ratio, egg weight and albumen quality. Mohan *et al.* (1995) found a 5% improvement in egg production in layers fed a diet supplemented with 100 mg/kg probiotic.

Goodling *et al.* (1987) reported no increase in hen-day egg production, feed efficiency, and egg size in the case of layers fed a liquid non-viable *Lactobacillus* product, a dried non-viable *Lactobacillus* product, or a viable *Lactobacillus* product during a 48 week experimental period. The reasons for the ineffectiveness of *Lactobacillus* were because of the non-host specific *Lactobacillus* used and the fact that the birds were being kept under relatively ideal conditions.

2.3.2 Prebiotics

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. They occur naturally in some feedstuffs of plant origin and in lower organisms like yeast cells (Fenster, 2001).

In principle only non-digestible, fermentable feed components are prebiotics. Most of these prebiotics are carbohydrates. These carbohydrates are divided in groups such as mono-, di-, oligo- and polysaccharides, based on their molecular length (Van Immerseel *et al.*, 2002; Wenk, 2003). Carbohydrates have been used as prebiotics to influence the composition of the bacterial populations in the large intestine (Guo *et al.*, 2004b).

2.3.2.1 Modes of action

Prebiotics are always feed ingredients that are not digested by the host, none or little used and/or metabolised as they pass through the upper portion of the intestinal tract, so that they can reach the flora of the large intestine. Secondly, prebiotics have to be able to serve as a substrate for one or more bacterial species and should also have a potentially beneficial effect on the host, and finally, they have to be able to cause a shift in the microflora in the gut that improves the health of the host (Gibson & Roberfroid, 1995; Van Immerseel *et al.*, 2002; Patterson & Burkholder, 2003).

The prebiotics most predominantly studied are fructo-oligosaccharide products (FOS, oligofructose, inulin) but research into the use of other possible compounds has been conducted (Gibson & Roberfroid, 1995). Fructo-oligosaccharide products (FOS) have demonstrated some potential for improving the health and growth rates of poultry (Collet & Dawson, 2001). Prebiotics can bind with fimbria of pathogenic Gram-negative bacteria, such as *E.coli* and *Salmonella*. Mannan-oligosaccharides (MOS), derived from yeast cell walls, also bind the fimbria of pathogenic bacteria (e.g. *E.coli*, *Salmonella*) to prevent them from attaching to, and therefore colonizing on, the mucosa of the small intestine. Diets supplemented with MOS affect a chicken's intestinal microflora (i.e. increasing villus height; improving uniformity and integrity) and reduce susceptibility to *S. enteritidis* colonization (Fenster, 2001; Patterson & Burkholder, 2003; Sun *et al.*, 2005).

2.4 Synbiotics

Prebiotics and probiotics are only two of quite a few approaches that have the potential to reduce enteric diseases in poultry. The combination of these two additives is known as synbiotics (Patterson & Burkholder, 2003). Synbiotics can also be defined as products produced by fermentation. The combination could improve the survival of the probiotic organism, because its specific substrate is available for fermentation. The advantages offered by both the live microorganism and the prebiotic can be beneficial to the host. Examples of synbiotics are FOS, *Bifidobacteria*, lactitol and *Lactobacilli* (Van Immerseel *et al.*, 2002).

2.5 Essential oils, herbs and botanicals

Herbs, spices and various plant extracts have received increased attention as possible antibiotic growth promoter replacements. The mere fact that plant or herbal extracts are natural makes it a preferred choice as an alternative to antibiotics for consumers (Hernandez *et al.*, 2004).

Herbs are non-woody, flowering plants famous for its' medicinal properties, flavour and aroma. Spices are defined as any of a class of spicy or aromatic substances of vegetable origin such as pepper, cinnamon and cloves used e.g. for seasoning and as preservatives. A drug made from a part of a plant, is known as a botanical. Plant extracts or essential oils possessing a distinct aroma are used mainly in the production of perfumes, flavourings and pharmaceuticals, but some are also of interest in animal nutrition because of their antimicrobial and antioxidative properties (Wenk, 2003).

Essential oils, herbs and botanicals used in animal feed are reported to have a beneficial effect by promoting feed intake and digestive secretions, stimulating the immune system. They may also have antibacterial, coccidiostatic, antiviral, anti-inflammatory and antioxidant properties (Wenk, 2003). Plant extracts could control and limit the growth of numerous pathogenic and non-pathogenic species of bacteria in the gut (Hernandez *et al.*, 2004). Plants are rich in a wide array of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties *in vitro* (Cowan, 1999; Lewis *et al.*, 2004). The fragrance of plants is carried in the so-called essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure, and they are called terpenes. When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Terpenoids activate against multiple types of microorganisms such as Gram-positive - and Gram-negative bacteria, fungi and protozoa (Cowan, 1999).

These type of additives can be difficult to regulate, because they may contain unacceptable levels of pesticides, heavy metals and other contaminants. It is also difficult to measure the activity of the relevant compounds in these additives and variation in activity may be due to differences in geographical growing areas, season, harvesting time, storage conditions and extraction method.

2.5.2 Studies

A few studies have been conducted to test the possible antimicrobial properties in several plant extracts.

Lewis *et al* (2004) tested broiler performance between 7 and 27 days of age. In this study garlic (*Allium vineale*), horseradish (*Armoracia rusticana*), juniper (*Juniperus species*), milk thistle (*Silybum marianum*), oregano (*Oreganum vulgare*) and yarrow (*Achillea millefolium*) were tested. The birds were fed a basal diet (control) which was supplemented with either an acid blend or one of the botanical extracts. All of the plant extracts were allocated at 2 (higher and lower) concentrations. Feed consumption and weight gain were measured. No treatment differences were observed in any of the performance parameters measured during day 7 to day 17 of age. The differences only became significant from day 17 to day 27 of age. The birds that were fed on the higher levels of garlic and yarrow showed the best feed conversion efficiency (FCE) ($P < 0.05$). Birds fed on the higher level of garlic also tended ($P = 0.079$) to have higher weight gains. Another study investigated the effect of five herbal natural feed additives i.e. Nor-Spice, Oregano Powder, Du-Sacch C Powder (FOS), Quiponin S Powder (products from the *Quillaia saponaria* tree), Nor-Spice S Garlic Powder and Nor-Spice Thyme Powder as alternatives for antimicrobial growth promoters. Growth performance and some intestinal traits in broilers were tested. Garlic resulted in higher body weight gains at 14 days ($P < 0.05$). Birds fed garlic, quiponin, thyme and antibiotic in their diets consumed more feed than the broilers fed Du-Sacch ($P < 0.05$). Between days 0 to 42 the supplements had no effect on body weight gain, feed intake and feed:gain ratio ($P > 0.05$). However, from days 0-14 the chickens fed on quiponin, garlic and thyme

gained more body weight than the chickens fed on the diets supplemented with an antibiotic (Demir *et al.*, 2005).

In a study a Chinese herbal medicine (CHM) formulation was tested and compared to an antibiotic (virginiamycin). The CHM consisted of 14 herbs and was allocated at four different concentrations. The herbs were processed and ground prior to addition to the feed. The CHM dietary treatments produced increased body weight gain at days 7-21, compared to the VRG groups. However, no increase in body weight gain was detected at days 21-28. The CHM groups also had a higher feed intake (FI) and feed conversion ratio (FCR) than the VRG groups between days 21-28. It was concluded that the only considerable increase in growth performance with the CHM groups happened between days 7-21 but not afterwards (Guo *et al.*, 2004a). Another study was conducted to compare the effect of an organic acid (formic acid) and plant extracts (blend of oregano, cinnamon, and pepper essential oils) to that of an antibiotic (avilamycin). It was found that the effect of formic acid and the plant extract were similar to avilamycin, and that these compounds were beneficial for improving growth traits and nutrient apparent ileal digestibility. A positive effect of formic acid on intestine mucosa was also observed (García *et al.*, 2007). Broiler chicks were also fed four levels of dried garlic for 35 days. It has proved to increase average daily weight gain ($P < 0.05$) during the first 21 days (Horton *et al.*, 1992).

Another study proved that garlic can also be beneficial in other species, such as pigs. A study was conducted with growing-finishing pigs. A herb mixture containing great nettle (*Urtica dioica*), garlic (*Allium sativum*) and wheat-grass (*Agropyron repens*) were included in the feed. These herb mixture supplementations have improved ($P < 0.05$) daily gains in the growing and finishing periods by 6% and 5% respectively. The herb mixture treatment resulted in a 10% lower ($P < 0.05$) feed conversion ratio (FCR) than the control diet. (Grela *et al.*, 1998).

2.6 Organic acids

Supplementing diets with organic acids can also cause enhanced growth, improved performance and increased productivity. Organic acids can suppress pathogenic bacteria in the intestine by lowering the pH, and therefore providing an unfavourable acidic environment for their functioning and proliferation. The faster the acids are absorbed, the smaller the pH lowering effect. This effect is directly influenced by the acid binding capacity of the diet. Furthermore, the digestibility of nutrients can be improved by organic acids (Wenk, 2003). By adding organic acids to the drinking water, it was shown to decrease *Campylobacter* numbers in the caeca (Sun *et al.*, 2005).

A mixture of dietary organic acids lowered the mortality rates during the first two weeks of the birds' life. It was also indicated that, in general, dietary organic acids improve growth performance and nutrient digestibility when fed to pigs (Pirgozliv *et al.*, 2007). The practice of acidifying poultry starter diets and drinking water systems was initially introduced as a means of improving animal health through the prevention of pathogen-induced intestinal upset. A study done with broilers, with acidification of drinking water with a mixture of organic and inorganic acids decreased mortality and

improved feed conversion. Results are shown in Table 2.4. The salt content and the buffering capacity of the diet can influence the effect of the acids (Collet & Dawson, 2001).

Table 2.4. Effects of acidification on mortality and feed efficiency of broilers (Collet & Dawson, 2001).

	Control	Acidification of water
Flock #1		
Number of birds	20 000	20 000
Mortality	3.1	2.9
Feed conversion	1.96	1.91
Flock #2		
Number of birds	20 000	20 000
Mortality	3.5	3.0
Feed conversion	1.95	1.91

Organic acids can be metabolised, and therefore also represent an energy source. Finally they can also improve the hygienic quality of meat with the suppression of undesired microorganisms like *Salmonella* or *Campylobacter* (Wenk, 2003).

2.7 Medium-Chain Triglycerides (MCT)

Medium-chain triglycerides (MCT) have fatty acids 6 to 12 carbon atoms long and can be easily utilized by pigs and chickens as an energy source. Medium-chain triglycerides can be more easily digested and absorbed than long-chain fatty acids, mainly because of their size and solubility (Lee & Chiang, 1994; Turner *et al.*, 1999). A study with neonatal pigs indicated that MCT was effective as an energy source, but did not improve growth or survival from birth to weaning (Lee & Chiang, 1994).

2.8 Aim of study

There are limited reports available to support the beneficial effects of alternatives on production performance in broilers. Studies revealed conflicting results; therefore there is still much scope for more research and new developments in terms of finding alternative solutions to replace antibiotics in feed rations.

In order to be a true alternative to growth promoting antibiotics, the proposed products must show a similar mode of action. This is said for some probiotics and prebiotics, herbal extracts and organic and inorganic acids. The poultry industry has become aware of the use of probiotics and prebiotics as a potential alternative for antibiotics, and it seems to be the most popular candidates in the quest for finding alternatives for the use of antimicrobials. However, further insight is needed.

The objective of the study was to evaluate various alternatives, such as garlic, Bio-Mos® and Acid-Pak 4-way®, in an attempt to find a suitable candidate that can be used as an alternative to antibiotics in poultry diets.

Chapter 3

The effect of fresh garlic and Bio-Mos[®], a commercial prebiotic, on nitrogen (N)-and amino acid digestibilities in broilers

3.1 Introduction

The concern surrounding the use of antibiotics as growth promoters in intensive agriculture systems has resulted in producers searching for possible alternatives to antibiotics. The suitability of these substances as substitutes for antibiotics, and more specifically the effect of these substances have not been intensively investigated.

Garlic (*Allium sativum*) has been prescribed as a traditional medicine for many years. Recent investigations have shown several therapeutic and prophylactic properties in garlic (Horton *et al.*, 1992). Early steps in identifying the active constituents of garlic were the discovery that the compound allicin (allyl 2-propene thiosulfinate) is formed when garlic cloves are crushed and that its formation depends upon the action of the enzyme aliinase of the bundle sheath cells upon the alliin of mesophyll cells (Ross *et al.*, 2001). Mannan oligosaccharides (MOS), for instance Bio-Mos[®], are derived from a specific strain of yeast. It consists of a mannan and a glucan component. The structure of the mannan component resembles that of the carbohydrates on the wall of the intestine. Pathogenic, growth-inhibiting microbes normally adhere to the mannans on the wall of the intestine, but bind to the mannan component of the Bio-Mos[®] instead. Because these pathogens do not attach to the gut wall, they are flushed from the upper gut. Therefore Bio-Mos[®] plays an essential role in animal nutrition and performance in that it prevents pathogens from adhering to the intestinal wall (Sun *et al.*, 2005). In poultry Bio-Mos[®] has a positive effect on flock health and viability, feed efficiency and weight gain, profitable egg production, improved chick quality and better meat and egg marketability (Waldroup *et al.*, 2003).

The objective of this trial was to evaluate the effect of fresh garlic and Bio-Mos[®] on apparent nitrogen (AND) and amino acid (AAD) digestibilities in broilers, and to compare their effects to that of an antibiotic (doxycyclin).

3.2 Materials and methods

One hundred and forty-four day-old female broiler chicks (Cobb 500) were placed in wire-mesh cages (three chicks per cage). The experimental units/cages were allocated at random to the 16 (A to P) experimental treatments with three replicates per treatment. The birds were vaccinated at day-old against Newcastle disease and Infectious bronchitis. The environmental temperature within the house was 30°C for the first day and was then decreased by 0.5°C every second day until 20°C was

reached. Continuous light (24L:0D) was provided for the duration of the trial. Birds were offered *ad libitum* access to feed and water. The experiment had a completely randomised design with main effect of dietary treatment. The three experimental trials were approved by the University of Stellenbosch Animal Ethics Committee.

3.3 Experimental design

A starter (0 to 21 days) and a finisher (22 to 35 days) broiler diet were formulated using the EFG Broiler Nutrition Optimizer Program (Winfeed 2 Feed Formulator, 2005). The ingredients used in the diets, as well as the formulated nutrient composition of the diets are shown in Table 3.1.

Table 3.1. Composition of starter and finisher diets based on as fed basis (g/kg).

Ingredient	Starter	Finisher
Maize	441.85	587.39
Maize gluten 60	19.52	58.52
Soybean full fat	50.00	50.00
Soybean oil cake meal (46%CP)	382.38	218.38
L-lysine HCl	2.06	3.67
DL-methionine	1.27	0.85
L-threonine	0.13	0.40
Choline chloride 60%	1.51	0.51
Vit + min premix	1.50	1.50
Limestone	16.69	17.70
Salt	1.72	1.75
Monocalcium phosphate	16.58	16.64
Sodium bicarbonate	4.78	2.70
Sunflower oil	60.00	40.00
<i>Calculated nutrient content(*)</i>		
AMEn (MJ/kg)	11.88	13.00
Crude protein (%)	24.70	20.92
Lysine (%)	1.52	1.25
Methionine (%)	0.50	0.44
Methionine + Cystine (%)	0.91	0.82
Threonine (%)	0.96	0.82
Tryptophan (%)	0.29	0.21
Arginine (%)	1.67	1.24
Isoleucine (%)	1.14	0.92
Leucine (%)	2.20	2.19
Histidine (%)	0.66	0.55
Phenylalanine (%)	1.17	1.00
Tyrosine (%)	1.00	0.84
Phenylalanine + Tyrosine (%)	2.17	1.85
Valine (%)	1.25	1.05
Calcium (%)	1.00	1.00
Available Phosphorus (%)	0.50	0.50

(*) Amino acids expressed on a digestible basis

The starter and finisher diets were fed as either mash or pellets. Fresh garlic was included at three levels of 8g/kg, 13g/kg and 18g/kg. Bio-Mos® was included at three levels of 1g/kg, 2g/kg and 3g/kg for the starter diet, and 0.5g/kg, 1g/kg and 1.5g/kg for the finisher diet. Celite® was included at 20g/kg as an indigestible marker in all dietary treatments. The antibiotic, doxycyclin, was added to treatments B and D (positive control) at 0.3 g/kg. Treatments A and C were the control diets (negative control). A summary of the dietary treatments is shown in Table 3.2.

Table 3.2 A description of the dietary treatments used throughout the trial.

Treatment	Description
A	Control diet without antibiotic (mash)
B	Control diet with antibiotic (mash)
C	Control diet without antibiotic (pelleted)
D	Control diet with antibiotic (pelleted)
E	Control + Bio-Mos® L1 (mash)
F	Control + Bio-Mos® L2 (mash)
G	Control + Bio-Mos® L3 (mash)
H	Control + Bio-Mos® L1 (pelleted)
I	Control + Bio-Mos® L2 (pelleted)
J	Control + Bio-Mos® L3 (pelleted)
K	Control + garlic L1 (mash)
L	Control + garlic L2 (mash)
M	Control + garlic L3 (mash)
N	Control + garlic L1 (pelleted)
O	Control + garlic L2 (pelleted)
P	Control + garlic L3 (pelleted)

All starter diets were removed on the morning of day 14 and the finisher diets were allocated to the respective cages. On the morning of day 21, one bird per cage was stunned and killed by cervical dislocation (Ten Doeschate *et al.*, 1993). Immediately after the chickens were killed the stomach was opened up and the ileum exposed. A 15cm segment of the gastrointestinal tract (GIT) of the terminal ileum, 2cm anterior to the ileo-caecal junction (to avoid contamination with urine), was removed. Each segment was emptied by gently squeezing the segment between the thumb and the forefinger so as to prevent damage to the intestinal mucosa. After most of the contents have been removed, each segment was flushed with distilled water (Ten Doeschate *et al.*, 1993; Sun *et al.*, 2005). Digesta samples from individual birds were pooled within a treatment and immediately frozen in a freezer at -20°C to avoid bacterial contamination of the samples. The samples were then freeze-dried and finely ground to pass through a 0.75 mm sieve and then stored at -20°C for later chemical analysis. The procedure was repeated on the mornings of day 28 and 35.

Duplicate samples of both dietary treatments and the excreta samples were analyzed for nitrogen (N) content with a Leco N analyzer. A Leco N analyzer determines the % N content from a 0.1g sample by means of a combustion process (AOAC, 1990). Digesta and feed were hydrolysed for 24 hours with 6N hydrochloric acid at 110°C. This was done for the determination of AA by HPLC using the Pico-Tag® system from Waters Chromatography Systems.

The apparent ileal digestibility coefficients were calculated for nitrogen (N) and amino acids (AA) using the following equations (Ravindran *et al.*, 1999).

Apparent nitrogen digestibility (AND)

$$\text{AND} = \frac{(\text{N/AIA})_d - (\text{N/AIA})_i}{(\text{N/AIA})_d}$$

where $(\text{N/AIA})_d$ = ratio of N to acid-insoluble ash in the diet

and $(\text{N/AIA})_i$ = ratio of N to acid-insoluble ash in the ileal digesta

Apparent amino acids digestibility (AAD)

$$\text{AAD} = \frac{(\text{AA/AIA})_d - (\text{AA/AIA})_i}{(\text{AA/AIA})_d}$$

where $(\text{AA/AIA})_d$ = ratio of AA to acid-insoluble ash in the diet

and $(\text{AA/AIA})_i$ = ratio of AA to acid-insoluble ash in the ileal digesta

Levene's test was used to test for homogeneity of variance and the Kolmogorov-Smirnov test was used to test for normal distribution of the data. All data of equal variance and normal distribution were analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0 (Waldroup *et al.*, 2003; Angel *et al.*, 2005). The Bonferroni (Dunn) t-test was used to test for differences between treatment means where the treatment effect was found to be significant. The probability level was set at 5%.

3.4. Results and discussion

Results

The results of AND coefficients for the entire experimental period are presented in Table 3.3. At days 21 and 28 none of the treatments had any significant effect ($P=0.207$ and $P=0.405$ respectively) on AND. At day 35, the treatments supplemented with garlic (pellet), as well as the highest level Bio-Mos® (1.5g/kg, mash), showed a negative effect ($P<0.0001$) on AND, when compared to the rest of the treatments. The rest of the treatments showed no significant differences in their effects on AND, when compared to the treatments with (positive control) or without (negative control) doxycyclin supplementation. The only differences between feeding a mash or pellet were seen with the treatments supplemented with garlic at the lowest (8g/kg) and middle (13g/kg) inclusion levels. It showed that the pelleted treatments had a negative effect ($P<0.0001$) on AND.

It can be concluded that none of the treatments improved AND, when compared to the positive or negative controls. It seems that feeding a pelleted feed had a negative effect on AND, because the

negative effects were more pronounced. The reason for this could be that the temperature involved in the pelleting process can have a negative influence on the activity of the products, garlic and Bio-Mos®.

Table 3.3 Apparent Nitrogen digestibility (AND) coefficients (\pm SE) for 21, 28 and 35 days.

Period Treatment	D21		D28		D35	
	N	AND	N	AND	N	AND
C+Ma	2	0.652 (0.109)	2	0.715 (0.020)	2	0.726 (0.004) ^a
C+Pe	2	0.756 (0.003)	2	0.699 (0.001)	2	0.782 (0.001) ^a
C-Ma	2	0.759 (0.004)	2	0.744 (0.026)	2	0.774 (0.001) ^a
C-Pe	2	0.715 (0.045)	2	0.723 (0.008)	2	0.698 (0.013) ^a
GARL1Ma	2	0.665 (0.032)	2	0.719 (0.017)	2	0.728 (0.004) ^a
GARL1Pe	2	0.710 (0.000)	2	0.674 (0.024)	2	0.222 (0.146) ^c
GARL2Ma	2	0.592 (0.044)	2	0.600 (0.009)	2	0.680 (0.014) ^a
GARL2Pe	2	0.616 (0.018)	2	0.699 (0.027)	2	0.327 (0.027) ^{dc}
GARL3Ma	2	0.562 (0.098)	2	0.742 (0.006)	2	0.180 (0.010) ^c
GARL3Pe	2	0.629 (0.056)	2	0.645 (0.050)	2	0.222 (0.007) ^c
MOSL1Ma	2	0.706 (0.029)	2	0.639 (0.054)	2	0.734 (0.007) ^a
MOSL1Pe	2	0.772 (0.055)	2	0.606 (0.175)	2	0.777 (0.043) ^a
MOSL2Ma	2	0.724 (0.008)	2	0.770 (0.011)	2	0.691 (0.022) ^a
MOSL2Pe	2	0.732 (0.011)	2	0.706 (0.026)	2	0.729 (0.003) ^a
MOSL3Ma	2	0.741 (0.044)	2	0.730 (0.021)	2	0.578 (0.030) ^{ab}
MOSL3Pe	2	0.712 (0.000)	2	0.776 (0.012)	2	0.663 (0.104) ^a
P-value ¹	2	0.207	2	0.405	2	<0.0001

¹ P-value. ^{a-c} Means within a column with different superscripts differ significantly

C+ = positive control; C- = negative control; Ma = mash; Pe = pellets; GAR = garlic; MOS = Bio-Mos®; L = levels

The different treatments were compared to the treatments with (positive control) or without (negative control) antibiotic (doxycyclin) supplementation for days 21, 28 and 35.

The AAD at day 21 are presented in Table 3.4. The following significant ($P < 0.05$) differences, between treatments, were found. When compared to the negative control (mash), the AAD for glycine improved with the treatments that were supplemented with Bio-Mos® (1g/kg, pellet) and garlic (8g/kg, mash). The treatment that was supplemented with garlic (13g/kg, pellet) had a negative effect on the AAD for three amino acids (tyr, phen and leu) and the treatment with garlic (18g/kg, mash) had a negative effect on the AAD for two amino acids (arg, met). When compared to the negative control (pellet), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for 13 amino acids (ser, arg, val, his, lys, pro, met, tyr, cys, ileu, phen, leu and gly), the treatment with garlic (13g/kg, pellet) had a negative effect on the AAD for four amino acids (met, tyr, phen and leu) and the treatment with Bio-Mos® (1.5g/kg, pellet) had a negative effect on the AAD for only one amino acid (gly). When compared to the positive control (mash), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for 16 amino acids (ala, thr, ser, arg, val, his, asp,

lys, pro, met, tyr, cys, ileu, phen, leu and gly), the treatment with garlic (13g/kg, pellet) had a negative effect on the AAD for five amino acids (ala, met, tyr, phen and leu) and the treatment with Bio-Mos® (1.5g/kg, pellet) had a negative effect on the AAD for one amino acid (gly). When compared to the positive control (pellet), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on AAD for 10 amino acids (ala, ser, arg, val, his, lys, pro, met, tyr and cys), the treatment with garlic (13g/kg, pellet) had a negative effect on the AAD for five amino acids (ala, met, tyr, phen and leu) and the treatment with Bio-Mos® (1.5g/kg, pellet) had a negative effect only on glycine.

The AAD at day 28 are presented in Table 3.5. The only significant ($P < 0.05$) difference was found between the negative control (mash) and the treatments that were supplemented with Bio-Mos® (0.5g/kg, pellet) and garlic (13g/kg, mash). These treatments had a negative effect on AAD for glycine, when compared to the negative control (mash).

The AAD at day 35 are presented in Table 3.6. The following significant ($P < 0.05$) differences, between treatments, were found. When compared to the negative control (mash), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for 16 amino acids (ala, thr, ser, arg, glut, val, his, asp, lys, pro, met, tyr, cys, phen, leu and gly). The treatment with garlic (18g/kg, pellet) had a negative effect on the AAD for four amino acids (met, tyr, phen and leu). The treatment with Bio-Mos® (1.5g/kg, pellet) had a negative effect on the AAD for four amino acids (met, cys, phen and gly). The treatment with garlic (8g/kg, pellet) had a negative effect on the AAD for three amino acids (tyr, cys and phen), and the treatment with garlic (13g/kg, pellet) had a negative effect on AAD for two amino acids (tyr and phen). When compared with the negative control (pellet), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for 16 amino acids (ala, thr, ser, arg, glut, val, his, asp, lys, pro, met, tyr, cys, phen, leu and gly), the treatment with garlic (18g/kg, pellet) had a negative effect on the AAD for three amino acids (glut, tyr and phen), and the treatment with garlic (13g/kg, pellet) and Bio-Mos® (1.5g/kg, pellet) had a negative effect on the AAD for amino acid (asp and gly respectively). When compared to the positive control (mash), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for 16 amino acids (ala, thr, ser, arg, glut, val, his, asp, lys, pro, met, tyr, cys, phen, leu and gly), the treatment with garlic (18g/kg, pellet) had a negative effect on the AAD for four amino acids (glut, met, tyr and phen), and the treatments with Bio-Mos® (0.5g/kg, 1g/kg, mash and 0.5g/kg, pellet) and garlic (13g/kg, mash) had a negative effect on the AAD for one amino acid (tyr). When compared with the positive control (pellet), the treatment that was supplemented with garlic (18g/kg, mash) had a negative effect on the AAD for all 17 amino acids, the treatment with garlic (18g/kg, pellet) had a negative effect on the AAD for four amino acids (glut, met, tyr and phen), the treatment with Bio-Mos® (1.5g/kg, pellet) had a negative effect on the AAD for four amino acids (met, ileu, phen and gly), the treatment with garlic (13g/kg, pellet) had a negative effect on the AAD for three amino acids (asp, tyr and phen), and the treatment with garlic (8g/kg, pellet) had a negative effect on the AAD for two amino acids (tyr, phen).

Discussion

In general most of the treatments did not show any improvement in AAD at any determination period (day 21, 28 or 35). Yang *et al.*, (2008) supported similar results by proving that Bio-Mos® had no effect on apparent total tract digestibility of nutrients. The lack of response could be the result of the absence of any real health challenge for the birds or the facilities were too clean. At day 21 and day 35, the diet supplemented with 18g/kg garlic (mash) has shown to have a negative effect on AAD, when compared to the negative and positive control. Some opposing results from Adibmoradi *et al* (2006) found that garlic meal, as a feed additive in chickens, resulted in some small intestinal morphological changes (enhanced villus height and crypt depth, decreased epithelial thickness), which in turn demonstrate that absorptive process could be activated. Sarica *et al* (2005) found that supplementation with garlic increased the length of the small intestine. This can have a positive influence on digestibility of nutrients. Yang *et al* (2007) also observed a numerical increase in the digestibility of nutrients in the small intestine in the young birds, when fed Bio-Mos® diets. It doesn't seem that feeding either a mash or a pellet had an influence on the effects exerted by the different treatments.

3.5 Conclusions

Besides statistical proof that garlic has no beneficial effects on AAD and AND, it is also a very unpractical alternative. There is the risk of destroying the active ingredient, allicin, with exposure to high temperatures. Therefore, the garlic needs to be freeze-dried. Freeze-drying is a much more cumbersome and more complicated process of removing moisture than oven-drying. Because garlic mainly constitutes moisture, an enormous amount of the weight of the garlic gets lost in the drying process. Therefore one needs to include much more fresh garlic, in order to get the correct weight of dry product needed. Its hygroscopic nature (tendency to retain moisture and form lumps), is another unattractive characteristic of garlic. This makes it difficult to store and be mixed with other feed ingredients, in other words processed.

Table 3.4 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either garlic or Bio-Mos® at 21 days.

Treatment									
Amino acid	C-Ma	C+Ma	C-Pe	C+Pe	MOSL1Ma	MOSL2Ma	MOSL3Ma	MOSL1Pe	P-value
Alanine	0.823 (0.050) ^{abc}	0.914 (0.002) ^a	0.866 (0.003) ^{abc}	0.917 (0.014) ^a	0.863 (0.015) ^{abc}	0.883 (0.001) ^{ab}	0.923 (0.012) ^a	0.861 (0.034) ^{abc}	0.0004
Threonine	0.761 (0.068) ^{abc}	0.880 (0.002) ^{ab}	0.873 (0.003) ^{abc}	0.821 (0.029) ^{abc}	0.875 (0.014) ^{abc}	0.750 (0.002) ^{abc}	0.914 (0.014) ^a	0.830 (0.041) ^{abc}	0.0002
Serine	0.787 (0.061) ^{abc}	0.881 (0.002) ^{ab}	0.872 (0.003) ^{ab}	0.897 (0.017) ^{ab}	0.820 (0.020) ^{ab}	0.845 (0.001) ^{ab}	0.927 (0.011) ^a	0.852 (0.036) ^a	0.0006
Arginine	0.969 (0.009) ^a	0.977 (0.000) ^a	0.965 (0.001) ^a	0.975 (0.004) ^a	0.951 (0.005) ^a	0.958 (0.000) ^a	0.982 (0.003) ^a	0.957 (0.011) ^a	0.0002
Glutamic acid	0.878 (0.035) ^{ab}	0.909 (0.002) ^{ab}	0.864 (0.003) ^{ab}	0.896 (0.017) ^{ab}	0.900 (0.011) ^{ab}	0.896 (0.001) ^{ab}	0.954 (0.007) ^a	0.882 (0.029) ^{ab}	0.0029
Valine	0.792 (0.059) ^{abc}	0.890 (0.002) ^{ab}	0.851 (0.004) ^{ab}	0.896 (0.017) ^{ab}	0.826 (0.019) ^{ab}	0.842 (0.001) ^{ab}	0.923 (0.012) ^a	0.823 (0.043) ^{ab}	0.0010
Histidine	0.838 (0.046) ^{ab}	0.937 (0.001) ^a	0.917 (0.002) ^a	0.939 (0.010) ^a	0.895 (0.012) ^{ab}	0.894 (0.001) ^{ab}	0.913 (0.014) ^a	0.890 (0.0027) ^{ab}	0.0018
Aspartic acid	0.765 (0.067) ^{ab}	0.822 (0.004) ^{ab}	0.807 (0.005) ^{ab}	0.827 (0.028) ^{ab}	0.826 (0.019) ^{ab}	0.806 (0.002) ^{ab}	0.939 (0.010) ^a	0.800 (0.048) ^{ab}	0.0054
Lysine	0.895 (0.030) ^{ab}	0.923 (0.002) ^a	0.911 (0.002) ^a	0.929 (0.012) ^a	0.904 (0.011) ^a	0.899 (0.001) ^{ab}	0.964 (0.006) ^a	0.896 (0.025) ^{ab}	0.0025
Proline	0.823 (0.050) ^{abc}	0.896 (0.002) ^{ab}	0.852 (0.004) ^{ab}	0.894 (0.017) ^{ab}	0.841 (0.018) ^{abc}	0.855 (0.000) ^{abc}	0.947 (0.008) ^a	0.860 (0.034) ^{abc}	0.0010
Methionine	0.952 (0.014) ^{ab}	0.961 (0.001) ^a	0.909 (0.002) ^a	0.937 (0.010) ^{ab}	0.915 (0.009) ^{ab}	0.949 (0.000) ^{ab}	0.972 (0.004) ^a	0.936 (0.016) ^{ab}	<0.0001
Tyrosine	0.855 (0.041) ^{abc}	0.912 (0.002) ^{ab}	0.845 (0.004) ^{ab}	0.891 (0.018) ^{ab}	0.884 (0.013) ^{ab}	0.891 (0.001) ^{ab}	0.954 (0.007) ^a	0.850 (0.036) ^{abc}	<0.0001
Cysteine	0.943 (0.016) ^{ab}	0.976 (0.000) ^a	0.974 (0.001) ^a	0.977 (0.004) ^a	0.965 (0.004) ^{ab}	0.965 (0.000) ^{ab}	0.976 (0.004) ^a	0.953 (0.011) ^{ab}	0.0035
Isoleucine	0.841 (0.045) ^{abc}	0.901 (0.002) ^{ab}	0.840 (0.004) ^{ab}	0.877 (0.020) ^{abc}	0.848 (0.017) ^{abc}	0.854 (0.001) ^{abc}	0.929 (0.011) ^a	0.838 (0.039) ^{abc}	0.0027
Phenylalanine	0.911 (0.025) ^{ab}	0.932 (0.001) ^a	0.889 (0.003) ^a	0.907 (0.015) ^{ab}	0.905 (0.011) ^{ab}	0.882 (0.001) ^{abc}	0.951 (0.008) ^a	0.870 (0.031) ^{abc}	0.0005
Leucine	0.910 (0.026) ^{ab}	0.937 (0.001) ^a	0.890 (0.003) ^a	0.922 (0.013) ^{ab}	0.915 (0.010) ^{ab}	0.899 (0.001) ^{abc}	0.954 (0.007) ^a	0.904 (0.023) ^{abc}	0.0003
Glycine	0.736 (0.075) ^{bcde}	0.905 (0.002) ^{abc}	0.887 (0.003) ^{abc}	0.847 (0.025) ^{abcd}	0.898 (0.011) ^{abcd}	0.720 (0.002) ^{cde}	0.894 (0.017) ^{abcd}	0.810 (0.046) ^{abcde}	<0.0001
Amino acid	MOSL2Pe	MOSL3Pe	GARL1Ma	GARL2Ma	GARL3Ma	GARL1Pe	GARL2Pe	GARL3Pe	P-value
Alanine	0.837 (0.003) ^{abc}	0.788 (0.012) ^{abc}	0.887 (0.009) ^a	0.904 (0.009) ^a	0.703 (0.070) ^c	0.853 (0.000) ^{abc}	0.717 (0.015) ^{bc}	0.855 (0.018) ^{abc}	0.0004
Threonine	0.934 (0.001) ^a	0.681 (0.018) ^{bc}	0.939 (0.005) ^a	0.893 (0.010) ^a	0.673 (0.077) ^c	0.771 (0.000) ^{abc}	0.906 (0.005) ^a	0.748 (0.031) ^{abc}	0.0002
Serine	0.833 (0.003) ^{ab}	0.810 (0.011) ^{abc}	0.894 (0.008) ^{ab}	0.874 (0.012) ^{ab}	0.598 (0.095) ^c	0.817 (0.000) ^{ab}	0.704 (0.016) ^{bc}	0.806 (0.024) ^{abc}	0.0006
Arginine	0.941 (0.001) ^{ab}	0.968 (0.002) ^a	0.969 (0.002) ^a	0.960 (0.004) ^a	0.893 (0.025) ^b	0.969 (0.000) ^a	0.945 (0.003) ^a	0.976 (0.003) ^a	0.0002

Glutamic acid	0.881 (0.002) ^{ab}	0.822 (0.010) ^b	0.900 (0.008) ^{ab}	0.902 (0.009) ^{ab}	0.812 (0.044) ^b	0.893 (0.000) ^{ab}	0.801 (0.012) ^b	0.852 (0.018) ^{ab}	0.0029
Valine	0.823 (0.003) ^{ab}	0.769 (0.013) ^{abc}	0.844 (0.012) ^{ab}	0.850 (0.014) ^{ab}	0.617 (0.090) ^c	0.805 (0.000) ^{abc}	0.685 (0.017) ^{bc}	0.800 (0.025) ^{abc}	0.0010
Histidine	0.896 (0.002) ^{ab}	0.855 (0.008) ^{ac}	0.920 (0.006) ^a	0.925 (0.007) ^a	0.755 (0.058) ^b	0.882 (0.000) ^{ab}	0.821 (0.009) ^{ab}	0.856 (0.018) ^{ab}	0.0018
Aspartic acid	0.859 (0.002) ^{ab}	0.728 (0.015) ^{ab}	0.878 (0.009) ^a	0.868 (0.012) ^{ab}	0.662 (0.080) ^b	0.833 (0.000) ^{ab}	0.744 (0.014) ^{ab}	0.800 (0.025) ^{ab}	0.0054
Lysine	0.905 (0.002) ^a	0.872 (0.007) ^{ab}	0.917 (0.006) ^a	0.909 (0.008) ^a	0.784 (0.051) ^b	0.900 (0.000) ^{ab}	0.853 (0.008) ^{ab}	0.882 (0.015) ^{ab}	0.0025
Proline	0.858 (0.002) ^{abc}	0.784 (0.012) ^{ab}	0.887 (0.009) ^{ab}	0.897 (0.010) ^{ab}	0.707 (0.069) ^c	0.844 (0.000) ^{abc}	0.737 (0.014) ^{bc}	0.847 (0.019) ^{abc}	0.0010
Methionine	0.894 (0.002) ^{ab}	0.854 (0.008) ^{abc}	0.934 (0.005) ^{ab}	0.956 (0.004) ^{ab}	0.783 (0.051) ^{cd}	0.946 (0.000) ^{ab}	0.754 (0.013) ^d	0.873 (0.016) ^{abc}	<0.0001
Tyrosine	0.828 (0.003) ^{abc}	0.816 (0.010) ^{bcd}	0.830 (0.013) ^{abc}	0.839 (0.015) ^{abc}	0.680 (0.075) ^{cd}	0.809 (0.000) ^{acdb}	0.641 (0.019) ^d	0.749 (0.031) ^{bcd}	<0.0001
Cysteine	0.968 (0.001) ^{ab}	0.943 (0.003) ^{abcd}	0.966 (0.003) ^{ab}	0.968 (0.003) ^{ab}	0.919 (0.019) ^b	0.949 (0.000) ^{ab}	0.945 (0.003) ^{ab}	0.952 (0.006) ^{ab}	0.0035
Isoleucine	0.857 (0.002) ^{abc}	0.794 (0.011) ^{ab}	0.871 (0.010) ^{abc}	0.851 (0.014) ^{abc}	0.708 (0.070) ^c	0.807 (0.000) ^{abc}	0.731 (0.014) ^{bc}	0.816 (0.023) ^{abc}	0.0027
Phenylalanine	0.895 (0.002) ^{abc}	0.858 (0.008) ^{abc}	0.917 (0.006) ^a	0.883 (0.011) ^{abc}	0.790 (0.050) ^{bc}	0.865 (0.000) ^{abc}	0.777 (0.012) ^c	0.844 (0.019) ^{abc}	0.0005
Leucine	0.906 (0.002) ^{abc}	0.876 (0.007) ^{abc}	0.926 (0.006) ^a	0.913 (0.008) ^{abc}	0.817 (0.043) ^{bc}	0.893 (0.000) ^{abc}	0.789 (0.011) ^c	0.877 (0.015) ^{abc}	0.0003
Glycine	0.837 (0.001) ^a	0.788 (0.021) ^e	0.887 (0.004) ^a	0.904 (0.008) ^{abc}	0.703 (0.072) ^{ed}	0.777 (0.000) ^{abcde}	0.717 (0.003) ^{ab}	0.855 (0.030) ^{abcde}	<0.0001

^{a-e} Means within a row with different superscripts are significantly different

C+ = positive control; C- = negative control; Ma = mash; Pe = pellets; GAR = garlic; MOS = Bio-Mos®; L = levels

Table 3.5 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either garlic or Bio-Mos® at 28 days.

Treatment									
Amino acid	C-Ma	C+Ma	C-Pe	C+Pe	MOSL1Ma	MOSL2Ma	MOSL3Ma	MOSL1Pe	P-value
Alanine	0.780 (0.042)	0.853 (0.002)	0.819(0.015)	0.832(0.018)	0.748 (0.079)	0.789 (0.073)	0.881 (0.011)	0.268 (0.432)	0.2431
Threonine	0.895 (0.021)	0.856 (0.032)	0.828 (0.037)	0.713 (0.095)	0.683 (0.078)	0.805 (0.007)	0.832 (0.047)	0.453 (0.279)	0.1835
Serine	0.713 (0.062)	0.739 (0.077)	0.716 (0.125)	0.767 (0.076)	0.599(0.211)	0.786 (0.059)	0.812 (0.086)	0.264 (0.490)	0.7667
Arginine	0.773 (0.161)	0.661 (0.235)	0.809 (0.135)	0.656 (0.257)	0.696 (0.191)	0.811 (0.116)	0.884 (0.091)	0.647 (0.288)	0.9728
Glutamic acid	0.316 (0.603)	0.618 (0.305)	0.275 (0.594)	0.302 (0.594)	0.196 (0.698)	0.533 (0.390)	0.524 (0.412)	-0.192 (1.094)	0.9984
Valine	0.887 (0.033)	0.899 (0.035)	0.894 (0.033)	0.901 (0.021)	0.869 (0.025)	0.899 (0.035)	0.903 (0.011)	0.780 (0.010)	0.1196
Histidine	0.770 (0.003)	0.801 (0.015)	0.769 (0.031)	0.785 (0.055)	0.759 (0.033)	0.779 (0.006)	0.828 (0.051)	0.523 (0.179)	0.0660
Aspartic acid	0.432 (0.417)	0.438 (0.418)	0.318 (0.515)	0.299 (0.560)	0.208 (0.639)	0.463 (0.399)	0.662 (0.252)	-0.525 (1.367)	0.9629
Lysine	0.909 (0.026)	0.921 (0.012)	0.913 (0.024)	0.917 (0.026)	0.886 (0.036)	0.923 (0.016)	0.905 (0.044)	0.842 (0.082)	0.2001
Proline	0.825 (0.052)	0.881 (0.026)	0.798 (0.054)	0.825 (0.063)	0.785 (0.076)	0.857 (0.024)	0.852 (0.074)	0.547 (0.245)	0.5021
Methionine	0.968 (0.013)	0.968 (0.015)	0.953 (0.026)	0.957 (0.022)	0.967 (0.012)	0.970 (0.013)	0.970 (0.009)	0.934 (0.003)	0.9863
Tyrosine	0.358 (0.577)	0.580 (0.357)	0.464 (0.436)	0.320 (0.600)	0.589 (0.342)	0.535 (0.405)	0.372 (0.534)	0.134 (0.802)	0.9604
Cysteine	0.896 (0.052)	0.945 (0.031)	0.838 (0.093)	0.875 (0.080)	0.860 (0.089)	0.824 (0.092)	0.838 (0.129)	0.875 (0.085)	0.7263
Isoleucine	0.721 (0.171)	0.721 (0.179)	0.606 (0.257)	0.626 (0.251)	0.591 (0.271)	0.712 (0.180)	0.779 (0.121)	0.283 (0.550)	0.9529
Phenylalanine	0.888 (0.078)	0.908 (0.035)	0.855 (0.053)	0.858 (0.063)	0.839 (0.075)	0.884 (0.035)	0.857 (0.074)	0.748 (0.162)	0.4085
Leucine	0.817 (0.027)	0.836 (0.078)	0.799 (0.055)	0.803 (0.007)	0.801 (0.030)	0.777 (0.045)	0.528 (0.181)	0.820 (0.031)	0.2550
Glycine	0.926 (0.078) ^a	0.906 (0.004) ^{ab}	0.888 (0.004) ^{ab}	0.841 (0.013) ^{ab}	0.769 (0.022) ^{ab}	0.828 (0.044) ^{ab}	0.829 (0.022) ^{ab}	0.681 (0.075) ^{ab}	0.0031
Amino acid	MOSL2Pe	MOSL3Pe	GARL1Ma	GARL2Ma	GARL3Ma	GARL1Pe	GARL2Pe	GARL3Pe	P-value
Alanine	0.798 (0.008)	0.808 (0.055)	0.719 (0.017)	0.784 (0.035)	0.840 (0.000)	0.825 (0.023)	0.780 (0.026)	0.868 (0.029)	0.2431
Threonine	0.807 (0.014)	0.822 (0.001)	0.613 (0.105)	0.438 (0.246)	0.721 (0.000)	0.780 (0.004)	0.778 (0.006)	0.831 (0.029)	0.1835
Serine	0.591 (0.246)	0.854 (0.030)	0.160 (0.570)	0.362 (0.358)	0.767 (0.000)	0.747 (0.049)	0.417 (0.375)	0.787 (0.062)	0.7667
Arginine	0.863 (0.074)	0.898 (0.073)	0.657 (0.253)	0.503 (0.356)	0.918 (0.000)	0.837 (0.102)	0.844 (0.082)	0.829 (0.137)	0.9728
Glutamic acid	0.547 (0.357)	0.332 (0.575)	-0.269 (1.038)	-0.334 (1.166)	0.896 (0.000)	0.171 (0.724)	0.085 (0.772)	0.390 (0.507)	0.9984
Valine	0.885 (0.053)	0.914 (0.041)	0.744 (0.057)	0.812 (0.025)	0.833 (0.000)	0.881 (0.043)	0.874 (0.053)	0.905 (0.023)	0.1196
Histidine	0.791 (0.011)	0.852 (0.010)	0.715 (0.003)	0.694 (0.047)	0.795 (0.000)	0.846 (0.026)	0.829 (0.028)	0.851 (0.000)	0.0660
Aspartic acid	0.514 (0.393)	0.491 (0.400)	-0.873 (1.582)	-0.918 (1.705)	0.825 (0.000)	0.295 (0.570)	0.371 (0.483)	0.435 (0.453)	0.9629
Lysine	0.926 (0.011)	0.939 (0.006)	0.746 (0.074)	0.808 (0.079)	0.918 (0.000)	0.914 (0.019)	0.908 (0.015)	0.916 (0.020)	0.2001

Proline	0.852 (0.026)	0.876 (0.018)	0.658 (0.122)	0.679 (0.144)	0.864 (0.000)	0.821 (0.040)	0.797 (0.041)	0.848 (0.064)	0.5021
Methionine	0.955 (0.025)	0.965 (0.020)	0.963 (0.019)	0.945 (0.012)	0.960 (0.000)	0.957 (0.022)	0.937 (0.042)	0.963 (0.019)	0.9863
Tyrosine	0.590 (0.293)	0.649 (0.270)	-0.908 (1.553)	-0.379 (1.213)	0.915 (0.000)	0.196 (0.667)	0.514 (0.307)	0.561 (0.315)	0.9604
Cysteine	0.868 (0.055)	0.896 (0.043)	0.582 (0.223)	0.730 (0.156)	0.930 (0.000)	0.846 (0.056)	0.882 (0.041)	0.876 (0.043)	0.7263
Isoleucine	0.651 (0.220)	0.744 (0.145)	0.135 (0.545)	0.253 (0.539)	0.869 (0.000)	0.660 (0.194)	0.579 (0.258)	0.678 (0.206)	0.9529
Phenylalanine	0.850 (0.046)	0.876 (0.039)	0.568 (0.162)	0.708 (0.124)	0.905 (0.000)	0.860 (0.035)	0.816 (0.032)	0.852 (0.046)	0.4085
Leucine	0.727 (0.086)	0.808 (0.045)	0.518 (0.159)	0.627 (0.054)	0.740 (0.000)	0.663 (0.126)	0.776 (0.064)	0.753 (0.060)	0.2550
Glycine	0.798 (0.031) ^a	0.808 (0.034) ^a	0.719 (0.030) ^{ab}	0.784 (0.076) ^b	0.765 (0.000) ^{ab}	0.825 (0.039) ^{ab}	0.780 (0.027) ^{ab}	0.868 (0.005) ^{ab}	0.0031

^{a-b} Means within a row with different superscripts are significantly different

C+ = positive control; C- = negative control; Ma = mash; Pe = pellets; GAR = garlic; MOS = Bio-Mos®; L = levels

Table 3.6 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either garlic or Bio-Mos® at 35 d.

Treatment									
Amino acid	C-Ma	C+Ma	C-Pe	C+Pe	MOSL1Ma	MOSL2Ma	MOSL3Ma	MOSL1Pe	P-value
Alanine	0.803 (0.002) ^a	0.881 (0.002) ^a	0.899 (0.007) ^a	0.867 (0.001) ^a	0.857 (0.004) ^a	0.822 (0.007) ^a	0.701 (0.023) ^a	0.847 (0.035) ^a	<0.0001
Threonine	0.801 (0.003) ^a	0.830 (0.002) ^a	0.875 (0.006) ^a	0.883 (0.001) ^a	0.821 (0.006) ^a	0.741 (0.011) ^a	0.681 (0.024) ^a	0.835 (0.038) ^a	<0.0001
Serine	0.793 (0.002) ^a	0.869 (0.002) ^a	0.888 (0.006) ^a	0.879 (0.001) ^a	0.819 (0.006) ^a	0.826 (0.007) ^a	0.756 (0.018) ^a	0.898 (0.023) ^a	<0.0001
Arginine	0.978 (0.001) ^a	0.968 (0.000) ^a	0.971 (0.003) ^a	0.950 (0.000) ^a	0.897 (0.003) ^a	0.934 (0.003) ^a	0.930 (0.005) ^a	0.963 (0.009) ^a	<0.0001
Glutamic acid	0.905 (0.001) ^a	0.925 (0.001) ^a	0.933 (0.005) ^a	0.903 (0.000) ^a	0.929 (0.002) ^a	0.929 (0.003) ^a	0.866 (0.010) ^a	0.932 (0.016) ^a	<0.0001
Valine	0.839 (0.002) ^a	0.879 (0.001) ^a	0.906 (0.006) ^a	0.886 (0.001) ^a	0.865 (0.004) ^a	0.836 (0.007) ^a	0.746 (0.019) ^a	0.842 (0.036) ^a	<0.0001
Histidine	0.812 (0.002) ^a	0.890 (0.002) ^a	0.895 (0.005) ^a	0.896 (0.001) ^a	0.890 (0.003) ^a	0.872 (0.005) ^a	0.851 (0.011) ^a	0.889 (0.025) ^a	<0.0001
Aspartic acid	0.839 (0.002) ^{ab}	0.850 (0.001) ^{ab}	0.870 (0.007) ^a	0.867 (0.001) ^a	0.881 (0.004) ^a	0.869 (0.005) ^a	0.848 (0.011) ^a	0.882 (0.027) ^a	<0.0001
Lysine	0.931 (0.001) ^a	0.942 (0.001) ^a	0.933 (0.003) ^a	0.948 (0.000) ^a	0.944 (0.002) ^a	0.939 (0.002) ^a	0.904 (0.007) ^{ab}	0.941 (0.014) ^a	<0.0001
Proline	0.854 (0.002) ^a	0.901 (0.001) ^a	0.905 (0.006) ^a	0.889 (0.001) ^a	0.892 (0.003) ^a	0.861 (0.006) ^a	0.808 (0.014) ^a	0.888 (0.026) ^a	<0.0001
Methionine	0.947 (0.001) ^a	0.964 (0.000) ^{ab}	0.964 (0.003) ^{abc}	0.934 (0.000) ^a	0.937 (0.002) ^{abc}	0.947 (0.002) ^{ab}	0.894 (0.008) ^{abcd}	0.948 (0.012) ^{abc}	<0.0001
Tyrosine	0.928 (0.001) ^a	0.954 (0.001) ^b	0.903 (0.003) ^a	0.942 (0.001) ^a	0.942 (0.002) ^a	0.935 (0.003) ^a	0.841 (0.012) ^{abc}	0.938 (0.014) ^a	<0.0001
Cysteine	0.928 (0.000) ^a	0.975 (0.001) ^{ab}	0.958 (0.003) ^{ab}	0.948 (0.000) ^{ab}	0.959 (0.001) ^{ab}	0.959 (0.002) ^{ab}	0.930 (0.005) ^{ab}	0.947 (0.012) ^{ab}	<0.0001
Isoleucine	0.886 (0.002) ^{ab}	0.904 (0.001) ^{ab}	0.926 (0.005) ^{ab}	0.897 (0.000) ^a	0.885 (0.004) ^{ab}	0.877 (0.005) ^{ab}	0.757 (0.018) ^{ab}	0.883 (0.027) ^{ab}	<0.0001
Phenylalanine	0.936 (0.001) ^a	0.946 (0.001) ^{ab}	0.955 (0.003) ^{ab}	0.934 (0.000) ^a	0.934 (0.002) ^{ab}	0.910 (0.004) ^{ab}	0.849 (0.011) ^{abc}	0.933 (0.015) ^{ab}	<0.0001
Leucine	0.918 (0.001) ^a	0.933 (0.001) ^{ab}	0.922 (0.004) ^{ab}	0.921 (0.000) ^{ab}	0.924 (0.002) ^{ab}	0.887 (0.005) ^{ab}	0.831 (0.013) ^{ab}	0.930 (0.016) ^{ab}	<0.0001
Glycine	0.803 (0.002) ^a	0.861 (0.002) ^{ab}	0.851 (0.005) ^a	0.903 (0.001) ^a	0.833 (0.005) ^a	0.709 (0.012) ^{ab}	0.681 (0.024) ^{ab}	0.776 (0.052) ^{ab}	<0.0001
Amino acid	MOSL2Pe	MOSL3Pe	GARL1Ma	GARL2Ma	GARL3Ma	GARL1Pe	GARL2Pe	GARL3Pe	P-value
Alanine	0.785 (0.003) ^a	0.763 (0.105) ^a	0.869 (0.005) ^a	0.847 (0.006) ^a	0.406 (0.105) ^b	0.686 (0.035) ^a	0.684 (0.003) ^a	0.880 (0.012) ^a	<0.0001
Threonine	0.770 (0.003) ^a	0.698 (0.134) ^a	0.834 (0.006) ^a	0.839 (0.006) ^a	0.187 (0.144) ^b	0.627 (0.041) ^a	0.594 (0.003) ^a	0.696 (0.000) ^a	<0.0001
Serine	0.782 (0.003) ^a	0.816 (0.082) ^a	0.872 (0.005) ^a	0.863 (0.005) ^a	0.250 (0.133) ^b	0.730 (0.030) ^a	0.692 (0.002) ^a	0.781 (0.000) ^a	<0.0001
Arginine	0.866 (0.002) ^a	0.945 (0.024) ^a	0.948 (0.002) ^a	0.958 (0.002) ^a	0.575 (0.075) ^b	0.916 (0.009) ^a	0.872 (0.001) ^a	0.954 (0.000) ^a	<0.0001
Glutamic acid	0.885 (0.002) ^a	0.894 (0.047) ^a	0.912 (0.003) ^a	0.929 (0.003) ^a	0.742 (0.046) ^b	0.839 (0.018) ^a	0.825 (0.001) ^a	0.676 (0.000) ^b	<0.0001
Valine	0.809 (0.003) ^a	0.766 (0.103) ^a	0.836 (0.006) ^a	0.829 (0.006) ^a	0.366 (0.112) ^b	0.670 (0.037) ^a	0.711 (0.002) ^a	0.831 (0.000) ^a	<0.0001
Histidine	0.819 (0.003) ^a	0.855 (0.064) ^a	0.841 (0.006) ^a	0.844 (0.006) ^a	0.443 (0.098) ^b	0.763 (0.026) ^a	0.723 (0.002) ^a	0.706 (0.000) ^a	<0.0001
Aspartic acid	0.876 (0.002) ^a	0.847 (0.068) ^{ab}	0.888 (0.004) ^a	0.889 (0.004) ^a	0.489 (0.090) ^c	0.784 (0.024) ^{ab}	0.786 (0.002) ^b	0.666 (0.000) ^{ab}	<0.0001
Lysine	0.917 (0.001) ^{ab}	0.916 (0.037) ^{ab}	0.931 (0.002) ^a	0.940 (0.002) ^a	0.720 (0.049) ^c	0.884 (0.013) ^{ab}	0.882 (0.001) ^{ab}	0.820 (0.000) ^{ab}	<0.0001
Proline	0.827 (0.003) ^a	0.823 (0.078) ^a	0.888 (0.004) ^a	0.896 (0.004) ^a	0.574 (0.075) ^b	0.755 (0.027) ^a	0.744 (0.002) ^a	0.820 (0.000) ^a	<0.0001

Methionine	0.940 (0.001) ^{abc}	0.917 (0.037) ^{bcd}	0.947 (0.002) ^{ab}	0.954 (0.002) ^{ab}	0.853 (0.026) ^d	0.902 (0.011) ^{abc}	0.897 (0.001) ^{abcd}	0.867 (0.000) ^{cd}	<0.0001
Tyrosine	0.882 (0.002) ^{ab}	0.896 (0.046) ^{abc}	0.880 (0.004) ^{ab}	0.894 (0.004) ^a	0.678 (0.057) ^d	0.732 (0.023) ^{bc}	0.764 (0.002) ^{bc}	0.736 (0.000) ^{cd}	<0.0001
Cysteine	0.909 (0.001) ^{ab}	0.885 (0.051) ^b	0.952 (0.002) ^{ab}	0.942 (0.002) ^{ab}	0.671 (0.058) ^c	0.818 (0.020) ^b	0.855 (0.001) ^{ab}	0.872 (0.000) ^{ab}	<0.0001
Isoleucine	0.839 (0.002) ^{ab}	0.799 (0.089) ^b	0.863 (0.005) ^{ab}	0.853 (0.005) ^{ab}	0.552 (0.079) ^{cb}	0.696 (0.034) ^{ab}	0.751 (0.002) ^{ab}	0.781 (0.000) ^{ab}	<0.0001
Phenylalanine	0.880 (0.002) ^{abc}	0.865 (0.060) ^{bc}	0.908 (0.003) ^{ab}	0.893 (0.004) ^{ab}	0.698 (0.053) ^d	0.776 (0.025) ^{bc}	0.801 (0.002) ^{bc}	0.753 (0.000) ^{cd}	<0.0001
Leucine	0.884 (0.002) ^{ab}	0.874 (0.056) ^{ab}	0.912 (0.003) ^{ab}	0.902 (0.004) ^{ab}	0.715 (0.050) ^c	0.800 (0.022) ^{ab}	0.805 (0.001) ^{ab}	0.795 (0.000) ^b	<0.0001
Glycine	0.773 (0.003) ^{ab}	0.637 (0.161) ^b	0.828 (0.006) ^a	0.805 (0.007) ^{ab}	0.253 (0.132) ^c	0.544 (0.051) ^{ab}	0.636 (0.003) ^{ab}	0.709 (0.000) ^a	<0.0001

^{a-d} Means within a row with different superscripts are significantly different

C+ = positive control; C- = negative control; Ma = mash; Pe = pellets; GAR = garlic; MOS = Bio-Mos®; L = levels

Chapter 4

The effect of addition of Bio-Mos®, Acid-Pak 4-way® and MCT on nitrogen (N)- and amino acid digestibilities in broilers

4.1. Introduction

From Trial 1 the conclusion was that garlic, as well as Bio-Mos® had no beneficial effects on AND and AAD in broilers. It was found that feeding a mash or a pellet did not have any significant effect on AND or AAD. It was also concluded from the previous trial that garlic was a very unattractive alternative, due to the difficulty of being handled and processed. Bio-Mos® is already a commercially accepted product, therefore it was decided to use it again in Trial 2. Bio-Mos® and two substances, Acid-Pak 4-way® (Alltech) and a medium-chain triglyceride, MCT (Aveve, Belgium) were used and evaluated in terms of their effects on AND and AAD.

Acid-Pak 4-way® is a blend of organic acids (i.e. citric acid and sorbic acids), probiotics (beneficial bacteria such as *Lactobacillus* and *Streptococcus*) and electrolytes. It lowers the pH in the crop (in the absence of any feed) and allows lactic acid producing organisms' passage through the intestinal tract, while discouraging the passage of pathogenic bacteria which prefer higher pH. The most effective way to use this product is addition to the drinking water (0.5g/l water), but due to the difficulty regarding determination of intake, it was included in the feed instead (Sun *et al.*, 2005). Medium-chain triglycerides (MCT) have fatty acids 6 to 12 carbon atoms long (Lee & Chiang, 1994). Medium-chain fatty acids are more easily digested and absorbed than long-chain fatty acids. Synthetic sources of medium-chain fatty acids have been reported to improve overall growth of the chick after the first few weeks post-hatch (Turner *et al.*, 1999).

The aim of this trial was to evaluate the effect of Bio-Mos®, Acid-Pak 4-way® and MCT on AND and AAD in broilers, and to compare their effects to that of an antibiotic (doxycyclin).

4.2. Materials and methods

Two hundred and sixty four day-old female Cobb 500 broiler chicks were placed in wire-mesh cages (six chicks per cage). The experimental units/cages were allocated at random to the 11 (A to K) experimental treatments with four replicates per treatment. The birds were vaccinated at day-old against Newcastle disease and Infectious bronchitis. The environmental temperature within the house was 30°C for the first day and was then decreased by 0.5°C every second day until 20°C was reached. Continuous light (24L:0D) was provided for the duration of the trial. Birds were offered *ad libitum* access to feed and water. The experiment had a completely randomised design with main effect of dietary treatment. The three experimental trials were approved by the University of Stellenbosch Animal Ethics Committee.

4.3 Experimental design

Starter (0-14 days) and a finisher (15-35 days) broiler diets were formulated with the EFG Broiler Nutrition Optimizer Program (Winfeed 2 Feed Formulator, 2005). A detailed list of the ingredients used in the diets and the formulated nutrient composition of the diets are shown in Table 4.1. The starter and finisher diets were fed as mash.

Table 4.1. Composition of starter and finisher diets for broiler chickens based on as fed basis (g/kg).

Ingredient	Starter	Finisher
Maize	549.85	708.73
Soybean oil cake meal (46%)	270	251.34
Soybean full fat	125.23	
Choline chloride 60%	0.67	0.53
DL-methionine	2.36	1.34
L-threonine	0.03	
L-lysine HCL	7.68	1.46
Limestone	21.69	18.13
Salt	4.03	4.07
Monocalcium phosphate	11.78	7.41
Sodium bicarbonate	4.08	4.18
Vitamin E		0.40
Vit + min premix	0.50	1.3
<i>Calculated nutrient content (*)</i>		
AMEn (MJ/kg)	12.49	12.68
Crude protein (%)	22.00	17.50
Lysine (%)	1.27	0.95
Methionine (%)	0.56	0.41
Methionine + Cystine (%)	0.93	0.72
Threonine (%)	0.88	0.69
Tryptophan (%)	0.27	0.20
Arginine (%)	1.51	1.12
Isoleucine (%)	1.00	0.79
Leucine (%)	1.95	1.65
Histidine (%)	0.62	0.50
Phenylalanine + tyrosine (%)	1.88	1.51
Valine (%)	1.05	0.84
Calcium	1.10	0.90
Total Phosphorous	0.67	0.54

(*) Amino acids expressed on a digestible basis.

AMEn: nitrogen corrected Apparent Metabolisable Energy

Bio-Mos® was included at 1g/kg, 2g/kg, and 3g/kg for the starter diet, and at 0.5g/kg, 1g/kg, 1.5g/kg for the finisher diet. Acid-Pak 4-way® was included at 0.4g/kg, 1g/kg and 1.6g/kg for both the starter and finisher diets. The MCT was included at 3g/kg, 3.6g/kg, 4.2g/kg for the starter diet and 2.1g/kg, 2.7g/kg and 3.4g/kg for the fisher diet. The antibiotic was added to the control at 0.3g/kg. Celite® was included at 20g/kg as an indigestible marker in all dietary treatments. A summary of the dietary treatments is shown in Table 4.2.

Table 4.2. Dietary treatments used throughout the trial.

Treatment	Description
A	Control + Bio-Mos® L1
B	Control + Bio-Mos® L2
C	Control + Bio-Mos® L3
D	Acid-Pak 4way L1
E	Acid-Pak 4way L2
F	Acid-Pak 4way L3
G	MCT L1
H	MCT L2
I	MCT L3
J	Control diet without antibiotic
K	Control diet with antibiotic

All starter diets were removed on the morning of day 14 and the finisher diets were allocated to the respective cages. On the morning of day 21, one bird per cage was stunned and killed by cervical dislocation (Ten Doeschate *et al.*, 1993). Immediately after the chickens were killed the stomachs were opened up and the ileum exposed. A 15cm segment of the terminal ileum, 2cm anterior to the ileo-caecal junction (to avoid contamination with urine), was removed. The segment was emptied by gently squeezing the segment between the thumb and the forefinger so as to prevent damage to the intestinal mucosa. After most of the contents have been removed, each segment was flushed with distilled water (Ten Doeschate *et al.*, 1993; Sun *et al.*, 2005). Digesta samples from individual birds were pooled within a treatment and immediately frozen at -20°C to avoid bacterial contamination of the samples. The samples were then freeze-dried and finely ground to pass through a 0.75 mm sieve. It was then stored at -20°C awaiting chemical analysis. The procedure was repeated on the mornings of day 28 and 35.

Duplicate samples of both the dietary treatments and the excreta samples were analyzed for N content with a Leco N analyser. A Leco N analyzer determines the % N content from a 0.1g sample by means of a combustion process (AOAC, 1990). The digesta and the feed were hydrolysed for 24 hours with 6N hydrochloric acid at 110°C. This was done for determination of amino acids (AA) by HPLC using the Pico-Tag system from Waters Chromatography Systems.

Apparent excreta digestibility coefficients for N and AA were calculated using the following equations (Ravindran *et al.*, 1999).

Apparent nitrogen digestibility (AND)

$$\text{AND} = \frac{(\text{N/AIA})_d - (\text{N/AIA})_i}{(\text{N/AIA})_d}$$

where $(\text{N/AIA})_d$ = ratio of N to acid-insoluble ash in the diet
and $(\text{N/AIA})_i$ = ratio of N to acid-insoluble ash in the ileal digesta

Apparent amino acid digestibility (AAD)

$$\text{AAD} = \frac{(\text{AA/AIA})_d - (\text{AA/AIA})_i}{(\text{AA/AIA})_d}$$

where $(\text{AA/AIA})_d$ = ratio of AA to acid-insoluble ash in the diet
and $(\text{AA/AIA})_i$ = ratio of AA to acid-insoluble ash in the ileal digesta

Levene's test was used to test for homogeneity of variance and the Kolmogorov-Smirnov test was used to test for normal distribution of the data. All data of equal variance and normal distribution were analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0. The Bonferroni (Dunn) t-test was used to test for differences between treatment means where the treatment effect was found to be significant. The probability level was set at 5%.

4.4. Results and discussion**Results**

The results of AND coefficients for the entire experimental period are presented in Table 4.3. Due to the absence of some results, it was difficult to evaluate the effects of the different treatments at day 21. The lack of results was mainly because the amount ileum contents collected for those treatments were too small, and it further decreased with the drying process. At days 28 and 35 there were no significant ($P > 0.05$) differences between the different treatments. It can therefore be concluded that no treatments had any beneficial or negative effect on AND, when compared to each other.

Table 4.3. Apparent Nitrogen digestibility (AND) coefficients (\pm SE) for 21, 28 and 35 days.

Period Treatment	D21		D28		D35	
	N	AND	N	AND	N	AND
MOSL1	2	0.803 (0.005)	2	0.797 (0.008)	2	0.829 (0.006)
MOSL2	2	0.813 (0.000)	2	0.791 (0.003)	2	0.805 (0.004)
MOSL3	2	0.725 (0.045)	2	0.741 (0.008)	2	0.823 (0.009)
APL1	2	-	2	0.726 (0.004)	2	0.776 (0.010)
APL2	2	-	2	0.830 (0.000)	2	0.798 (0.000)
APL3	2	0.770 (0.024)	2	0.796 (0.019)	2	0.755 (0.025)
MCTL1	2	0.804 (0.044)	2	0.823 (0.000)	2	0.801 (0.000)
MCTL2	2	-	2	0.734 (0.000)	2	0.737 (0.044)
MCTL3	2	-	2	0.823 (0.009)	2	0.770 (0.017)
C-	2	-	2	0.731 (0.000)	2	0.722 (0.002)
C+	2	-	2	0.781 (0.000)	2	0.721 (0.000)
P-value	2	0.3446	2	0.0040	2	0.0211

– Could not be determined.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; ACP4 = Acid-Pak 4-way®; MCT = Medium chain triglycerides; L = levels

The different treatments were compared to the treatments with (positive control) or without (negative control) the antibiotic (doxycyclin) supplementation for days 21, 28 and 35.

The AAD at day 21 are presented in Table 4.4. When compared to the negative control, the treatment supplemented with MCT (3.4g/kg) have improved AAD for 12 amino acids (ala, thr, ser, val, his, asp, pro, tyr, cys, ileu, leu and gly), but had a negative effect ($P < 0.05$) on AAD for five amino acids (arg, glu, lys, met and phen). The treatments with Bio-Mos® (0.5g/kg and 1g/kg), Acid-Pak 4-way® (1g/kg and 1.6g/kg) and MCT (2.1g/kg) had a negative effect on AAD for glutamic acid and the treatment with Bio-Mos® (1.5g/kg) had a negative effect on AAD for three amino acids (his, lys and met). When compared to the positive control, the treatment supplemented with MCT (3.4g/kg) have improved AAD for 14 amino acids (ala, thr, ser, val, his, asp, lys, pro, tyr, cys, ileu, phen, leu and gly) and had a negative effect on three amino acids (arg, glu and met). The treatments with Bio-Mos® (0.5, 1 and 1.5g/kg), Acid-Pak 4-way® (1.6g/kg) and MCT (2.1g/kg) improved AAD for two amino acids (ileu and phen), and the treatment with Acid-Pak 4-way® (1g/kg) had a negative effect on nine amino acids (thr, ser, val, asp, pro, tyr, ileu, phen and leu).

The AAD at day 28 are presented in Table 4.5. The following significant ($P < 0.05$) differences, between treatments, were found. When the treatments were compared to the negative control, the treatment supplemented with MCT (2.7g/kg) had a negative effect on AAD only for valine. For the rest of the treatments, there were no significant ($P > 0.05$) effects on AAD when compared to the negative control. When the treatments were compared to the positive control, the treatment supplemented with Bio-Mos® (1.5g/kg) improved AAD ($P < 0.05$) for 13 amino acids (ala, thr, ser, arg, val, his, pro, met, tyr, cys, ileu, leu and gly), the treatment with Bio-Mos® (0.5g/kg) improved AAD ($P < 0.05$) for 11 amino acids (ala, thr, ser, arg, val, his, pro,

met, tyr, cys and gly). The treatment with Acid-Pak 4-way® (0.4g/kg) improved AAD ($P < 0.05$) for 10 amino acids (ala, thr, ser, val, met, tyr, cys, phen, leu and gly). The treatment with Bio-Mos® (1g/kg) improved AAD ($P < 0.05$) for six amino acids (ala, val, met, tyr, cys and gly) and the treatment with MCT (3.4g/kg) have also improved AAD for six amino acids (thr, arg, met, tyr, cys and gly). The treatment with Acid-Pak 4-way® (1.6g/kg) improved AAD ($P < 0.05$) for four amino acids (met, tyr, cys and gly) and the treatment with Acid-Pak 4-way® (1g/kg) and MCT (2.1g/kg) improved AAD ($P < 0.05$) for three amino acids (met, tyr and cys). The treatment supplemented with MCT (2.7g/kg) improved AAD for 11 amino acids (ala, thr, ser, arg, val, his, asp, lys, pro, ileu and gly), but had a negative effect on the AAD for three amino acids (met, tyr and cys).

The AAD at day 35 are presented in Table 4.6. No treatments had any beneficial or negative effect ($P < 0.05$) on AAD, when compared to the negative and positive controls.

Discussion

The most significant effects were seen at days 21 and 28. At day 21, the treatment supplemented with MCT (3.4g/kg) had the most significant beneficial effect on AAD, when compared to the negative- and positive control, as it increased AAD for the majority of the amino acids. The treatment with Acid-Pak 4-way® (1g/kg) had the most significant negative effect on AAD when compared to the positive control. At day 28, the treatments with Bio-Mos® (0.5g/kg and 1.5g/kg) and Acid-Pak 4-way® (0.4g/kg) have shown to have the most significant beneficial effect on AAD when compared to the positive control. It increased AAD for more than half of the 17 amino acids. The treatment supplemented with MCT (2.7g/kg) has shown the most significant negative effect on AAD, when compared to the positive control.

4.5 Conclusions

It can be concluded that MCT had the greatest beneficial effect at day 21, although Akiba *et al.*, (1993) found no influence on true amino acid availability with MCT inclusion. Bio-Mos® and Acid-Pak 4-way® had the more pronounced beneficial effect at day 28. These results agree with Adibmoradi *et al.*, (2006) which found that garlic meal indirectly activate the absorptive process, as well as Yang *et al.*, (2007) that observed a numerical increase in the nutrient digestibility in the small intestine. Although addition to water is recommended for Acid-Pak 4-way, it has also proven that it is possible to be included in the feed.

Table 4.4 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either Bio-Mos®, Acid-Pak 4-way® or MCT at 21 days.

Treatment												
Amino acid	MOSL1	MOSL2	MOSL3	APL1	APL2	APL3	MCTL1	MCTL2	MCTL3	C-	C+	P-value
Anine	0.823 (0.004) ^{bc}	0.797 (0.003) ^{bc}	0.602 (0.061) ^c	0.810 (0.055) ^{bc}	0.490 ^b	0.838 (0.021) ^{bc}	0.838 (0.039) ^{bc}	0.776 ^{bc}	1.037 ^a	0.851 (0.037) ^{bc}	0.661 (0.048) ^{bc}	<0.0001
Threonine	0.774 (0.005) ^{bc}	0.853 (0.002) ^{bc}	0.602 (0.061) ^{bc}	0.758 (0.070) ^{bc}	0.492 ^b	0.713 (0.037) ^{bc}	0.813 (0.045) ^{bc}	0.640 ^{bc}	1.228 ^a	0.784 (0.053) ^{bc}	0.589 (0.058) ^c	<0.0001
Serine	0.772 (0.005) ^{bc}	0.852(0.002) ^{bc}	0.602 (0.061) ^c	0.780 (0.064) ^{bc}	0.494 ^b	0.800 (0.026) ^{bc}	0.819 (0.044) ^{bc}	0.650 ^{bc}	1.253 ^a	0.814 (0.045) ^{bc}	0.612 (0.055) ^c	0.0002
Arginine	0.853 (0.003) ^{bc}	0.889 (0.002) ^{bc}	0.602 (0.061) ^c	0.745 (0.074) ^{bc}	0.497 ^b	0.912 (0.011) ^b	0.936 (0.015) ^b	0.854 ^{bc}	0.719 ^a	0.889 (0.027) ^{bc}	0.748 (0.036) ^{bc}	0.0002
Glutamic acid	0.896 (0.002) ^b	0.891 (0.002) ^b	0.602 (0.061) ^c	0.844 (0.045) ^{bc}	0.492 ^b	0.887 (0.014) ^b	0.893 (0.026) ^b	0.824 ^{bc}	0.642 ^a	0.912 (0.022) ^c	0.822 (0.025) ^{bc}	<0.0001
Valine	0.795 (0.004) ^{bc}	0.823 (0.002) ^{bc}	0.602 (0.061) ^c	0.762 (0.069) ^{bc}	0.489 ^b	0.821 (0.023) ^{bc}	0.825 (0.042) ^{bc}	0.690 ^{bc}	1.241 ^a	0.833 (0.041) ^{bc}	0.586 ^c (0.058) ^c	<0.0001
Histidine	0.855 (0.003) ^{bc}	0.885 (0.002) ^b	0.602 (0.061) ^c	0.834 (0.048) ^{bc}	0.494 ^b	0.881 (0.015) ^b	0.884 (0.028) ^b	0.831 ^{bc}	0.952 ^a	0.897 (0.025) ^b	0.810 (0.027) ^{bc}	<0.0001
Aspartic acid	0.840 (0.003) ^{bc}	0.854 (0.002) ^{bc}	0.602 (0.061) ^{bc}	0.782 (0.063) ^{bc}	0.486 ^b	0.816 (0.024) ^{bc}	0.844 (0.038) ^{bc}	0.717 ^{bc}	0.831 ^a	0.750 (0.061) ^{bc}	0.493 (0.071) ^c	<0.0001
Lysine	0.856 (0.003) ^{bc}	0.906 (0.001) ^b	0.602 (0.061) ^c	0.815 (0.054) ^{bc}	0.495 ^b	0.872 (0.016) ^{bc}	0.891 (0.026) ^b	0.772 ^{bc}	0.822 ^a	0.896 (0.026) ^b	0.732 (0.038) ^{bc}	<0.0001
Proline	0.840 (0.003) ^{bc}	0.856 (0.002) ^{bc}	0.602 (0.061) ^c	0.801 (0.058) ^{bc}	0.494 ^b	0.853 (0.019) ^{bc}	0.853 (0.035) ^{bc}	0.753 ^{bc}	1.047 ^a	0.809 (0.047) ^{bc}	0.621 (0.053) ^c	<0.0001
Methionine	0.943 (0.001) ^b	0.967 (0.000) ^b	0.602 (0.061) ^c	0.933 (0.019) ^b	0.500 ^b	0.972 (0.004) ^b	0.969 (0.008) ^b	0.929 ^b	0.654 ^a	0.953 (0.011) ^b	0.801 (0.028) ^{bc}	<0.0001
Tyrosine	0.851 (0.003) ^{bc}	0.871 (0.002) ^{bc}	0.602 (0.061) ^c	0.812 (0.054) ^{bc}	0.500 ^b	0.881 (0.015) ^{bc}	0.890 (0.026) ^{bc}	0.754 ^{bc}	0.926 ^a	0.870 (0.032) ^{bc}	0.628 (0.052) ^c	<0.0001
Cysteine	0.758 (0.005) ^{bc}	0.915 (0.001) ^{bc}	0.602 (0.061) ^{bc}	0.678 (0.093) ^{bc}	0.500 ^b	0.735 (0.034) ^{bc}	0.928 (0.017) ^{bc}	0.521 ^c	1.098 ^a	0.633 (0.090) ^{bc}	0.625 (0.053) ^{bc}	<0.0001
Isoleucine	0.874 (0.003) ^{bc}	0.881 (0.002) ^{bc}	0.602 (0.061) ^{bc}	0.804 (0.057) ^{bcd}	0.494 ^b	0.846 (0.020) ^{bc}	0.892 (0.026) ^{bc}	0.766 ^{bcd}	1.051 ^a	0.837 (0.040) ^{bcd}	0.497 (0.071) ^d	<0.0001
Phenylalanine	0.912 (0.002) ^b	0.937 (0.001) ^b	0.602 (0.061) ^{bc}	0.846 (0.044) ^{bcd}	0.494 ^b	0.893 (0.014) ^{bc}	0.947 (0.013) ^b	0.840 ^{bcd}	0.829 ^a	0.861 (0.034) ^{bcd}	0.587 (0.058) ^d	<0.0001
Leucine	0.870 (0.003) ^{bc}	0.889 (0.002) ^{bc}	0.602 (0.061) ^c	0.829 (0.049) ^{bc}	0.494 ^b	0.884 (0.015) ^{bc}	0.891 (0.026) ^{bc}	0.803 ^{bc}	0.918 ^a	0.868 (0.032) ^{bc}	0.665 (0.047) ^c	<0.0001
Glycine	0.808 (0.004) ^{bc}	0.854 (0.002) ^{bc}	0.602 (0.061) ^c	0.778 (0.064) ^{bc}	0.988 ^b	0.777 (0.028) ^{bc}	0.849 (0.036) ^{bc}	0.783 ^{bc}	2.004 ^a	0.846 (0.038) ^{bc}	0.735 (0.037) ^{bc}	<0.0001

^{a-d} Means within a row with different superscripts are significantly different.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides; L = levels

Table 4.5 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either Bio-Mos®, Acid-Pak 4-way® or MCT at 28 days.

Treatment	MOSL1	MOSL2	MOSL3	APL1	APL2	APL3	MCTL1	MCTL2	MCTL3	C-	C+	P-value
Alanine	0.772 (0.001) ^{bc}	0.716 (0.003) ^c	0.754 (0.007) ^c	0.811 (0.004) ^{bc}	0.943 (0.057) ^{ab}	0.854 (0.015) ^{abc}	0.428 ^{abc}	0.372 ^c	0.877 (0.007) ^{abc}	0.854 (0.005) ^{abc}	0.500 ^a	0.0007
Threonine	0.676 (0.002) ^c	0.808 (0.002) ^{abc}	0.757 (0.007) ^{bc}	0.711 (0.006) ^{bc}	0.927 (0.073) ^{ab}	0.777 (0.022) ^{abc}	0.422 ^{abc}	0.326 ^c	0.771 (0.013) ^{bc}	0.768 (0.009) ^{bc}	0.500 ^a	0.0021
Serine	0.663 (0.002) ^c	0.807 (0.002) ^{abc}	0.742 (0.007) ^{bc}	0.640 (0.007) ^{bc}	0.923 (0.077) ^{ab}	0.776 (0.023) ^{abc}	0.425 ^{abc}	0.319 ^c	0.806 (0.011) ^{abc}	0.822 (0.007) ^{abc}	0.500 ^a	0.0011
Arginine	0.808 (0.001) ^{bc}	0.869 (0.001) ^{abc}	0.834 (0.005) ^{bc}	0.859 (0.003) ^{abc}	0.949 (0.051) ^{ab}	0.899 (0.010) ^{abc}	0.472 ^{ab}	0.395 ^c	0.838 (0.009) ^{bc}	0.883 (0.004) ^{abc}	0.500 ^a	0.0037
Glutamic acid	0.909 (0.000) ^a	0.918 (0.001) ^a	0.893 (0.003) ^a	0.940 (0.001) ^a	0.952 (0.048) ^a	0.931 (0.007) ^a	0.459 ^a	0.442 ^a	0.925 (0.004) ^a	0.920 (0.003) ^a	0.500 ^a	0.1659
Valine	0.780 (0.001) ^{bc}	0.791 (0.002) ^{bc}	0.787 (0.006) ^{bc}	0.811 (0.004) ^{bc}	0.940 (0.060) ^{ab}	0.848 (0.015) ^{abc}	0.426 ^{abc}	0.374 ^c	0.845 (0.009) ^{abc}	0.837 (0.006) ^{ab}	0.500 ^a	0.0045
Histidine	0.887 (0.001) ^b	0.906 (0.001) ^{ab}	0.887 (0.003) ^b	0.914 (0.002) ^{ab}	0.964 (0.036) ^{ab}	0.915 (0.009) ^{ab}	0.461 ^{ab}	0.430 ^b	0.913 (0.005) ^{ab}	0.911 (0.003) ^{ab}	0.500 ^a	0.0135
Aspartic acid	0.729 (0.001) ^{ab}	0.791 (0.002) ^{ab}	0.718 (0.008) ^{ab}	0.797 (0.004) ^{ab}	0.903 (0.097) ^{ab}	0.758 (0.024) ^{ab}	0.383 ^{ab}	0.327 ^b	0.747 (0.014) ^{ab}	0.746 (0.010) ^{ab}	0.500 ^a	0.0240
Lysine	0.873 (0.001) ^{ab}	0.914 (0.001) ^{ab}	0.874 (0.004) ^{ab}	0.868 (0.003) ^{ab}	0.950 (0.049) ^{ab}	0.907 (0.009) ^{ab}	0.457 ^{ab}	0.423 ^b	0.895 (0.006) ^{ab}	0.905 (0.004) ^{ab}	0.500 ^a	0.0412
Proline	0.740 (0.001) ^{bc}	0.786 (0.002) ^{abc}	0.748 (0.007) ^{bc}	0.804 (0.004) ^{abc}	0.928 (0.072) ^{ab}	0.826 (0.018) ^{abc}	0.419 ^{abc}	0.340 ^c	0.819 (0.010) ^{abc}	0.808 (0.007) ^{abc}	0.500 ^a	0.0052
Methionine	0.954 (0.000)	0.944 (0.001)	0.926 (0.002)	0.935 (0.001)	0.969 (0.031)	0.956 (0.004)	0.485	0.458	0.965 (0.002)	0.951 (0.002)	0.500	0.0745
Tyrosine	0.872 (0.001)	0.859 (0.002)	0.811 (0.005)	0.823 (0.004)	0.925 (0.075)	0.889 (0.011)	0.451	0.418	0.912 (0.005)	0.905 (0.004)	0.500	0.0776
Cysteine	0.923 (0.000)	0.924 (0.001)	0.828 (0.005)	0.826 (0.003)	0.913 (0.087)	0.911 (0.009)	0.448	0.425	0.912 (0.005)	0.861 (0.005)	0.500	0.1670
Isoleucine	0.828 (0.001) ^{ab}	0.811 (0.002) ^{ab}	0.787 (0.006) ^b	0.814 (0.004) ^{ab}	0.935 (0.065) ^{ab}	0.873 (0.013) ^{ab}	0.438 ^{ab}	0.402 ^b	0.852 (0.008) ^{ab}	0.823 (0.007) ^{ab}	0.500 ^a	0.0159
Phenylalanine	0.877 (0.001) ^{ab}	0.893 (0.001) ^{ab}	0.840 (0.004) ^{ab}	0.814 (0.004) ^b	0.944 (0.056) ^{ab}	0.902 (0.010) ^{ab}	0.451 ^{ab}	0.426 ^{ab}	0.869 (0.007) ^{ab}	0.867 (0.005) ^{ab}	0.500 ^a	0.0231
Leucine	0.861 (0.001) ^{ab}	0.863 (0.002) ^{ab}	0.828 (0.005) ^b	0.828 (0.003) ^b	0.942 (0.058) ^{ab}	0.899 (0.010) ^{ab}	0.445 ^{ab}	0.415 ^{ab}	0.888 (0.006) ^{ab}	0.885 (0.004) ^{ab}	0.500 ^a	0.0255
Glycine	0.768 ^c (0.001) ^c	0.823 (0.002) ^{bc}	0.807 (0.005) ^{bc}	0.787 (0.004) ^{bc}	0.948 (0.052) ^{ab}	0.831 (0.017) ^{bc}	0.831 ^{abc}	0.378 ^c	0.834 (0.009) ^{bc}	0.827 (0.006) ^{bc}	0.500 ^a	0.0018

^{a-c} Means within a row with different superscripts are significantly different. – Could not be determined.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides; L = levels

Table 4.6 Mean apparent digestibility coefficients (\pm SE) for individual amino acids in broiler chickens on a diet supplemented with either Bio- Mos®, Acid-Pak 4-way® or MCT at 35 days.

Treatment												
Amino acid	MOSL1	MOSL2	MOSL3	APL1	APL2	APL3	MCTL1	MCTL2	MCTL3	C-	C+	P-value
Alanine	0.873 (0.001)	0.792 (0.003)	0.804 (0.007)	0.827 (0.005)	0.883 (0.117)	0.773 (0.030)	0.830	0.793 (0.034)	0.800 (0.014)	0.781 (0.002)	0.825 (0.001)	0.6474
Threonine	0.820 (0.002)	0.843 (0.002)	0.812 (0.007)	0.791 (0.007)	0.905 (0.095)	0.763 (0.031)	0.842	0.742 (0.042)	0.749 (0.018)	0.701 (0.002)	0.755 (0.001)	0.0673
Serine	0.803 (0.002)	0.848 (0.002)	0.846 (0.006)	0.778 (0.007)	0.908 (0.092)	0.758 (0.032)	0.812	0.715 (0.047)	0.787 (0.015)	0.742 (0.002)	0.788 (0.001)	0.0877
Arginine	0.853 (0.002) ^{ab}	0.900 (0.001) ^{ab}	0.913 (0.003) ^{ab}	0.874 (0.004) ^{ab}	0.954 (0.046) ^a	0.892 (0.014) ^{ab}	0.930 ^{ab}	0.827 (0.028) ^a	0.851 (0.011) ^{ab}	0.857 (0.001) ^{ab}	0.834 (0.001) ^{ab}	0.0102
Glutamic acid	0.941 (0.001)	0.919 (0.001)	0.916 (0.003)	0.929 (0.002)	0.942 (0.058)	0.899 (0.013)	0.921	0.840 (0.026)	0.886 (0.008)	0.887 (0.001)	0.896 (0.001)	0.1394
Valine	0.849 (0.002)	0.836 (0.002)	0.824 (0.007)	0.827 (0.005)	0.899 (0.101)	0.772 (0.030)	0.836	0.776 (0.037)	0.749 (0.018)	0.773 (0.002)	0.780 (0.001)	0.2612
Histidine	0.925 (0.001)	0.923 (0.001)	0.918 (0.003)	0.917 (0.003)	0.955 (0.045)	0.893 (0.014)	0.926	0.868 (0.021)	0.880 (0.009)	0.893 (0.001)	0.901 (0.001)	0.1223
Aspartic acid	0.796 (0.002)	0.832 (0.002)	0.790 (0.008)	0.772 (0.007)	0.877 (0.123)	0.737 (0.035)	0.799	0.658 (0.056)	0.684 (0.023)	0.697 (0.002)	0.697 (0.002)	0.1023
Lysine	0.905 (0.001)	0.913 (0.001)	0.915 (0.003)	0.908 (0.003)	0.932 (0.068)	0.868 (0.017)	0.889	0.830 (0.028)	0.817 (0.013)	0.841 (0.001)	0.847 (0.001)	0.0677
Proline	0.847 (0.002)	0.840 (0.002)	0.821 (0.007)	0.824 (0.006)	0.901 (0.099)	0.791 (0.027)	0.856	0.727 (0.045)	0.771 (0.016)	0.776 (0.002)	0.778 (0.001)	0.1869
Methionine	0.973 (0.000) ^a	0.956 (0.001) ^{ab}	0.966 (0.001) ^{ab}	0.965 (0.001) ^{ab}	0.947 (0.053) ^{ab}	0.845 (0.020) ^b	0.926 ^{ab}	0.939 (0.010) ^{ab}	0.864 (0.010) ^{ab}	0.911 (0.001) ^{ab}	0.926 (0.000) ^{ab}	0.0087
Tyrosine	0.894 (0.001)	0.884 (0.002)	0.861 (0.005)	0.937 (0.002)	0.922 (0.078)	0.838 (0.021)	0.891	0.808 (0.031)	0.845 (0.011)	0.868 (0.001)	0.842 (0.001)	0.1661
Cysteine	0.915 (0.001) ^{ab}	0.933 (0.001) ^{ab}	0.861 (0.005) ^{ab}	0.980 (0.001)	0.914 (0.086) ^{ab}	0.822 (0.023)	0.877 ^{ab}	0.799 (0.033) ^{ab}	0.859 (0.010) ^{ab}	0.765 (0.002) ^b	0.826 (0.001) ^{ab}	0.0140
Isoleucine	0.874 (0.001)	0.852 (0.002)	0.821 (0.007)	0.867 (0.004)	0.890 (0.110)	0.778 (0.029)	0.851	0.790 (0.034)	0.704 (0.021)	0.763 (0.002)	0.757 (0.001)	0.0974
Phenylalanine	0.895 (0.001)	0.907 (0.001)	0.883 (0.004)	0.908 (0.003)	0.928 (0.072)	0.835 (0.022)	0.893	0.834 (0.027)	0.768 (0.017)	0.829 (0.001)	0.803 (0.001)	0.0233
Leucine	0.902 (0.001)	0.884 (0.001)	0.871 (0.004)	0.899 (0.003)	0.924 (0.076)	0.840 (0.021)	0.883	0.831 (0.028)	0.817 (0.013)	0.833 (0.001)	0.835 (0.001)	0.2031
Glycine	0.860 (0.001)	0.868 (0.001)	0.864 (0.005)	0.821 (0.006)	0.921 (0.079)	0.821 (0.024)	0.890	0.823 (0.029)	0.805 (0.014)	0.780 (0.002)	0.819 (0.001)	0.1428

^{a-b} Means within a row with different superscripts are significantly different. – Could not be determined.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides; L = levels

Chapter 5

The effect of addition of Bio-Mos®, Acid-Pak 4-way and MCT on growth performance in broilers

5.1. Introduction

In terms of AAD and AND, the addition of Bio-Mos®, Acid-Pak 4-way® and MCT has proven to have some beneficial effects.

In this trial Bio-Mos®, MCT and Acid-Pak 4-way® were tested again, but this time to evaluate its potential to increase broiler production performance (growth performance and feed efficiency). The studies, as seen in Chapter 3 and 4, with Bio-Mos®, MCT and Acid-Pak 4-Way® have shown some positive results and some non-significant results. Additional research was therefore needed to determine their effect on broiler production performance. The inclusion levels that proved the most effective at the time, in terms of AND and AAD, were used. The main production criteria's were body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) (Whitehead, 2002).

5.2 Materials and methods

Nine hundred day-old Cobb 500 broiler chicks (as-hatched) were placed in thirty floor pens, each pen containing thirty birds. The experimental units/pens were allocated at random to five experimental treatments with six replicates per treatment. The birds were vaccinated at day-old against Newcastle disease and Infectious bronchitis. The environmental temperature within the house was 30°C for the first day and was then decreased by 0.5°C every second day to 20°C. Continuous light (24L:0D) was provided for the duration of the trail. Birds were offered *ad libitum* access to feed and water. The experiment had a completely randomised design with main effect of dietary treatment.

The three experimental trials were approved by the University of Stellenbosch Animal Ethics Committee.

5.3 Experimental design

A control starter diet and control finisher diet were formulated by the EFG Broiler Nutrition Optimizer Program (Winfeed 2 Feed Formulator). A detailed list of the ingredients used in the experimental diet and the formulated nutrient composition of the diets are presented in Table 5.1.

Table 5.1 Composition of starter and finisher diets for broiler chickens based on as fed basis (g/kg).

Ingredient	Starter	Finisher
Maize	549.85	708.73
Soybean 46	270	251.34
Soybean full fat	125.23	
Choline chloride 60%	0.67	0.53
DL-methionine	2.36	1.34
L-threonine	0.03	
L-lysine HCL	7.68	1.46
Limestone	21.69	18.13
Salt	4.03	4.07
Monocalcium phosphate	11.78	7.41
Sodium bicarbonate	4.08	4.18
Vitamin E		0.40
Vit + min premix	0.50	1.3
<i>Calculated nutrient content (*)</i>		
AMEn (MJ/kg)	12.49	12.68
Crude protein (%)	22.00	17.50
Lysine (%)	1.27	0.95
Methionine (%)	0.56	0.41
Methionine + Cystine (%)	0.93	0.72
Threonine (%)	0.88	0.69
Tryptophan (%)	0.27	0.20
Arginine (%)	1.51	1.12
Isoleucine (%)	1.00	0.79
Leucine (%)	1.95	1.65
Histidine (%)	0.62	0.50
Phenylalanine + tyrosine (%)	1.88	1.51
Valine (%)	1.05	0.84
Calcium	1.10	0.90
Total Phosphorous	0.67	0.54

(*) Amino acids expressed on a digestible basis. AMEn: Nitrogen corrected Apparent Metabolisable Energy

The starter and finisher diets were fed as a mash. Bio-Mos®, MCT and Acid-Pak 4-way® were included at 3.0g/kg, 4.2g/kg and 1.6g/kg respectively. A summary of the dietary treatments are presented in Table 5.2. The starter diet was provided from day one to day thirteen and the finisher diet from day fourteen to the end of the trial at day 35. Birds were weighed (per pen) on arrival and on days seven, 14, 21, 28, 35. Food intake per pen was measured at days seven, 14, 21, 28 and 35 and mortality was recorded daily. Feed conversion ratio (FCR) is equal to gram feed, divided by the weight (g) gained. Body weight gain (BWG) is equal to the total weight (g) gained during the entire experimental trial.

Table 5.2. Dietary treatments used throughout the trial.

Treatment	Description
A	Control -
B	Control +
C	Control + Bio-Mos®
D	Control + MCT
E	Control + Acid-Pak 4-way®

Data were tested for homogeneity of variance using Levene's test and for normal distribution using the Kolmogorov-Smirnov test. All data of equal variance and normal distribution were analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0 (Waldroup *et al.*, 2003; Angel *et al.*, 2005). The Bonferroni (Dunn) t-test was used to test differences between treatment means when the treatment effect was found to be significant. The probability level was set at 5%.

5.4. Results and discussion

The treatment means for body weight (BW) for the entire experimental period are presented in Table 5.3. No treatments had any significant effects on BW ($P=0.05$) for days 0-7, 8-14, 15-21, 22-28 and 29-35 of the experimental period.

Table 5.3. Mean bodyweight (BW) (g/bird) (\pm SE) on the dietary treatments for the periods 0-7d, 8-14d, 15-21d, 22-28d and 29-35d, respectively.

Period		0-7d	8-14d	15-21d	22-28d	29-35d
Treatment	N	BW	BW	BW	BW	BW
C-	5	169.33 (6.018)	435.39 (13.850)	822.87 (20.494)	1377.31 (34.289)	1845.91 (39.011) ^{ab}
C+	5	173.08 (6.018)	438.79 (13.850)	839.07 (20.494)	1395.56 (34.289)	1870.11 (39.011) ^{ab}
MOS	5	169.95 (6.018)	433.08 (13.850)	822.38 (20.494)	1384.74 (34.289)	1870.85 (39.011) ^{ab}
MCT	5	171.76 (6.018)	435.04 (13.850)	811.07 (20.494)	1346.25 (34.289)	1814.19 (39.011) ^b
AP	5	174.39 (6.018)	443.27 (13.850)	851.78 (20.494)	1413.46 (34.289)	1911.00 (39.011) ^a
P-value		0.910	0.953	0.329	0.401	0.188

^{a-b} Means within a column with different superscripts are significantly different.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides

The treatment means for body weight gain (BWG) for the entire experimental period are presented in Table 5.4. There were no significant ($P \geq 0.05$) differences between treatments for days 0-7, 8-14, 22-28 and 29-35 of the experimental period. At days 15-21, the treatment supplemented with Acid-Pak 4-way® had a significantly ($P=0.017$) higher BWG, when compared to the negative control, as well as the treatments supplemented with Bio-Mos® and MCT. The treatment with MCT performed poorer, in terms of BWG, when compared to the positive control.

Table 5.4. Mean bodyweight gain (BWG) (g/bird/period) (\pm SE) of the dietary treatments for the periods 0-7d, 8-14d, 15-21d, 22-28d and 29-35d, respectively.

Period		0-7d	8-14d	15-21d	22-28d	29-35d
Treatment	N	BWG	BWG	BWG	BWG	BWG
C-	5	124.46 (5892)	266.06 (9.204)	387.48 (9.164) ^{bc}	554.44 (19.712)	468.60 (20.221)
C+	5	129.94 (5892)	265.71 (9.204)	400.29 (9.164) ^{ab}	556.48 (19.712)	474.55 (20.221)
MOS	5	125.97 (5892)	263.13 (9.204)	389.29 (9.164) ^{bc}	562.36 (19.712)	486.11 (20.221)
MCT	5	127.67 (5892)	263.28 (9.204)	376.03 (9.164) ^c	535.18 (19.712)	467.94 (20.221)
AP	5	132.03 (5892)	268.88 (9.204)	408.52 (9.164) ^a	561.68 (19.712)	497.54 (20.221)
P-Value		0.714	0.970	0.017	0.646	0.544

^{a-c} Means within a column with different superscripts are significantly different.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides

The treatment means for feed intake (FI) for the entire experimental period are presented in Table 5.5. There were no significant ($P \geq 0.05$) differences between treatments for the entire experimental period.

Table 5.5 Mean feed intake (FI) (g/bird/period) (\pm SE) of the dietary treatments for the periods 0-7d, 8-14d, 15-21d, 22-28d and 29-35d, respectively.

Period		0-7d	8-14d	15-21d	22-28d	29-35d
Treatment	N	FI	FI	FI	FI	FI
C-	5	204.78 (10.892) ^{ab}	429.09 (19.194) ^{ab}	1305.30 (83.623) ^{ab}	1046.61 (24.127)	1042.14 (22.680)
C+	5	204.87 (10.892) ^{ab}	410.60 (19.194) ^b	1354.06 (83.623) ^a	1050.92 (24.127)	1033.81 (22.680)
MOS	5	196.22 (10.892) ^{ab}	418.79 (19.194) ^{ab}	1143.15 (83.623) ^c	1045.27 (24.127)	1052.98 (22.680)
MCT	5	187.54 (10.892) ^b	454.85 (19.194) ^a	1165.96 (83.623) ^{bc}	1036.80 (24.127)	1047.99 (22.680)
AP	5	214.66 (10.892) ^a	436.70 (19.194) ^{ab}	1316.03 (83.623) ^{ab}	1078.41 (24.127)	1059.92 (22.680)
P-value		0.168	0.210	0.060	0.500	0.814

^{a-c} Means within a column with different superscripts are significantly different.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides

The treatment means for feed conversion ratio (FCR) for the entire experimental period are presented in Table 5.6. There were no significant ($P \geq 0.05$) differences between treatments for days the entire experimental period.

Table 5.6 Feed conversion ratio (FCR) (\pm SE) of the dietary treatments for the periods 0-7d, 8-14d, 15-21d, 22-28d and 29-35d, respectively.

Period		0-7d	8-14d	15-21d	22-28d	29-35d
Treatment	N	FCR	FCR	FCR	FCR	FCR
C-	5	1.65 (0.062) ^a	1.62 (0.074) ^{ab}	3.37 (0.227)	1.90 (0.054)	2.23 (0.079)
C+	5	1.58 (0.063) ^{ab}	1.55 (0.074) ^b	3.39 (0.227)	1.90 (0.054)	2.19 (0.079)
MOS	5	1.56 (0.063) ^{ab}	1.59 (0.074) ^{ab}	2.94 (0.227)	1.86 (0.054)	2.17 (0.079)
MCT	5	1.47 (0.063) ^b	1.73 (0.074) ^a	3.10 (0.227)	1.94 (0.054)	2.25 (0.079)
AP	5	1.63 (0.063) ^a	1.62 (0.074) ^{ab}	3.22 (0.227)	1.92 (0.054)	2.14 (0.079)
P-value		0.059	0.207	0.267	0.663	0.690

^{a-b} Means within a column with different superscripts are significantly different.

C+ = positive control; C- = negative control; MOS = Bio-Mos®; AP = Acid-Pak 4-way®; MCT = Medium chain triglycerides

None of the treatments had any significant effect on BW, but Zulkifli *et al.* (2006) found opposing results which proved that Acid-Pak 4-way® improved BW. In terms of BWG, Acid-Pak 4-way® had a higher BWG, when compared to the negative control, Bio-Mos® and MCT. Sun *et al.* (2005) also found that the effects of Acid-Pak 4-way® on BWG was comparable with the effects of antibiotic supplementation. In terms of FI and FCR, no treatments proved to have an effect.



Chapter 6

General Conclusion

The search for effective alternatives to antibiotic feed additives has intensified as a result of the ban on antibiotic growth promoters in Europe. Several strategies are currently available that can have significant impacts on poultry production. The basic mechanisms that explain the beneficial effects of these strategies are often clearly different from those that explain the effects of antimicrobial growth promoters. There is limited data to support the efficacy of many of the AGP alternatives that are commercially available. Often field trials are conducted without the inclusion of positive and negative controls; variation is almost impossible to rule out. In addition, the exact mode of action is frequently not known and research to clarify these factors is expensive.

The primary objective of this thesis was to evaluate whether alternative feed additives, such as fresh garlic, Bio-Mos®, Acid-Pak 4-way® and MCT, may improve N and AA digestibility and thus performance of a commercial diet. In both of the digestibility trials, none of the treatments (garlic, Bio-Mos®, Acid-Pak 4-way® and MCT), improved AND. In the first trial, neither garlic nor Bio-Mos® showed any improvement in AAD at any determination period (day 21, 28 or 35). In the second trial, all three treatments (Bio-Mos®, Acid-Pak 4-way® and MCT) showed some improvement in terms of AAD. Acid-Pak 4-way® proved to have some beneficial effects in terms of BWG.

It can be concluded that Bio-Mos®, Acid-Pak 4-way®, as well as MCT can be a possible alternatives to antibiotic supplementation. These three treatments did not necessary prove to be more effective than antibiotics, but are definitely competitive alternatives. There were more prominent effects with the production performance trial with Acid-Pak 4-way®. The lack of response, especially seen with AAD, could be the result of no real health challenge for the birds; for example facilities were too clean or the correct diet composition was formulated.

Whether or not we, as an industry, are convinced that there is a scientific case for the withdrawal of subtherapeutic antibiotics, it is clear that the consumer has the last say. It is therefore important that alternative strategies for improving poultry production are identified. The use of alternatives to AGP's will not provide a single solution to AGP removal from animal feed. One of the most important lessons to be learned is that AGP removal necessitates improved general husbandry and management, hygiene and health status are essential for minimising the losses from in-feed AGP removal.

REFERENCES

- ACVM Group, 2000. Review of animal health antibiotic products and their potential for contributing to the development of antibiotic resistant strains of human bacterial pathogens. *Ministry of Agriculture and Forestry, New Zealand*.
- Adibmoradi, M., Navidshad, B. Seifdavati, J. & Royan, M., 2006. Effect of dietary garlic meal on histological Structure of small intestine in broiler chickens. *J. Poult. Sci.* 43: 378-383
- Akiba, Y., Hah, T.W., Murakami, H., Horiguchi, M. & Yamazaki, M., 1993. Metabolizable energy and effect on amino acid availability of medium-chain triglycerides in diets fed to chickens of different ages. *Anim. Feed Sci. Tech.* 43: 259-268
- Angel, R., Dalloul, R.A. & Doerr, J., 2005. Performance of broiler chickens fed diets supplemented with a direct-fed microbial. *Poult. Sci.* 84: 1222-1231.
- AOAC, 1990. Association of analytical chemists: Methods of analysis (15th edition). Association of Analytical Chemists, Washington DC.
- Cencic, A, Noya, B.R. & Botic, T., 2006. Can probiotics have a beneficial impact on the health status of farmed animals? *World Nutrition Forum*: 59-74.
- Collett, S., Carl, R. & Dawson, A., 2001. Alternatives to sub-therapeutic antibiotics: What are the options? How effective are they? *Proc. of the 2nd Internat. Poult. Broiler Nutritionists' Conf.*: 267-281.
- Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12: 564-582.
- Cromwell, G.L., 1999. Safety issues, performance benefits of antibiotics for swine examined. *Feedstuffs*, 18 pp.
- Demir E., Sarica, S., Ozcan, M.A. & Suicmez, M., 2005. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *Archiv fur Geflugelkunde* 69: 110-116.
- Dibner, J.J. & Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. *Poult. Sci.* 84: 634-643.
- Dibner, J.J. & Richards, J.D., 2004. The digestive system: Challenges and opportunities. *J. of App. Poult. Res.* 13: 86-93.
- EFG Broiler Nutrition Optimizer Program, Winfeed 2 Feed Formulator, 2005.
- Fenster, R., 2001. Feed additives: A global market study. *PJB publications Ltd.*: 82.
- Fritts, C.A. & Waldroup, P.W., 2003. Evaluation of Bio-Mos® mannan oligosaccharide as a replacement for growth promoting antibiotics in diets for turkeys. *Int. J. Poult. Sci.* 1: 19-22.
- Fuller, R., 1989. Probiotics in man and animals. *J. App. Bacteriol.* 66: 365-378.
- García, V., Catalá-Gregori, P., Hernández, F., Megías, M.D. & Madrid, J., 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. *J. Appl. Poult. Res.* 16: 555-562.
- Gibson, G.R. & Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125: 1401-1412.
- Goodling, A.C., Cerniglia, G.J. & Heber, J.A., 1987. Production performance of White Leghorn layers fed *Lactobacillus* fermentation products. *Poult. Sci.* 66: 480-486.

- Grela, E.R, Krusinski, R. & Matras, J., 1998. Efficacy of diets with antibiotic and herb mixture additives in feeding of growing-finishing pigs. *J. Anim. Feed Sci.* 7: 171-175.
- Guo, F.C., Kwakkel, R.P., Soede, J., Williams, B.A. & Verstegen, M.W.A., 2004a. Effect of a Chinese herb medicine formulation, as an alternative for antibiotics, on performance of broilers. *Br. Poult. Sci.* 45: 793-797.
- Guo, F.C., Williams, B.A., Kwakkel, R.P., Li, H.S., Li, X.P., Luo, J.Y., Li, W.K. & Verstegen, M.W.A., 2004b. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poult. Sci.* 83:175-182.
- Hernandez, F, Madrid, J., Garcia, V., Orengo, J. & Megias, M.D., 2004. Influence of two plant extracts on broiler performance, digestibility, and digestive organ size. *Poult. Sci.* 83: 169-174.
- Horton, G.M.J, Fennell, M.J. & Prasad, B.M., 1992. Effect of dietary garlic on performance, carcass composition and blood chemistry changes in broiler chickens. *Can. J. Anim. Sci.* 71: 939-942.
- Jin, L.Z., Ho, Y.W., Abdullah, N. & Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World Poult. Sci. J.* 53: 351-368.
- Leary A.M. & Partridge, I., 2008. Popular alternatives to antibiotic feed additives in monogastric production systems. *Zootecnica* 28.
- Lee, H.F. & Chiang, S.H., 1994. Energy value of medium-chain triglycerides and their efficacy in improving survival of neonatal pigs. *J. Anim. Sci.* 72: 133-138.
- Lewis M.R., Rose, S.P., Mackenzie, A.M. & Tucker, L.A., 2004. Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. *Vet. and Comp. Oncol.* 2: 82-90.
- Maritz, J. 2005. Antibiotics in poultry production: Can South Africa afford to follow global trends? *Worlds' Poult. Sci. Assoc., South African Branch, Proc. of the 24th Scientific Day*, pp. 131-139.
- Mohan, B., Kadirvel, R., Bhaskaran, M. & Notarajan, A., 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br. Poult. Sci.* 36: 799-803.
- Moore, P.R., Evenson, A., Luckey, T.D., McCoy, E., Elvehjam, C.A. & Hart, E.B., 1946. Use of sulfasuxidine, streptothricin and streptomycin in nutritional studies with chick. *J. Biol. Chem.* 165:437-441.
- Nahashon, S.N., Nakaue, H.S. & Mirosh, I.W., 1996. Performance of single comb white leghorn fed a diet supplemented with a live microbial during the growth and egg laying phases. *Anim. Feed. Sci. Tech.* 57: 25-38.
- North, M.O. & Bell, D.D., 1990. Commercial chicken production manual, 4th edition, Van Nostrand Reinhold, New York, U.S.A., 913 pp.
- Olnood, C.G., Mikkelsen, L.L., Choct, M. & Iji, P.A., 2007. Antagonistic activity of novel probiotics and their effect on growth performance of broiler chickens. *Aust. Poult. Sci. Symp.*
- Partridge, I.G., 1991. Growth Promoters in animal production: Status and prospects. *Recent advances in Animal Nutrition in Australia.* 229-238.
- Patterson, J.A., and K.M. Burkholder. 2003. Application of Prebiotics and Probiotics in Poultry Production. *Poult. Sci.*, 82: 627-631
- Pirgozliev, V., Murphy, T.C., Owens, B., George, J. & McCann, M.E.E., 2007. Fumaric acid and sorbic acid as feed additives in broiler feed. *Res. Vet. Sci.* 84: 387-394.

- Priyankarage, N., Silva, S.S.P., Gunartne, S.P., Kothalawala, H., Palliyaguru, M.W.C.D. & Gunawardana, G.A., 2003a. Efficacy of probiotics and their effects on performance, carcass characteristics, intestinal microflora and *Salmonella* incidence in broilers. *Br. Poult. Sci.* 44: S26.
- Priyankarage N., Silva, S.S.P., Gunartne, S.P., Palliyaguru, M.W.C.D., Weerasinghe, W.M.P.B. & Fernando, P.S., 2003b. Comparison of the efficacies of different probiotics for broiler chickens. *Br. Poult. Sci.* 44: S42-S43.
- Ravindran, V, Hew, L.I., Ravindran, G. & Bryden, W.L., 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. *Br. Poult. Sci.* 40: 266-274.
- Ross, Z.M, O'Gara, E.A., Hill, D.J., Sleightholme, H.V. & Maslin, D.J., 2001. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Appl. and Environm. Microbiol.* 67: 475-480.
- Sarica, S., Ciftci, A., Demir, E., Kilinc, K. & Yildirim, Y., 2005. Use of an antibiotic growth promoter a two herbal natural feed additives with and without exogenous enzymes in wheat-based broiler diets. *S. Afr. J. Anim. Sci.* 35: 61-72.
- Sun, X., Mcelroy, A., Webb, K.E., Sefton, A.E. & Novak, C., 2005. Broiler Performance and Intestinal alterations when feeding drug-free diets. *Poult. Sci.* 84: 1294-1302.
- Ten Doeschate, R.A.H.M., Scheele, C.W., Schreurs, V.V.A.M. & Van der Klis, J.D., 1993. Digestibility studies in broiler chickens: Influence of genotype, age, sex and method of determination. *Br. Poult. Sci.* 34: 131-146.
- Tortuero, F. & Fernandez, E., 1995. Effect of inclusion of microbial culture in barley-based diets fed to laying hens. *Anim. Feed. Sci. Tech.* 53: 255-256.
- Turner, K.A., Applegate, T.J. & Lilburn, M.S., 1999. Effects of feeding high carbohydrate or fat diets. 2. Apparent digestibility and apparent metabolizable energy of the posthatch poult. *Poult. Sci.* 78: 1581-1587.
- Van Immerseel, F, Cauwerts, K., Devriese, L.A., Haesebrouck, F. & Ducatelle, R., 2002. Feed additives to control *Salmonella* in poultry. *World Poult. Sci. J.* 58: 501-513.
- Waldroup, P.W., Oviedo-Rondon Edgar, O. & Fritts, C.A., 2003. Comparison of Bio-Mos® and antibiotic feeding programs in broiler diets containing copper sulphate. *Int. J. Poult. Sc.*, 2: 28-31.
- Wenk, C., 2003. Growth promoter alternatives after the ban on antibiotics. *Pig News and Information* 24: 11N-12N.
- Whitehead, C.C., 2002. Nutrition and poultry welfare. *World Poult. Sci. J.* 58: 349-355.
- Yang, Y., Iji, P.A. & Choct, M., 2007. Effects of different dietary levels of mannanoligosaccharide on growth performance and gut development of broiler chickens. *Asia-Australas. J. Anim. Sci.* 20:1084-1091.
- Yang, Y., Iji, P.A., Kocher, A., Thomson, E., Mikkelsen, L.L. & Choct, M., 2008. Effects of mannanoligosaccharides in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *Br. Poult. Sci.* 49: 186-194.
- Yeo, J & Kim, K., 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. *Poult. Sci.* 76: 381-385.

Zulkifli, I., Juriah, K., Htin, N.N. & Norazlina, I., 2006. Responses of heat stressed broiler chickens to dietary supplementation of virginiamycin and Acid-Pak 4-Way™, and early age feed restriction. *Eur. Poult Sci.* 70:119-126.

