Effect of Alternatives to Antibiotic Growth Promoters on Broiler Performance

Nthoto V. Mosoeunyane

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(National University of Lesotho)

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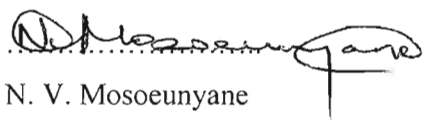
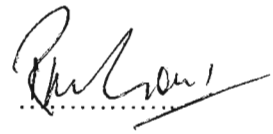
Declaration

We hereby declare that the research reported in this thesis does not contain material that has been accepted for the award of any other degree or diploma in another University and, to the best of our knowledge, does not contain material previously published or written by another person, except where due reference is made in the text.



N. V. Mosoeunyane

(Student)



R.M. Gous

(Supervisor)

Dedication

This thesis is dedicated to my late father, Khethang Mosoeunyane. May his soul rest in peace.

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General Introduction

Over the past decades the poultry industry has intensified production in order to meet the world's ever-increasing demand for animal protein. Poultry feeds have commonly been supplemented with a number of feed additives and trace elements aimed at improving performance and health of the birds (Jackson *et al.*, 2003). One of the most common of these feed additives, antibiotic growth promoters (AGPs) have, until recently, been incorporated in poultry feeds to increase the digestion and absorption of carbohydrates and fats (Eyssen and De Somer, 1963), improve growth and feed efficiency, and control diseases (Woodward *et al.*, 1988; Hilton *et al.*, 2002; Waldroup *et al.*, 2003b). However, these AGPs have come under increasing scrutiny by some scientists, consumers and government regulators because of the potential development of antibiotic-resistant bacteria that may cross the species-barrier to humans after continuous use (Ratcliff, 2000). The sub-therapeutic use of antibiotics also leads to development of antibiotic resistance in animal pathogens so that it becomes less effective when treating common infections.

These concerns over the emergence of resistant bacteria have led to many countries having to ban or to regulate the use of in-feed antibiotics. In 1999 the European Union (EU) banned four AGPs (virginiamycin, spiramycin, tylosin and Zinc bacitracin), which are commonly used in livestock and poultry feeds (Huyghebaert, 2003). Subsequently, and with effect from January 1, 2006, the EU has banned the use of all AGPs in animal and poultry feeds (Halfhide, 2003). These actions have encouraged nutritionists and feed manufacturers all over the world to search for alternatives to AGPs that would help maintain efficient poultry production and ensure the production of safe poultry meat and eggs. The possible alternatives to AGPs include feeding prebiotic compounds, probiotic

organisms, enzymes, herbs and essential oils, and acidifying the feed using organic acids (Huyghebaert, 2003).

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already in the colon (Gibson and Roberfroid, 1995). The use of prebiotics in poultry feeds has been shown to have the potential to suppress enteric diseases in poultry and subsequent contamination of poultry products (Patterson and Burkholder, 2003). They also modulate the immune response, improve the integrity of the intestine mucosa and promote improved growth and feed conversion in chickens and turkeys (Ammerman *et al.*, 1988, 1989; Olsen, 1996; Savage and Zakrzewska, 1997; Sonmez and Eren, 1999; Spring, 1999; Iji *et al.*, 2001). Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its microbial balance (Fuller, 1989). Dietary supplementation with probiotics has been reported to increase daily gain and feed intake (Yeo and Kim 1997), increase the numbers of lactobacilli and decrease the numbers of *E. coli* (Xuan *et al.*, 2001).

Organic acids are known to have strong antibacterial effects. They have been used as *Salmonella* control agents in feed and water supplies for livestock and poultry (Nursey, 1997). Inclusion of organic acids in broiler feeds has resulted in improved feed conversion and carcass quality (Izat *et al.*, 1988; Denli, 2003).

Enzyme inclusion in poultry feeds has shown great value by increasing digestibility, decreasing intestinal viscosity and inactivating anti-nutritional factors. Their benefits in monogastric feeds include not only enhanced growth performance and feed conversion, but also reduced environmental problems due to reduced output of excreta (Choct, 2004).

The benefits of supplementing poultry feeds with a mixture of herbal essential oils and plant extracts are well documented (William and Losa, 2002; Hernández *et al.*, 2004; Alçiçek *et al.*, 2004). These products are commonly used in poultry feeds due to their anti-microbial effects. The anti-microbial activity of spices and essential oils has been attributed to a number of substituted aromatic molecules, such as eugenol, cinnamaldehyde, and carvacrol (Juven *et al.*, 1994; Bergonzelli *et al.*, 2003). Eugenol has been reported to inhibit the growth of *E. coli* (Blaszyk and Holley, 1998) while cinnamaldehyde inhibited the growth of *E. coli* and *Salmonella typhimurium* (Helander, 1998). A blend of capsicum, cinnamaldehyde and carvacrol has been found to lower the number of *E. coli* and *Clostridium perfringens* in caeca (Jamroz and Kamel, 2002). Thus, feed additives with anti-microbial activity have been considered as potential alternatives to antibiotics, as they improve performance of broilers through the control of the microflora.

The major objective of this project was to evaluate the effect, on performance of broiler chickens, of various products as potential alternatives to AGPs. To this end, four broiler trials were conducted in which the efficacy of various feed supplements was compared with a commonly used AGP.

Chapter 1 is a review of the literature on the role of antibiotics in broiler production, and presents information on possible alternatives to AGPs, including prebiotic compounds, probiotic organisms, enzymes, organic acids and herbs and essential oils. Chapter 2 evaluates the use of feed supplementation with organic selenium (Selplex) and a prebiotic (Bio-Mos) on the fertility of male broiler breeders, as well as *E. coli* concentrations in the faeces as an indicator of transferal through cloacal contact during natural mating. In Chapter 3 a comparison is made between AGPs (Zinc bacitracin) and a prebiotic (Bio-

Mos) on the growth performance of broiler chickens. In Chapter 4 the effects of supplementing feed with an organic feed supplement, "Kick Start" (a product derived from a blend of "fresh" sprouts of wheat, barley, fenugreek, lupin and sunflower), on growth performance of broiler chickens is determined. The impact of supplementing feeds with AGPs (Zinc bacitracin), a prebiotic (Bio-Mos), a probiotic (All-Lac XCL 5x), and an organic-acid based product (Acid-pak 2x) on performance of broiler chickens challenged with *Clostridium perfringens* is compared in Chapter 5.

These trials were all conducted at Ukulinga, the University of KwaZulu-Natal research farm, over a period of two years. The primary objective of the trials was to find a suitable alternative to the AGPs commonly used in South Africa.

Chapter 1

Literature review

1.0 Introduction

The poultry industry for the past decades has given considerable attention to maximizing growth performance in poultry so as to provide for the ever-increasing nutritional needs of the human population. Improvements were possible because of genetic selection for growth, improved nutrition, management systems and better disease control measures.

Nutrition is the most critical factor when one considers the fact that the cost of feed alone constitutes between 65-70% of the total cost of production in poultry enterprise. Poultry diets are formulated to contain adequate amounts of nutrients to meet the birds' needs for maintenance, growth and production (eggs and meat). Poultry possess a limited natural resistance and immunity against colonisation infection by pathogenic microorganisms (Huyghebaert, 2003). Since the efficiency of poultry digestion depends on the microorganisms that inhabit the digestive tract, their diets are supplemented with feed additives and trace elements that create an environment conducive for digestion of feed, as well as improving performance and health of the birds.

Antibiotics have been used for more than five decades in commercial poultry enterprises in enhancing intestinal digestion and absorption of carbohydrates and fats (Miles and Harms, 1984). They are also incorporated into feeds to improve growth, feed efficiency and to control diseases. Although sub-therapeutic antibiotics have been used successfully for years, there is a growing concern about their continued use as performance enhancers with regard to build-up of resistance in the human population. Several approaches are now being considered as alternatives to antibiotic growth promoters. The possible alternatives

include among others probiotics; prebiotics, organic acids and essential oils, and their effects will be highlighted in this review.

1.1 Antibiotics and their uses

Antibiotics are natural, semi-synthetic and synthetic compounds with antimicrobial activity (Phillip *et al.*, 2004). They can be administered orally or topically. They are used in human and veterinary medicine to treat and prevent diseases and as growth promoters in animal feed.

Antibiotics kill or inhibit the growth of bacteria and related microorganisms that cause a reduction of physiological and metabolic performance of food producing animals. There are two types of antibiotics, bactericidal and bacteristatic (Zahner and Maas, 1972). Bactericidal antibiotics work by killing the bacteria that they are targeting. Bacteristatic antibiotics do not kill the organism, but prevent the existing cells from multiplying, keeping the population level constant (Zahner and Maas, 1972). These authors further indicated that many antibiotics work by binding to the active site of the cell's enzymes and thus rendering them ineffective. Therefore, by disabling an enzyme, an antibiotic can stop vital cellular processes or interrupt the synthesis of new proteins in the growth of the cell.

1.1.1 Mode of action

Since their discovery more than 50 years ago, antibiotics have been widely used in livestock and poultry industries when the management was changing rapidly from low-performance and free-range farming to a more controlled and intensive husbandry industry. Shortage of food following World War II also contributed to the widespread use of

antibiotics in the UK's livestock intensive farming (Compassion in World Farming Trust, 2001).

Ferket *et al* (2002) suggested that the mechanisms by which antibiotics influence microflora and promote growth are not well understood: nevertheless many modes of action have being proposed. Some antibiotics are reported to control and limit the growth of microbes (*Clostridium perfringens*) known to be harmful to poultry (Truscott and Al-Sheikhly, 1977). Tannock (1997) reported that growth-promoting antibiotics limit the growth and colonization of a number of non-pathogenic species of bacteria such as *Lactobacilli*, *Bifidobacteria*, *Bacteroides* and *Enterococci* in the gut. By having a favourable microbial composition, the lining of the intestinal wall remains healthy and can thus absorb nutrients from the feed effectively. As a result of enhanced nutrient utilization of their diets, supplemented birds need less feed and produce less waste. Likewise Sodano (1979) observed that stopping infection before it starts would reduce the time and energy needed to fight infection and keep the animals in optimal health. Rosen (1995) further indicated that reduction of bacterial growth-by-growth promoters has been shown to reduce the production of antagonistic microbial metabolites that adversely affect the physiology of the host animal.

1.1.2 Therapeutic use

Enteric diseases are reported to be of economic importance in the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (Patterson and Burkholder, 2003).

Veterinarians in the poultry industry use antibiotics to prevent and treat diseases and to enhance growth, which ultimately improves the bird's productivity. Use of antibiotics in poultry has facilitated efficient production, allowing the consumer to purchase good quality meat and eggs at a reasonable cost (Donoghue, 2003). This author also indicated that their use has enhanced the health and well being of poultry by reducing the incidence of diseases. Antibiotics are administered in feed or in drinking water to prevent, control and treat diseases, especially those that localize in the birds' intestinal tract. North and Bell (1990) indicated that an antibiotic would prevent multiplication of bacteria provided enough is present to attack all the bacteria that are present. It is therefore imperative to supply the correct level because if the amount of antibiotic is small and the number of bacteria is large, an antibiotic would not be effective to fight the disease. Bacitracin has been shown to have an effect in preventing and treating necrotic enteritis in broilers chickens in several studies. Engberg *et al.* (2000) found significantly reduced counts of *Clostridium perfringens* in the caeca of broilers treated with bacitracin at 20 ppm, while others (Stutz *et al.*, 1983; Stutz and Lawton, 1984; Hock *et al.*, 1997) found reduced *C. perfringens* numbers in the ileum when bacitracin was included in diets at a rate of 55 ppm. Supplementation of Zinc bacitracin also resulted in a lower *lactobacillus salivarius* count. Higher levels of *Lactobacilli* may influence broiler growth depression associated with competition in nutrient uptake (Engberg *et al*, 2000).

1.1.3 Sub-therapeutic use

The majority of broiler chickens raised for meat are confined in intensive conditions that compromise their health and well-being. Low-level doses of antibiotics are regularly fed to these birds to speed their growth and compensate for the unsanitary, crowded, and stressful conditions in which they are kept.

About 90% of the antibiotics used in agriculture are for growth promotion and prophylactic purposes rather than treating disease infections (Chadwick and Goode, 1997, cited by Khachatourians, 1998). It is estimated that more than 8 million kg of antibiotics (about one third of all antibiotics) are used sub-therapeutically for growth promotion (Levy, 1998) in the United States. The recommended levels of antibiotics were 510ppm for feeds in the 1950's but have been increased by 10- to 20-fold (Tenover and McGowan, 1996). In poultry, 1400g of bacitracin, chlortetracycline, erythromycin, lincomycin, neomycin, oxytetracycline, penicillin, streptomycin, tylosin or virginiamycin is added to each tonne of feed for growth promotion (Gillespie, 1997).

The growth-enhancing effect of antibiotics was first demonstrated in poultry when streptomycin was included in chicken feed (Moore *et al.*, 1946). These authors observed that the dietary supplements inhibited intestinal bacteria that were producing toxic materials or were rendering certain dietary vitamins unavailable to the animal. Supplementation of antibiotics to animal feed resulted in reduced thickness of the intestinal wall and the overall total mass of the gut (Yen *et al.*, 1985). Coates *et al.* (1955) reported thinner walls of the intestinal tract in chickens fed antibiotics and these authors concluded that antibiotics facilitated absorption and utilization of nutrients. Visek (1978) and Postma *et al.* (1999) reported that antibiotics reduce weight and length of the intestines in poultry; the decrease of the gut wall being associated with improved nutrient absorption (Catron *et al.*, 1953, Scott *et al.*, 1969; Maynard *et al.*, 1979).

Some antibiotics used for growth promotion have been shown to improve the growth and feed efficiency of broilers (Woodward *et al.*, 1988; Miles *et al.*, 1984) and turkey (Waibel *et al.*, 1991). Other studies indicated that antibiotics decrease flock variation (Miles and Harms, 1984) and increase the intestinal digestion and absorption of carbohydrates and fats

(Eyssen and De Somer, 1963). Due to a favourable microbial composition, the lining of the intestinal wall remains healthier and thus absorbs nutrients from the feed more effectively. Thus, Rosen (1995) indicated that to reduce animal production costs, antibacterials, as nutrition improvers are essential.

1.1.4 Antibiotic resistance

Sub-therapeutic use of antibiotics promotes growth and reduces the costs of production in livestock and poultry industries. Many of the antibiotics used in poultry have been used in human medicine as well (Edens, 2003). Antibiotics have been shown to be vital in curing infectious diseases. However, bacteria have the capacity to develop defence mechanisms against antibiotics and can become resistant to their effects. Certain species of bacteria become resistant when antibiotics are administered over a long period of time (North and Bell, 1990). When such resistance develops, the antibiotics no longer stop bacterial growth, and, thus, are no longer capable of treating or curing the disease. In most cases resistance develops only to those antibiotics e.g. bacitracin that are absorbed from the intestinal tract (North and Bell, 1990).

Poultry that are given antibiotics often carry antibiotic-resistant strains of *Salmonella*, which in the long run reach humans through poultry meat, eggs and other foods (Khachatourians, 1998). Ferket *et al.* (2002) further mentioned that after prolonged use, antibiotics have a potential of developing antibiotic resistant human pathogenic bacteria that can be transmitted via the food chain.

Some scientists, consumers and government regulators have been scrutinizing antibiotics because of the potential development of antibiotic-resistant human pathogenic bacteria

after long use (Phillips 1999; Ratcliff 2000). According to Wegener *et al.* (1994), some zoonotic (*Salmonella, Campylobacter*, etc.) and other bacteria are transmitted from animals to human beings via the food chain. These bacteria may possess antibiotic-resistance genes to existing susceptible normal human flora. As a result of the growing concern over the transmission and increase of resistant bacteria via the food chain, the European Union (EU) in June 1999 decided to ban four commonly used growth promoters, namely, Virginiamycin, Spiramycin, Tylosin and Zinc bacitracin (Huyghebaert, 2003). Halfhide (2003) predicted that by the beginning of 2006 the EU would officially ban all growth promoting antibiotics in poultry and livestock. In Sweden, the use of antimicrobial growth promoters was banned in 1986 (Wierup, 2001). Moreover, Fritts and Waldroup (2003) reported that the use of antibiotics for growth in poultry had been banned in several countries and the possibility of many parts of the world facing similar legislation is strong.

1.1.5 Effects of antibiotics on the growth of germ-free animals

The largest benefits with antibiotics are seen in animals that are stressed, exposed to large quantities of pathogenic bacteria or are raised under less than ideal conditions in animal husbandry (Hill *et al.*, 1952).

Animals grown in sterile environments received no benefit from being given the antibiotics (Tyler, 1965). Luckey (1952) observed that no growth response to antibiotics was obvious when chicks and turkeys were reared under germ-free conditions with or without antibiotic (including bacitracin) supplementation of sterile autoclaved diets. Coates *et al.* (1955) reported that well-nourished healthy chicks responded less to antibiotic supplementation when housed in a carefully cleaned and disinfected place. It can therefore be concluded that antibiotics have little or no benefit when good management practices are followed.

1.2 Alternatives for antibiotic use in poultry

The concern that antibiotics in livestock and poultry production limit the efficiency of antibiotics in human medicine has led to serious consideration of reducing their use. The incidence of necrotic enteritis associated with *Clostridium perfringens* in poultry has increased in countries that stopped using antibiotic growth promoters (Immerseel *et al.*, 2004). *Clostridium perfringens* is an important food-borne pathogen and is estimated to cause 248,000 cases of food-borne illness in the United States annually (Mead *et al.*, 1999). There is therefore a need to find alternatives to the use of antibiotics for preventing and treating diseases as well as improving the performance of poultry. On the other hand, Spring (2003) suggested that in order to control pathogens and to limit the impact of reduced use of antibiotic growth promoters, there is a need to change bio-security, management, housing and nutrition.

Some probiotic microorganisms and organic acids could be used as alternatives to antibiotic as growth stimulants and for improvement of feed conversion efficiency in farm animals (Esteive *et al.*, 1997). Huyghebaert (2003) reported that a number of alternatives, including organic acids, prebiotics, probiotics, and feed enzymes, have been used to maintain the general health status, growth rate and improve feed efficiency of poultry in Sweden after the ban of antibiotics. In this paper, probiotics, prebiotics, organic acids and essential oils will be briefly reviewed.

1.2.1 Probiotics

Fuller (1989) defined a probiotic as a live microbial feed supplement that affects the host animal beneficially by improving its microbial balance. Microbes, which have been used as

probiotics include *Lactobacilli, Streptococci, Bifido* bacteria, bacilli and yeasts (Shim, 2005). These microoganisms inhibit growth of potential pathogens by lowering the pH through production of acetic and lactic acid (Honma *et al.*, 1987). Probiotics improve the growth and development of the normal desirable microbial population in the gut, allowing them to dominate the undesirable organisms. They provide a dietary means of balancing the intestinal mucosa making it more difficult for pathogens to colonise and cause damage in the intestinal tract (Edens, 2003). Probiotics promote a balance of intestinal flora that produce such organic compounds as lactic acid, hydrogen peroxide and acetic acid. The increase in the acidity of the intestine inhibits the reproduction of many harmful bacteria.

Effects of probiotics on poultry performance

The use of probiotics in poultry industry is of great interest now that public concern about antibacterial growth promoting agents has increased and some farmers are using probiotics in preference to antibiotics (Fuller, 1989). The proposed benefits of probiotics include enhanced survival of newborns, reduction or prevention of diarrhea, increased growth rate, improved feed efficiency and enhanced immune response (Stavric and Kornegay, 1995). Tannock (2004) reported that probiotics are considered to exert a number of beneficial effects including immuno-stimulation, inhibition of enteric pathogens, and maintenance of a healthy intestinal microflora. Chapman and Lyons (1988) further observed that probiotics in poultry and pig feeds reduce symptoms of stress, act as natural growth promoters, and improve production and general health.

The effect of supplementation with probiotics in chicken diets has been inconsistent (Dilworth and Day, 1978). Fuller (1989) reported improved weight gain and feed conversion in broiler chickens, enhanced egg production and decreased cracked eggs in

breeders whose diet was supplemented with probiotics. Yeo and Kim (1997) also observed significant improvements in daily gain and feed intake in broiler chicks fed probiotics. Similarly, Cavazzoni *et al.* (1998) reported that a *Bacillus coagulans*-based probiotic product enhanced growth rate of broilers. Jin *et al.* (1998) observed a significant improvement in food conversion ratio (FCR) and weight gain of broiler over a 42-d trial period with a *Lactobacillus*-based probiotic supplement. Inclusion of *Bacillus subtilis* improved feed conversion and eggshell thickness in laying hens thereby decreasing the number of cracked eggs (Pedroso *et al.*, 1999). The addition of probiotics to poultry feed has been found to improve egg production, food conversion ratio (Tortuero and Fernandez, 1995; Abdulrahim *et al.*, 1996) as well as egg weight (Nahashon *et al.*, 1996). Nahashon *et al.* (1996) observed that when layers were fed 153g CP/kg diet containing *Lactobacillus* the birds produced larger eggs than those given a similar diet without *Lactobacillus*.

The enhanced performance of poultry supplemented with probiotics can be related to microstructures in the intestine where villus height is increased, goblet cell numbers are increased and crypt depth is reduced. Edens (2003) concluded that probiotics improve the morphology of the intestinal tract leading to improved absorption of nutrients. Thus, the cost of probiotics is competitive with the use of antibiotic growth promoters making them just as attractive as the growth promoters themselves. Han *et al.* (1984) studied the effect of supplementing chicken feed with an aerobic spore former (*Lactobacillus sporogenes*) and *Clostridium butyricum*. The supplements significantly improved weight gain and feed conversion of chickens besides suppressing the counts of *Staphylococci* and coliforms in both chickens and in pigs.

With regard to health, probiotics have potential benefits that include immuno-stimulation, anti-inflammatory reactions, exclusion and killing of pathogens in the intestinal tract and reduced bacterial contamination of processed broiler carcasses (Edens, 2003). Enteric diseases are of major concern in the poultry industry as contaminants of poultry products since they result in poor performance and increased mortality. Enteric bacterial infections also create a threat to intestinal health and can contribute to poor feed efficiency and liveability of a flock (Porter, 1998). Tollba *et al.* (2004) reported significant decreases ($P<0.05$) in the pH of the intestines (duodenum, jejunum, ileum and caecum) and total count of some pathogenic bacteria (*Escherichia coli* and *Salmonella pullorum*) when chicks were fed a commercial diet containing either *Lactobacillus* or *Pediococcus* concentrate. Thus Patterson and Burkholder (2003) concluded that probiotics were one of several approaches that have the potential to reduce enteric diseases in poultry and subsequent contamination of poultry products.

1.2.2 Prebiotics

These are health-promoting non-digestible food ingredients that affect the host (animal) beneficially by selectively stimulating the growth and/or activity of one or a limited number of bacterial species that are naturally present or introduced into the intestine (Gibson and Roberfroid, 1995). Classes of prebiotics include non-digestible oligosaccharides and FOS (Gibson and Roberfroid, 1995). Oligosaccharides have been shown to reduce risk of disease possibly by reducing the proliferation of pathogenic species (Bailey *et al.*, 1991) and improving the digestibility of various dietary fractions (Leske *et al.*, 1991). Common oligosaccharides used in animal diets include Mannan oligosaccharides (MOS) and FOS (Monsan and Paul, 1995). Most prebiotics have been

shown to exert their beneficial effects on the host by selectively feeding the good bacteria at the expense of the pathogenic ones. Prebiotics apparently increase the growth of non-pathogenic microorganisms whilst reducing colonization of bacteria such as *E. coli* and *Salmonella* (Yusrizal and Chen, 2003) thereby improving intestinal bacterial balance in broilers (Chung and Day, 2004). Newman (1994) observed that some prebiotics provide binding sites for pathogenic bacteria (*Salmonellae* and *E. coli*), which are then flushed out of the digestive tract with the faeces, while others promote the growth of beneficial bacteria by acting as a food source.

1.2.2.1 Frutooligosaccharides (FOS) in birds' performance

The Frutooligosaccharides (FOS) are naturally occurring compounds from a variety of plants (Fishbein *et al.*, 1988) and have been used as feed additives for poultry and swine. They have been extensively studied for their ability to improve animal health and performance (Tomomatsu, 1994). They may be used as alternatives for sub-therapeutic levels of antibiotics (Terada *et al.*, 1994).

Studies on the inclusion of FOS in poultry diets have indicated improvements in weight gain and feed efficiency, reduction in mortality and intestinal colonization by salmonella (Ammerman *et al.*, 1988 and 1989; Waldroup *et al.*, 1993). Bailey *et al.* (1991) investigated the influence of FOS on the ability of *Salmonella typhimurium* to grow and colonize the gut of chickens. Reduced susceptibility to Salmonella colonization in stressed chickens was reported in birds fed a FOS-supplemented diet. Choi *et al.* (1994) also noted a lower incidence of intestinal colonization with *Salmonella typhimurium* in chicks fed FOS supplemented diets than in those on the control diet. In contrast, Schoeni and Wong

(1994) challenged broiler chicks with *Campylobacter,* and only 8% of chicks fed FOS diet were colonized by the microbes compared to 80% of chicks on the control diet.

Yusrizal and Chen (2003) reported improved body weight gain, feed conversion, carcass weight, carcass percentage and increased small intestine length in female birds supplemented with FOS. Ammerman *et al.* (1989) reported that providing male broilers with a 0.375% level of FOS in their diet produced heavier birds at 47 days and improved carcass weight and breast weight whereas fat pad content was lowered. In agreement, Izat *et al.* (1990) reported improved body weight gain and a reduced level of salmonellae on processed carcasses when broilers were supplied with 0.375% FOS in their diets. In contrast, Waldroup *et al.* (1993) reported that the addition of 0.375% FOS in the diet had little consistent effect on growth rate, feed utilization, mortality, carcass dressing percentage and abdominal fat content of broilers. Oyarzabal and Conner (1996) observed no difference in the rate of intestinal colonization between chicks fed FOS and those that were on a control diet when both groups of birds were challenged with *Salmonella typhimurium* in drinking water.

1.2.2.2 Mannan oligosaccharides (Bio-Mos)

Bio-Mos is a natural extract derived from the cell wall of the yeast *Saccharomyces cerevisiae*. It is a complex carbohydrate product that was developed at Alltech and has been scientifically proven around the world to be beneficial to animals (Alltech, Inc., Nicholasville, Kentucky USA). Bio-Mos (MOS) was introduced as a feed additive for broiler chickens in 1993. Since then it has been reported to improve body weight, feed conversion ratio and liveability in broiler chickens (Hooge, 2004) and to help optimise gut health and bird performance (Spring, 2003). It has been claimed to provide specific

binding sites (D-Mannose) to enteric pathogens, thus reducing their chance of attaching to the intestinal tract (Finucane *et al.*, 1999a).

Effect of Bio-Mos on broiler performance

Several studies have been conducted with broiler chickens; however, some reports on the use of MOS in broiler diets show inconsistent results. Waldroup *et al.* (2003a) reported no significant influence on body weight and feed conversion in a study where the effects of combination of antibiotics, MOS and organic forms of copper in broiler feeds were evaluated. MOS was added at 1g/kg and 0.75g/kg in diet fed from 0-42days and 42-56 days, respectively. However, in another study where Waldroup *et al.* (2003b) evaluated the response of broiler to diets containing a combination of antibiotics, MOS, and organic forms of copper, a significant improvement in feed conversion was reported. MOS was included at the same rate (as in the previously mentioned experiment) except that inclusion rate of 0.75g/kg was fed from 42–63 days. Similarly, Parks *et al.* (2001) reported improved body weight gain and feed conversion upon MOS inclusion in broiler feeds. Kumprecht *et al.* (1997) observed improved live weight and feed conversion ratio when MOS was added to a starter and grower /finisher diet at levels from 0.5g/kg to 3g/kg.

In a study conducted by Iji *et al.* (2001) on broiler chickens, MOS showed potential in containing enteric pathogens, modulating the immune response, improving the reliability of the intestinal mucosa and promoting improved growth and feed conversion efficiency in chickens. Hooge (2003) compared MOS-fed birds to those on control diet and observed a significant improvement in weight gain, feed conversion and mortality in birds supplemented with MOS. However, when comparing MOS-treated birds with those on

antibiotic growth promoters, performance was similar, but MOS-fed birds showed a lower mortality.

Maintenance of gut health is important for high quality and profitable poultry production (Parks *et al.*, 2001). MOS has shown promise in suppressing enteric pathogens and modulating the immune system (Spring *et al.*, 2000). Fernandez *et al.* (2002) evaluated the protective effect against *Salmonella enteritidis* in the chick ceca when broiler chicks were either dosed with cecal contents of hen-fed MOS, palm kernel meal (PKM) or no supplement. It was concluded that hen caecal contents (HCC) from hens fed MOS or PKM were more effective against *Salmonella enteritidis* colonization in chicks than the HCC from hens given control feed.

MOS reduced the concentration of *S. typhimurium 29E* and *S. dublin* in challenged three-day-old chicks. In testing the effect of MOS on concentrations of bacteria that do not express Type 1 fimbriae, Spring *et al.* (2000) conducted a trial with S. *typhimurium* 27A. However, this strain did not colonize chicks sufficiently to evaluate if MOS affected the cecal concentration.

Effect of Bio-Mos on breeder and layer performance

The reports of research on breeder flocks suggest that MOS supplementation resulted in significant improvement in the antibody response in broilers and layers (Cotter *et al.*, 2000; Raju and Devegowda, 2002). Shashidhara and Devegowda (2003) observed that supplementation of MOS improved sperm density, antibody titers and all production traits except egg production, in broiler breeders. Several workers have noticed improved hatchability in broiler breeders when yeast was included in diets (McDaniel, 1991;

McDaniel and Sefton, 1991). Stanley *et al.* (1997) conducted an experiment to examine the effect of dietary MOS on serum, egg and liver cholesterol and egg production. They found that MOS significantly reduced egg yolk cholesterol, and slight differences were observed in serum and liver cholesterol between MOS-fed layers and those on control diet.

Cragoe and Olsen (1994) observed a higher egg production (83.75 vs. 85.39 %) and lower mortality in birds given MOS compared to controls. Berry and Lui (2000) on the other hand reported increased egg production and improved feed conversion when Bio-Mos was included in diets of molted broiler breeder hens. Stanley *et al.* (2000) observed no significant difference between Vegpro-supplemented hens (73%) and control hens (70%) over 105 days of production. A combination of Vegpro and MOS-treated feed had the highest egg production (82%) and MOS treated hens produced 79% of eggs. These authors concluded that layers supplemented with MOS and dietary protease (Vegpro) performed better than the control or those fed either MOS or Vegpro alone.

Effect of MOS on turkeys' performance

The inclusion of MOS in turkey feed was introduced commercially as an alternative growth promoter in 1993 (Bio-Mos, Alltech, Inc., Nicholasville, Kentucky USA). This has been demonstrated to improve live performance of turkeys (Hooge, 2004 and Parks *et al.*, 2001). This observation has been confirmed in several studies. Savage *et al.* (1997) noted improved weight gain and feed efficiency when MOS was included at a rate of 1kg/tonne. Hulet *et al.* (2000) observed improved feed efficiency and no difference in body weight in turkey hens fed 0.5g/kg MOS diet. Savage *et al.* (1997) observed maximum weight gain in poults fed starter diet containing 0.1% MOS. However, Olsen (1996) evaluated liveability, litter quality and serum titer levels in 15000 poults fed MOS supplemented diet and

observed no marked effect on body weights as opposed to improved feed conversion efficiency.

1.2.3 Organic acids

Supplementation of broiler feeds with organic acids generally lowers the pH and buffering capacity of the diet, reduces pH within the stomach, increases nutrient digestibility, promotes beneficial bacteria at the expense of pathogenic organisms and decreases intestinal bacterial growth. As a result there is an improvement in gastro-intestinal health, resulting in enhanced growth performance and improved feed efficiency. Organic acids penetrate the cell membrane of bacteria and once they are inside the cell, the acid dissociates and produces H^+ ions, which lower the pH of the cell causing the organism to use its energy to restore the normal balance (Nursey, 1997). A lower pH condition thus protects the animal from infection especially at a young age. Organic acids have also been utilized as food additives and preservatives for preventing food deterioration and prolonging the shelf life of perishable food ingredients (Ricke, 2003).

Dietary supplementation of organic acids has shown positive results in studies conducted on chicks and pigs (Patten and Waldroup, 1988). Thompson and Hinton (1997) indicated that the performance-enhancing effect of organic acids on feed efficiency in poultry is not so pronounced as in pigs. Fumaric acid improved feed efficiency by 3.5 to 4% in broilers and layers, although there was no significant effect on rate of lay (Vogt and Matthes, 1981). Skinner *et al.* (1991) observed marked improvements in body weight and feed utilization at 49d in male broilers supplemented with 0, 0.125, 0.25, or 0.5% fumaric acid.

Despite the many positive effects of organic acids, there are reports where no effect has been observed. For instance, Skinner *et al.* (1991) reported no effect on mortality rates, abdominal fat percentage and dressing percentage with supplementation of fumaric acids on male broilers. Runho *et al.* (1997) conducted a study in which 0.25 to 1.0% fumaric acid was compared with AGP (Nitrovin) and reported no effect on growth of broilers; however, feed consumption was reduced, resulting in a marked improvement in feed to gain.

With regard to the control of pathogens, organic acids are generally used in Europe to inhibit pathogens like salmonella in both raw material and finished feed (Radcliffe, 2000). They have also been used as fungistats in feed (Paster, 1979). Formic and propionic acids and various combinations have been explored for potential bactericidal activity in feed contaminated with food borne pathogens, particularly *Salmonella* species (Khan and Katamay, 1969; Mchan and Shotts, 1992). Dibner and Buttin (2002) reported that organic acids improved protein and energy digestibilities by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of sub-clinical infections and secretion of immune mediators and by reducing the production of ammonia and other growth-depressing microbial metabolites. Reductions in bacteria are related to feeding organic acids, which are particularly effective against acid-intolerant species of *E. coli*, *Salmonella* and *Campylobacter*.

The use of organic acids is also aimed at replacing antibiotic growth promoters in poultry feeds, and for prevention of necrotic enteritis and reduction of *Salmonella* and *Campylobacter* (Heres *et al.*, 2004). Organic acids have been used to control *salmonella* in feed and in water supplies for livestock and poultry. Birds drinking organic acid-supplemented water of pH 4 had decreased *Campylobacter* infection with intact intestinal

epithelial cells (Chaveerach *et al.*, 2004). Akbari *et al.* (2004) evaluated the effects of adding acetic acid to drinking water on chick performance and ileal microorganisms and found no significant effect on the performance and ileal microbial counts of chickens.

Revington (2002) reported that organic acids, both individual acids and blends of several acids, fall among the possible alternatives for antibiotics since they are known to have strong antibacterial effects.

1.2.4 Essential oils

Following the removal of some antibiotics in animal production the use of essential oils is becoming more common in animal nutrition. Antimicrobial activity of plant oils and extracts has been recognized for many years (Hammer *et al.*, 1999). The essential oil (EO) combination is a feed additive of natural origin, and may be considered as a potential growth promoter in broiler production (Alçiçek *et al.*, 2003).

Effects observed on supplementation of essential oils are either positive or non-significant. Alçiçek *et al.* (2003) reported a significant increase in liveweight, feed intake (except at day 42) and feed conversion in turkeys. Jang *et al.* (2004) observed a significant increase in digestive enzyme activity of the pancreas and intestinal mucosa, leading to an increase in growth performance. That happened when a blend of commercial essential oils combined with lactic acid were supplemented in broiler chicken diet. Supplementation of EO improved feed efficiency of broilers by 5% from 1-40 d of age, and significantly reduced digesta viscosity as well as the percentage of birds with sticky droppings (William and Losa, 2002). On the contrary, Botsoglou *et al.* (2004) investigated performance parameters and oxidation of body lipids of broiler chickens and concluded that the mixture

of herbal essential oils (Apacox) exerted no growth-promoting effect when incorporated in the chicken diet.

Allen *et al.* (1997) reported that two essential oils components, camphor and 1.8-cineole when supplemented at 119ppm, showed no clear effects on weight gains when chickens were reared without coccidia challenge, but significant weight gains were observed when birds were infected with coccidia.

Mitsch *et al.* (2004) conducted a study to find the effect of two different blends of essential oils on *Clostridium perfringens* in the intestine and faeces of broiler chickens. The results indicated that colonization and propagation of *C. perfringens* could be controlled in the gut by specific blends of essential oil components. Therefore essential oils may be of help to prevent problems with *C. perfringens* and necrotic enteritis.

Lee *et al.* (2003) reported no effect of essential oil constituents on growth performance in female broiler chickens. However, these authors indicated that positive effects may have been observed under less hygienic environmental conditions or when using a less digestible diet. This statement could be in line with studies of Bassett (2000) and Langhout (2000), who indicated that the effect of dietary essential oils on growth performance becomes apparent when chickens are subjected to sub-optimal conditions such as a less digestible diet and/or less clean environment.

1.3 Discussion and conclusion

The concern about antibiotic resistance in human pathogens continues to be of utmost importance in many countries. Now that pressure is mounting to reduce or eliminate antibiotic use in poultry diets, it is important that the poultry industry responds to demands

by consumers by providing alternatives that would improve efficiency of feed utilization and consequently reduce cost of production.

It is evident from this review that maintaining gut health is important for the production of high quality poultry products. Finding alternatives to AGPs in livestock and poultry production should be the top priority. Since South Africa exports some of its products to overseas markets, livestock and poultry producers in this country are faced with the challenge of eliminating the use of AGPs as fast as possible to retain good relations with their clients. Sweden ceased the use of antimicrobial growth promoters in 1986. The EU has officially banned all growth-promoting antibiotics in poultry and livestock with effect from January 2006. Therefore concerted efforts should be made to find viable alternatives to AGPs as urgently as possible.

This review indicates that further investigation is necessary for finding potential alternatives to antibiotic growth promoters and for identifying factors contributing to the inconsistency of results. The studies reported in this thesis are an attempt to address these issues.

Chapter 2

The effect of Bio-Mos and Selplex (organic selenium) on the fertility of broiler breeder males

2.1 Introduction

To meet the ever-increasing demand for animal protein, the poultry industry has to ensure that broiler breeders are fertile, productive and free from infections. Supplementing feed with additives and trace elements has been shown to improve fertility and minimize infections. Bacterial infections account for the majority of the identified veterinary problems in broiler breeders, *Escherichia coli* (*E. coli)* infections being recognized as one of the most common causes of mortality and morbidity (Leeson and Summers, 2000). In adult birds *E. coli* could infect the reproductive tract resulting in salpingitis and occasionally peritonitis (Monroy *et al.*, 2005). These infections usually occur in birds whose immunity is suppressed (Leeson and Summers, 2000).

Bio-Mos as a feed additive has been reported to promote growth of *Lactobacilli* and other beneficial bacteria species while inhibiting growth of *Salmonella* and *Escherichia coli* (*E. coli*) in the gut (Kumprecht *et al.*, 1997). Many studies (Hooge, 2004 and Šimpraga *et al.*, 2003) observed increased immunity and health of animals when Bio-Mos was included in the diets while Shashidhara and Devegowda (2003) reported improved antibody titres in birds. Fairchild *et al.* (2001) demonstrated that dietary Bio-Mos could improve the overall performance of poults, especially when they are challenged with *E. coli.*

Selenium (Se) is one of the essential trace elements required for enhancing performance in poultry. Since 1974, after being approved by the United States Food and Drug

Administration (FDA) as a feed supplement, Se has been demonstrated as an essential trace element for male fertility (Hansen and Deguchi, 1996). In 2000 the FDA approved organic Selenium (Selplex) as a source of Se supplementation in broiler and layer chickens. Supplementation of selenium in poultry diets enhances sperm numbers, and using an organic selenium source reduces production of defective sperm, thereby having a positive effect on the fertilizing potential of the male (Edens, 2002).

The deficiency in dietary Se can result in reduced numbers of normal spermatozoa per ejaculate and decreased motility and fertilizing capacity (Surai *et al.*, 2001). Cantor (1997) also indicated that Se deficiency depress egg production and hatchability in chickens and turkeys.

The present study was designed to evaluate the effect of Bio-Mos and Selplex on the fertility and *E. coli* levels of broiler breeder males.

2.2 Materials and methods

2.2.1 Animals and diets

The 144 Ross 788 male broiler breeders used in this experiment had been reared from day-old in 12 light-proofed rooms at Ukulinga Research Farm. They were placed in individual floor cages (Figure 2.1) at the start of the trial (33 weeks of age) and remained in these cages for the entire trial period of 34 weeks.

The commercial male breeder mash without antibiotics, used as the control feed, was obtained from Meadow Feed Mills (P.O. Box 426, Pietermaritzburg, South Africa). The nutrient composition of the feed is shown in Table 2.1.

Selplex and Bio-Mos were added in proportion recommended by the supplier (Alltech (PTY) LTD, Corner of Koelenhof and Bottleary Roads, Farm no: 1277, Stellenbosch, 7599) to this control feed to produce the four dietary treatments shown in Table 2.2. These dietary treatments were randomly allocated to three birds per room; hence there were 12 replicates per treatment with 3 birds per replicate. Each bird was allocated 125g of feed per day.

2.2.2 Measurements

2.2.2.1 Semen collection and assessment

Semen was collected and pooled from all the birds from rooms 1 to 6 and 7 to 12 on the same dietary treatment using the abdominal massage technique (Burrows and Quinn, 1937). Two persons were involved in collecting semen, one holding the rooster by the thighs and the other massaging the abdomen and milking semen into the flip top vials. Samples were collected in morning hours at 46, 51, 54, 56, 58, 62 and 66 weeks of age. The plan was to collect semen more frequent however; it was abandoned due to the severe decline in semen production resulting from heat stress experienced during the months of November 2004 to February 2005.

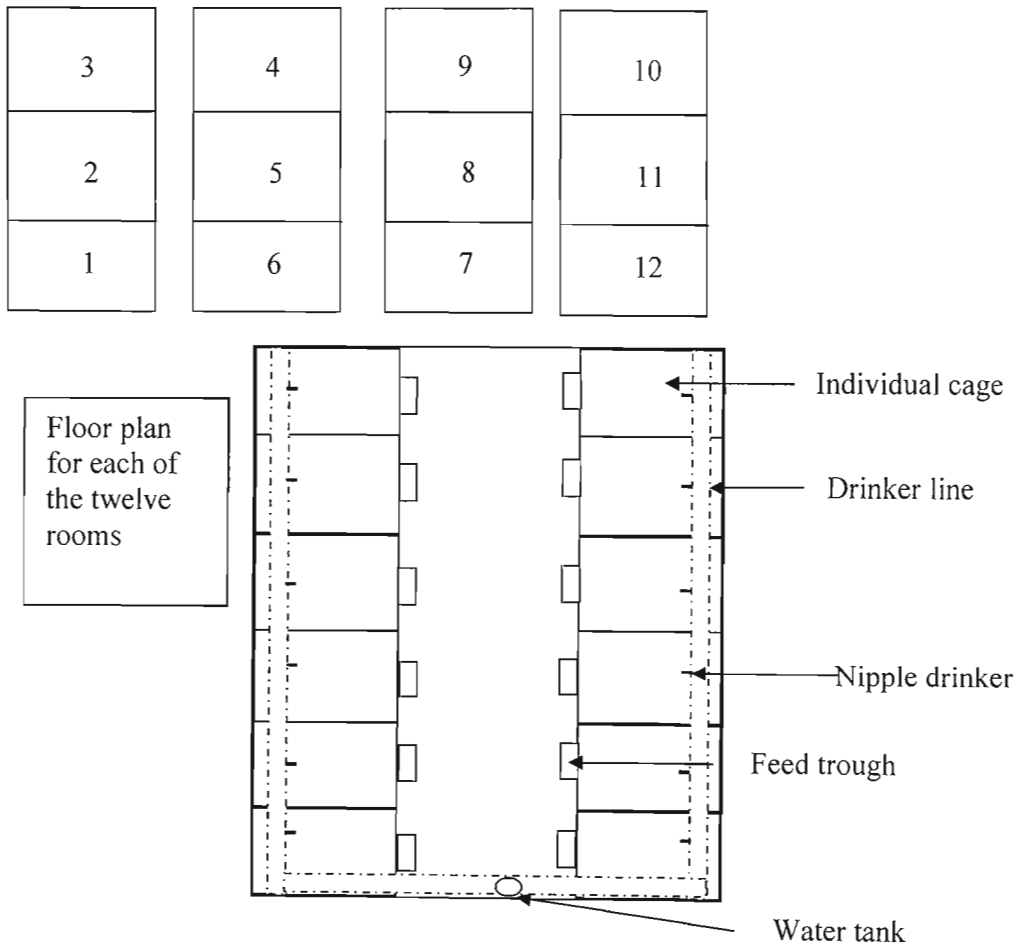
Table 2.1 *Ingredient and nutrient composition (g/kg) of the basal feed*

Ingredient (g/kg)	Content, g/kg
Yellow maize	600
Middlings	78.1
Full fat Soya	61
Sunflower oilcake	100
Soybean oilcake 47%	48.2
Salt	2
Lysine HCl	1.3
Methionine	1.1
Limestone fine	38
Limestone grit	40
Mono calcium phosphate.	10
Sodium bicarbonate	1.5
Molasses	17
Choline chloride 75	0.7
Vit / Mineral premix	1.5
Nutrient (g/kg)	
AME (MJ/kg)	11.7
Protein	145
Fibre	45.6
Lysine	7.2
Methionine	3.7
Arginine	9.5
Valine	6.9
Ca	29.0
Available phosphorus	4.0
Sodium	1.5

Table 2.2 *Dietary treatments and their description*

Treatment	Description
1	Male broiler breeder mash without additives (Control)
2	Control + Selplex (0.1mg/kg)
3	Control + Bio-Mos (1g/kg)
4	Control + Selplex (0.1mg/kg) + Bio-Mos (1g/kg)

Figure 2.1 *Diagrammatic representation of housing facilities*



Semen motility

A drop of semen was diluted with Tyrodes solution and the sample was assessed using a light microscope at 40x magnification. Since the assessment was subjective, two people were involved in taking the scores. Each person observed three areas of the sample to determine the percentage of progressive normal motile sperm, abnormal motile and dead sperm. As there was no significant difference between the motility scores of the individuals, their scores were used as replicates.

Sperm concentration

A volume of 100µl undiluted semen sample was added to a solution of Eosin and Nigrosin and thoroughly mixed. A 50µl amount of the mixture was then used to fill the two sides of a haemocytometer. Spermatozoa were counted using a light microscope at 40x magnification at five randomly chosen blocks on either side of the haemocytometer. The scores obtained, together with the corresponding sperm motility scores were then used to calculate the number of live sperm per mL using the formula as shown in Appendix A.

Sperm morphology

A drop of undiluted semen was placed in a 3% volume gluteraldehyde solution immediately after collection to fix and preserve the spermatozoa in their current state. The samples were examined at a later date. The morphology assessment was done using a light microscope with a 100x oil immersion lens. From each sample a total of 200 spermatozoa were counted at random and allocated to the following categories: normal, bent, coiled in heads, bulbs or knots in the head and bulbs at mid-piece region, as observed.

2.2.2.2 Escherichia coli counts

Fresh faecal samples were collected at 57, 61 and 66 weeks of age from 10 roosters selected at random from each of the four dietary treatments. Each rooster was placed in a cage with a tray underneath to collect the droppings. About 5g of freshly voided faecal samples were collected from the trays into the flip top vials. These samples were taken to Allerton Regional Veterinary Laboratories for *E. coli* content analysis.

2.2.3 Statistical analysis

Data used for semen morphology and motility analysis were collected from all the treatments and for *E. coli* counts data were collected from Bio-Mos and control treatments.

Semen morphology data were analyzed using the Chi squared test of Minitab (2000) statistical software. Semen motility data were analysed using Genstat (2002).

Data on *E. coli* counts were log transformed and were then transferred to Genstat (2002) statistical software where analysis of variance was performed to determine treatment means and standard errors.

2.3. Results

2.3.1 Escherichia coli counts

Dietary treatments had no effect ($P>0.05$) on the *E. coli* counts in faecal samples from broiler breeders that were on Bio-Mos and control diets (Table 2.3). Similarly the

interaction between the treatments and collection dates had no effect on the level of *E. coli* counts. However, the *E. coli* counts differed (P<0.001) on different collection dates.

2.3.2 Sperm motility

Mean sperm motility measurements assessed at different ages is shown in Tables 2.4. Dietary treatments had no effect (P>0.05) on sperm motility. Age of the birds had an effect (P<0.001) on the percentage of motile sperms, with the highest percentage of progressive normal motile sperm (80.63%) being obtained from birds aged 46 weeks. The lowest percentage (67.81%) was from the birds at 66 weeks.

2.3.3 Sperm concentration

Dietary treatments did not show any effect (P>0.05) on semen concentration (Table 2.5). Age and its interaction with dietary treatment affected (P<0.001) semen concentration. The highest sperm concentration was obtained from birds at 51 weeks of age while the lowest concentration was noted at 58 weeks.

2.3.4 Sperm morphology

The number of sperms in various categories from a total of 4400 observations of sperm morphology measured on each treatment compared with the control treatment using the Chi squared test of differences is shown in Table 2.6. Supplementation with both Bio-Mos and Selplex increased (P<0.05) the number of normal spermatozoa compared to control diets. Birds on the Bio-Mos supplemented diet had the highest number of total normal spermatozoa (3332 vs. 3244) compared to those on control diet and those on a combination of Selplex and Bio-Mos (3301 vs. 3244) had the lowest. A highly reduced (P<0.001)

number of bent spermatozoa and low (P<0.05) number of spermatozoa with coiled heads were observed on Bio-Mos supplemented birds. A lower number (P<0.05) of bent spermatozoa were observed on both Selplex and Selplex and Bio-Mos combination treatments. No differences (P>0.05) were observed in spermatozoa with knots in heads, bulb in heads or bulb at mid-piece.

Table 2.3 *Effect of dietary Bio-Mos on mean E. coli counts (log CFU/g) in faecal samples collected at different bird ages.*

Age at collection (weeks)	Mean *E. coli* count (log CFU/g)	
	Bio-Mos	Control
57	-0.12	0.42
61	-0.36	-0.08
66	5.15	4.18

S.E.M Treatments = 0.481 ^{ns} Age = 0.059*** Age x Treatments = 0. 834^{ns}

*** P = 0.001, ns = not significant, S.E.M. = Standard Error Means

Table 2.4 *Effect of dietary treatments on percentage sperm motility (% dead sperm) at different roosters' age.*

Treatment	Age (weeks)							
	46	51	54	56	58	62	66	Mean
Control	6.0	15.0	12.5	10.0	11.3	15.0	12.5	11.7
Selplex	15.0	15.0	7.5	17.5	12.5	6.3	11.3	12.1
Bio-Mos	7.5	10.0	11.3	12.5	12.5	7.5	16.3	11.1
Mos + Sel	7.5	20.0	10.0	20.0	8.3	10.0	12.5	12.6
Grand mean	9.0	15.0	10.3	15.0	11.1	9.7	13.1	11.4

S.E.M. Treatment = 0.962^{ns} Age = 2.203* Treatment x Age = 4.407^{ns}

P < 0.05 = * ns = not significant S.E.M. = Standard Error Means Mos + Sel = Bio-Mos and Selplex

Table 2.5 *Mean sperm concentration (µl) by feed treatment and age (weeks).*

Treatment	Ages (weeks)							
	46	51	54	56	58	62	66	Mean
Control	97.0	35.0	44.5	109.0	28.0	81.0	96.0	70.1
Selplex	67.0	53.0	76.0	100.0	31.5	93.0	50.5	67.3
Bio-Mos	38.0	200.0	90.0	61.0	32.5	65.0	40.0	75.2
Mos + Sel	57.0	72.0	105.5	85.0	24.0	76.0	45.5	66.4
Grand mean	64.8	90.0	79.0	88.8	29.0	78.8	58.0	69.8

S.E.M. Treatment = 4.8^{ns} Age = 10.9*** Treatment x Age = 21.9***

P<0.001 =*** ns = not significant S.E.M. = Standard Error Means Mos + Sel = Bio-Mos and Selplex

Table 2.6 *Number of sperms in various categories from a total of 4400 observations of each treatment versus control treatment, using Chi squared test of differences.*

	Sperm morphological categories					
	Normal	Bent	Coiled heads	Knots in heads	Bulb in heads	Bulb at mid-piece
Control	3244	865	62	61	107	61
Selplex	3326	779	80	65	100	50
Chi^2 value	4.039*	5.520*	1.816^{ns}	0.046^{ns}	0.430^{ns}	1.359^{ns}
Control	3244	865	62	61	107	0.627^{ns}
Bio-Mos	3332	720	93	66	177	72
Chi^2 value	4.660*	14.045***	5.275*	0.084^{ns}	0.212^{ns}	0.627^{ns}
Control	3244	865	62	61	107	61
Mos +Sel	3301	759	90	68	113	69
Chi^2 value	1.937*	7.121*	1.819^{ns}	0.263^{ns}	0.073^{ns}	0.365^{ns}

$p< 0.05$ = *, $p<0.001$ =***, ns = not significant

2.4 Discussion

This experiment was conducted to evaluate whether fertility and *E. coli* levels of broiler breeder males would be reduced by supplementation with Bio-Mos and/or Selplex. There was no statistically significant influence of dietary treatment on sperm motility or sperm concentration. This observation is in disagreement with findings of Surai *et al.* (2001) who reported that a deficiency in dietary selenium resulted in reduced numbers of normal spermatozoa per ejaculate and decreased motility and fertilizing capacity. Gallo *et al.* (2003) reported a significant increase in sperm concentration, motility and viability in broiler breeder males that were supplemented with selenium compared to those that were on the control diet. Furthermore, Galil and Samad (2004) indicated that supplementing selenium resulted in increased motility of the sperm and livability.

The higher number of normal spermatozoa ($P<0.05$) and reduced number of bent spermatozoa ($P<0.05$) observed on Selplex-supplemented roosters compared to those on control diets indicated that Selplex had a positive effect on semen quality. This agrees with findings of Sefton and Edens (2004), who reported that Selplex supplementation had superior semen quality when judged with the counts of normal against abnormal forms. A sperm with an abnormal mid-piece or head deformity is incapable of ovum fertilization (Froman *et al.*, 1999). Lake and Stewart (1978) further indicated that bent spermatozoa are motile when viewed in drops of semen placed under the microscope, although they are infertile. Production of fertile eggs depends on spermatozoa entering the sperm-storage tubules in the oviduct (Donoghue *et al.*, 1998). Only morphologically normal spermatozoa are capable of ascending through the hen's vagina to the region where these sperm-storage tubules are located (Bakst *et al.*, 1994).

The significant effect observed in sperm motility and concentration is not consistent with the increasing age of the birds. However, Chotesangasa (2001) found that the native cocks during 35-52 weeks of age had similar quality of semen though the semen volume and total sperm per ejaculation tended to be lower in the younger birds. Chotesangasa (2001) further concluded that cocks from the age of 38 weeks and older were able to produce high quality semen similar to the more mature chickens. On the other hand, Selvan *et al.* (1994) reported that semen collected at 36 weeks of age was superior to semen collected at 26 weeks, in respect of volume, gross motility, sperm concentration and percentages of live and abnormal spermatozoa.

Bio-Mos supplementation had no significant effect on reducing faecal *E. coli* counts in broiler breeders. These results contradict findings of Spring *et al.* (2000) who reported that Bio-Mos has the ability to suppress enteric pathogens such as *Salmonella* species and *E. coli* and to modulate the immune response in poultry. A reduced number of intestinal pathogenic microflora (*E. coli, Clostridium perfringens*) was observed in chickens supplemented with Bio-Mos in comparison to those on the control diet (Jamroz *et al.*, 2004). Finucane *et al.* (1999b) furthermore reported a reduced intestinal concentration of *Clostridium perfringens* when Bio-Mos was included into turkeys' diets. The lack of response to Bio-Mos supplementation may be an indication that the level of inclusion was not sufficient to influence reduction of *E. coli* on faecal samples. On the other hand the health status of the roosters might have been so good that there was no challenge for Bio-Mos to be effective.

Although there is no extensive investigation on the influence of Bio-Mos on fertility in broiler breeders, slight improvements in this experiment were observed where it was used

in the feed. Shashidhara and Devegowda (2003) reported that supplemental Bio-Mos improved sperm density and antibody titers in broiler breeders.

2.5 Conclusion

Supplementation of Selplex and Bio-Mos significantly increased the number of normal spermatozoa but had no significant influence on sperm concentration or motility. On the other hand Bio-Mos had no significant influence on the reduction of *E. coli* count. It is therefore recommended that in future experiments the inclusion rates might be increased to evaluate whether these would improve results.

Chapter 3

The influence of Mannan oligosaccharides (Bio-Mos) and Zinc bacitracin on growth of broiler chickens

3.1 Introduction

Antimicrobial feed additives have made a tremendous contribution in improving production in the poultry industry. By controlling and limiting the growth of microbes such as *Clostridium perfringens* that are known to be detrimental to the health of poultry (Truscott and Al-Sheikhly, 1977), antibiotic growth promoters (AGPs) improve growth and feed utilisation (Foster and Stevenson, 1983). This is achieved by inhibiting growth of microorganisms in the gut that compete for nutrients, hence making nutrients more available for birds to grow (Bunyan *et al.*, 1977)

Zinc bacitracin (ZnBac) is one of the AGPs that have been reported in several studies to improve growth rate and feed utilization in broiler chickens (Choi and Ryu, 1987; Waldroup *et al.,* 1999). Abdulrahim *et al.* (1999) investigated the effect of *Lactobacillus acidophilus* and Zinc bacitracin as dietary additives for broiler chickens. These authors observed an improvement in body weight of 10.8% with both additives in the diet over untreated diets; bacitracin alone induced a 9.1% improvement. Feed conversion was however reduced on ZnBac- supplemented feeds. Broiler chickens fed diets supplemented with salinomycin and Zinc bacitracin, individually or in combination, resulted in significantly lower *Clostridium perfringens* and *Lactobacillus salivarius* counts, the latter being a dominant lactic acid bacterium found in broiler intestinal contents (Engberg *et al.*, 2000). Despite the positive effects of the use of AGPs in poultry production, there are major concerns prevailing regarding the potential development of microbial resistance to the antibiotics. As a result, the AGPs are being placed under pressure due to consumers'

fear that their use in animal feed lead could to the formation of resistant strains of bacteria that are harmful to humans (Langhout, 2000). Many countries, as a result, have severely limited or eradicated use of AGPs in poultry and livestock and there is a strong possibility of other countries following suit. Given this scenario there is a need to find possible effective alternatives to antibiotic growth promoters for the feed industry. .

Prebiotics, which include Mannan oligosaccharides, are one of the classes of alternatives to AGPs that have received some attention (Shashidhara and Devegowda, 2003). Bio-Mos, a Mannan oligosaccharide (MOS) derived from the yeast *Saccharomyces cerevisiae,* has been used as a feed additive since 1993. In studies conducted on chickens and turkeys MOS has been reported to promote growth and feed conversion, improve the integrity of intestinal mucosa, suppress enteric pathogens and enhance immunity (Olsen, 1996; Iji *et al.*, 2001; Hooge, 2004). Gürbüz *et al.* (2004) however reported that Bio-Mos improves growth performance mainly when birds are challenged with enteric pathogens. When the birds are under less disease or crowding stresses, alternative growth promoters often perform similarly to control and antibiotics (Hooge, 2004).

The objective of this study was to evaluate the use of Mannan oligosaccharides (Bio-Mos) as an alternative to Zinc Bacitracin on the growth of broiler chickens.

3.2 Materials and methods

3.2.1 Birds and housing

Two thousand eight hundred and eighty (2880) day-old Ross 1 broilers (1440 males and 1440 females) used in this experiment were obtained from National Chicks Farms. Sixty sexed chicks were randomly assigned to each of 48 deep litter pens in a broiler house at the Ukulinga research farm. Each pen was equipped with two chick feeders and two water founts for the first seven days, in addition to nipple drinkers. On day 7, two tube feeders were introduced when the chick founts and feeders were removed. During the entire experimental period of 35 days, water and feed were offered *ad libitum*. Temperature was reduced stepwise from 31^0C at day-old to 21^0C at day 21, and maintained at this level for the rest of the experiment. Controlled gas brooders were used for the first two weeks to maintain temperature, and ventilation was controlled with fans and sidewall curtains. The humidity in the house was dependent on exchange of air provided by the tunnel ventilation system.

3.2.2 Feeds and treatments

A commercial broiler starter and grower feeds in a mash form, without antibiotics, were obtained from Meadow Feed Mills (P.O. Box 426, Pietermaritzburg, South Africa) and these basal feeds were used as control diets. Four levels of Bio-Mos (0, 0.75, 1.5 and 2.0g/kg) and two levels of Zinc Bacitracin (0 and 0.33g/kg) were added to the basal feed to produce the eight dietary treatments as shown in Table 3.1. The nutritional composition of the feeds is shown in Table3.2.

3.2.3 Experimental design

A completely randomised design with no blocking was used for this experiment. There were eight dietary treatments that were replicated six times (three replicates being with males and three using females); each pen of 60 birds represented a replicate.

Table 3.1 *Dietary treatments and their description.*

Dietary treatments	Diet description
1	Broiler mash without additives (Control)
2	Control + Bio-Mos (0.75g/kg)
3	Control + Bio-Mos (1.5g/kg)
4	Control + Bio-Mos (2.0g/kg)
5	Control + Zinc bacitracin (0.333g/kg)
6	Control + Bio-Mos (0.75g/kg) + Zinc bacitracin (0.333g/kg)
7	Control + Bio-Mos (1.5g/kg) + Zinc bacitracin (0.333g/kg)
8	Control + Bio-Mos (2.0g/kg) + Zinc bacitracin (0.333g/kg)

3.2.4 Measurements

Birds were group-weighed per pen weekly to determine mean body weight, from which weight gain was calculated. Feed consumption was calculated at the end of each week by weighing back the unconsumed food and subtracting this from the food allocated. Mortality was recorded when it occurred.

Table 3.2 *Ingredient and nutrient composition (g/kg) of the two basal feeds.*

Ingredient (g/kg)	Content, g/kg
Yellow maize	600
Middlings	78.1
Full fat Soya	61
Sunflower oilcake	100
Soybean oilcake 47%	48.2
Salt	2
Lysine HCl	1.3
Methionine	1.1
Limestone fine	38
Limestone grit	40
Mono calcium phosphate	1.0
Sodium bicarbonate	15
Molasses	17
Choline chloride 75	0.7
Vit / Mineral premix	1.5
Nutrient (g/kg)	
AME (MJ/kg)	11.7
Protein	145
Fibre	45.6
Lysine	7.2
Methionine	3.7
Arginine	9.5
Valine	6.9
Ca	29.0
Available phosphorus	4.0
Sodium	1.5
Potassium	6.3
Choline (mg/kg)	450

3.2.5 Statistical analysis

Body weight, food intake and feed conversion efficiency (FCE) data were processed using MS-Excel (2000) and were then transferred to Genstat (2002) statistical software. Analysis of variance was performed to determine treatment effects and standard errors of means. The performance data were regressed using simple linear regression with groups to determine the trends in the measures of performance.

3.3 Results

The mean body weight gain (g/d), food intake (g/d) and feed conversion efficiency (FCE, g gain/ kg food) of broilers to 35d of age is shown in Table 3.3. The regression coefficients are presented in Table 3.4 and 3.5.

3.3.1 Body weight gain

Body weight of broilers during the entire study period was not improved ($P>0.05$) by supplementation with Bio-Mos (MOS) when no Zinc bacitracin was present in the feed (Table 3.3); even in the presence of Zinc bacitracin there was no improvement in performance.

3.3.2 Food intake

The food intake at 21d was reduced ($P< 0.01$) by supplementation of Bio-Mos (Table 3.3 and 3.4). Zinc bacitracin alone also decreased intake, but in the presence of Bio-Mos, intake increased as level of Bio-Mos increased. Additions of Bio-Mos alone or when combined with Zinc bacitracin resulted in no difference ($P>0.05$) in food intake beyond 21d of age.

3.3.3 Feed conversion efficiency

Supplementary Bio-Mos in the absence of Zinc bacitracin increased FCE ($P<0.01$) while adding Bio-Mos in the presence of Zinc bacitracin brought no effect ($P>0.05$) to FCE (Table 2.3) during the starter phase (0-21d). No response ($P>0.05$) was observed due to addition of feed additives during the grower and finisher phases (21-35d).

3.4 Discussion

The objective of this experiment was to determine whether supplementation with Bio-Mos and Zinc bacitracin would have an effect on performance of broiler chickens. The results obtained are similar to the findings of Waldroup *et al.* (2003a). These authors reported no significant differences in body weight gain but improved feed conversion efficiency (FCE) on broilers fed maize-based diet supplemented with Bio-Mos. Flemming *et al.* (2004) incorporated Bio-Mos in maize-based feeds and observed significant improvements in daily weight gain, feed intake and feed conversion ratio. Mateo *et al.* (2000) similarly fed broilers maize-based feeds containing Bio-Mos and Zinc bacitracin and reported significant improvements in body weight gain and FCE during the first 3 weeks. Talay *et al.* (2004) observed significant improvement in body weight ($P<0.05$) on broilers fed a basal diet that consisted of corn (50%), wheat (10%) and soy (30%). Jamroz *et al.* (1997) reported no improved performance of broilers when Bio-Mos was added to diets containing over 60% wheat. However, Kocher *et al.* (2004) reported that inclusion of Bio-Mos in a broiler diet, based on 70% wheat and 17% soya bean meal, had a significantly different FRC compared to the negative control. Bozkurt *et al.* (2005) reported an improvement in body weight gain, feed intake and feed conversion in broilers fed wheat-soya-based diets supplemented with Bio-Mos and AGPs.

Table 3.3 *The effect of Zinc-bacitracin and Bio-Mos on mean body weight gain, feed intake and feed conversion efficiency of broilers to 35d of age.*

Inclusion level	Weight gain (g)				Feed intake (g)				Feed conversion efficiency (g/kg)			
	0-21d		0-35d		0-21d		0-35d		0-21d		0-35d	
Bio-Mos	-ZnBac	+ZnBac	-ZnBac	+ZnBac	-ZnBac	+ZnBac	-ZnBac	+ZnBac	-ZnBac	+ZnBac	-ZnBac	+ZnBac
0	32.8	30.1	47.4	46.0	53.6	50.0	94.4	88.9	613	604	502	517
0.75	32.6	32.6	46.3	47.5	53.5	53.3	90.9	91.4	616	613	511	520
1.5	32.0	30.3	47.4	46.1	46.6	52.6	92.1	92.2	698	570	514	501
2.0	32.2	33.4	46.1	48.2	46.1	53.6	89.1	93.7	707	625	518	515
SEM	**1.22**	**0.86**	**1.51**	**1.07**	**2.20**	**1.55**	**2.97**	**2.10**	**26.37**	**18.65**	**8.05**	**5.69**

Table 3.4 *Constant terms and regression coefficients for weight gain, feed intake and feed conversion efficiency on broilers given feeds supplemented with Bio-Mos and ZnBac from 0-21d age, using simple linear regression with groups.*

	Weight gain			Feed intake			Feed conversion efficiency		
Source	Coefficient	S.E.	P	Coefficient	S.E.	P	Coefficient	S.E.	P
Constant	32.78	1.05	<0.001	54.58	1.85	<0.001	600.7	22.7	<0.001
Bio-Mos	0.364	0.803	N.S.	-4.38	1.42	0.003	54.3	17.4	0.003
ZnBac[1]	-2.25	1.48	N.S.	-3.83	2.62	N.S.	1.1	32.1	N.S.
Bio-Mos*ZnBac[2]	1.37	1.14	N.S.	5.90	2.01	0.005	-53.4	24.6	0.035

[1] Effect of Zinc bacitracin addition to the feed on the constant term (e.g. for weight gain, 32.78 – 2.25 = 30.53)

[2] Effect of combining Zinc bacitracin and Bio-Mos on the regression coefficient (e.g. for weight gain, 0.364 + 1.37 = 2.00)

Table 3.5 *Constant terms and regression coefficients showing the effect of Bio-Mos and ZnBac[1] on weight gain feed intake and feed conversion efficiency.*

	Weight gain			Feed intake			Feed conversion efficiency		
Source	Coefficient	S.E.	P value	Coefficient	S.E.	P value	Coefficient	S.E.	P value
Constant	46.69	0.88	<0.001	91.49	1.75	<0.001	510.61	4.87	<0.001
Bio-Mos	0.17	0.67	N.S.	0.07	1.34	N.S.	1.56	3.73	N.S.

[1] ZnBac had no significant effect on the response to Bio-Mos

The addition of Bio-Mos and Zinc bacitracin in sorghum/soya-based feeds significantly increased body weight and improved feed conversion ratio (Ao *et al.*, 2004). Iji *et al.* (2001) however reported minor improvements in body weight but no improvement in feed conversion in broilers after feeding sorghum/lupin-based diets containing 5g Bio-Mos/kg. These responses to Bio-Mos therefore do not appear to be related to the cereal source being used. Most of the trials have been conducted on maize-based feeds and there are as many positive as negative responses observed.

The health status of the birds and the hygienic level in a house can greatly influence response to supplements (Talay *et al.*, 2004). The absence of significant differences in body weight gain in this trial may suggest that infection rates at the farm were low and thus did not permit the impact of additives to be shown. Hooge (2004) indicated that increased disease or crowding stresses usually result in greater improvement by beneficial additives as opposed to when the level of stress is low.

The improved FCE observed may be attributed to one or more of the following factors: nutrient digestibility, digestion rate and passage rate. With less feed consumed, Bio-Mos may have increased the availability of the nutrients that then resulted in increased growth of the birds. With regard to digestion rate, if nutrient availability were limiting, Bio-Mos may have increased digestion rate leading to improved FCE and growth. On the other hand, digestion rate might have been reduced if the rate of assimilation was limiting, thus resulting in fewer nutrients in the excreta and hence improvement in FCE. Bio-Mos might have reduced the passage rate resulting in a lower food intake and improved absorption of nutrients, as suggested by Kumprecht *et al.* (1997). Loddi *et al.* (2004) further stated that supplementary

Bio-Mos increased the height and perimeter of villi, thereby increasing the absorptive intestinal surface area resulting in improved feed conversion ratio. The interplay of the three factors is likely to be responsible for the improved FCE observed in this experiment.

3.5 Conclusion

This study demonstrated that the addition of Bio-Mos whether alone or in the presence of Zinc Bacitracin had no significant influence on body weight gain. Bio-Mos and Zinc bacitracin both depressed food intake in the early growing period, resulting in an enhanced efficiency of feed utilization. Young birds possess low immunity therefore it is possible for them to respond positively to feed additives, as these are known to increases the rate of growth of young animals and the efficiency with which they utilize food. The benefit however was not seen in the latter part of the period of growth. Overall there was no significant improvement in performance or FCE with the use of either of these feed additives. The early advantage in the use of the additives was therefore lost by the time the birds were marketed.

Chapter 4

Effect of an organic feed supplement (Kick start) on performance of broiler chickens

4.1 Introduction

Poultry feeds are often supplemented with feed additives, supplements and trace elements, with the aim of improving the nutritive value of the feed, enhancing the performance, and the health of the birds. Due to the fear of antibiotic resistant bacteria, there is a global demand for safe food and this has encouraged the search for natural and organic products that would enhance performance by the birds.

Kick Start, a 100% organic product derived from a blend of "fresh" sprouts of wheat, barley, fenugreek, lupin, sunflower and soya is one of the organic supplements that have been used to enhance performance of animals. Herbs, spices and various plant extracts are becoming more popular in the poultry industry as alternatives to antibiotic growth promoters (Revington, 2002 and Huyghebaert, 2003). Many companies have been investigating the growth-promoting and immunostimulating effects of a number of such extracts as a means of improving broiler performance (Eisa and Abd El-Hamied 2003).

In pursuit of improved chicken health and in order to fulfil consumer expectations in relation to food quality safety, poultry producers are increasingly making use of natural feed supplements, mainly herbs (Gardzielewska *et al.*, 2003), essential oils or extracts of aromatic plants (Botsoglou *et al.*, 2005).

Herbs and plant extracts have been proven to stimulate the growth of beneficial bacteria and to minimize pathogenic bacterial activity in the gastrointestinal tract of poultry (Gill, 1999; Langhout, 2000). Supplementation of a broiler feed with a herbal essential oil mixture made significant improvements to body weight gain, feed conversion ratio and carcass yield (Alçiçek *et al.*, 2004). Alçiçek *et al.* (2003) evaluated essential oils extracted from herbs growing in Turkey and found that these significantly improved the body weight, feed conversion ratio and carcass yield of broilers after a growing period of 42 days. Halle *et al.* (2005) further reported reduced feed intake and improved feed conversion efficiency in broilers supplemented with oregano and its essential oils. Jamroz and Kamel (2002) observed improvements of 8.1% in daily gain and 7.7% in feed conversion ratio in 17-day-old poults fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde and carvacrol at 300 ppm. Hernández *et al.* (2004) reported that plant extracts gave similar live performance to antibiotic growth promoter (avilamycin) when included in broiler diets.

The aim of this study was to determine effect of an organic feed supplement "Kick Start" on growth performance of broiler chickens.

4.2. Materials and methods

4.2.1. Birds and housing

Two hundred and eighty day old sexed Ross broiler chicks were obtained from National Chicks Farms. Ten chicks were randomly assigned to each of 28 cages in a brooder room at Ukulinga research farm, with males and females being kept in separate cages. Each cage was equipped with a feeding trough and two nipple drinkers. Water and feed were supplied *ad*

libitum. A gas heater/blower was used to provide heat to the room. The temperature was set at 29.5°C and was reduced by 1°C every 3d until 23°C was reached at 21d of age.

The trial started when the birds were seven days old and lasted for a period of two weeks. During the week before the trial started birds were on a commercial starter diet.

4.2.3. Feeds and treatments

This trial was run concurrently with another trial in which feeds varying in methionine content were fed to broilers. The seven feeds used in the methionine response trial were produced by blending a summit and a dilution feed (Table 4.1) in various proportions, with the methionine content varying across the feeds as follows 5.4, 4.8, 4.3, 3.8, 3.3 and 2.8g/kg (Table 4.2). Feed seven was the same dilution as feed six, but was supplemented with 41.2g DL-methionine/kg feed. The dilution feed contained no protein or amino acids.

Each of the seven methionine-limiting feeds was divided into two equal parts, and 124g of an organic feed supplement "Kick Start" was added to 41kg (3g/kg feed) to evaluate its effect on growth performance of broiler chicks. The nutritional analysis of Kick Start is shown in Table 4.3.

Table 4.1 *Composition (g/kg) of the summit and dilution feeds.*

Ingredients	Summit	Dilution
Maize	135	-
Soybean Full fat	-	-
Soybean 50	650	-
Sunflower 37	4.2	-
Fish meal 65	5.0	-
Sugar	-	600
Sunflower Oil	92.3	92.0
Filler	-	251
Salt	2.1	2.7
Vitamin and mineral premix	3.0	3.0
Choline-chloride 60	-	3.5
Limestone	11.6	18.3
Mono Calcium Phosphate	1.2	20.0
Potassium Carbonate	-	5.8
Sodium Bicarbonate	-	4.1
L-cysteine	0.09	-
L-lysine HCL	0.49	-
DL-Methionine	0.02	-
L-Threonine	1.22	-

4.2.4. Measurements

Birds were group-weighed per pen on a weekly basis to determine mean body weight, from which mean daily weight gain was calculated. Feed consumption was determined at the end of

each week by weighing back the unconsumed food and subtracting this from the food allocated. Mortality was recorded when it occurred.

4.2.5. Statistical analysis

Body weight, food intake and feed conversion efficiency (FCE) data were processed using MS-Excel (2000) and were then transferred to Genstat (2002) statistical software. Analysis of variance was performed to determine treatment effects and standard errors of means.

Table 4.2 *The proportions of summit and dilution feeds used in the trial, with the resultant calculated methionine contents.*

Treatment	Basal Feeds Blending Proportions		Methionine Content
	Summit	Dilution	g/kg
1	1.00	0.00	5.4
2	0.89	0.11	4.8
3	0.80	0.20	4.3
4	0.71	0.29	3.8
5	0.62	0.38	3.3
6	0.52	0.48	2.8
7[a]	0.52	0.48	2.8

[a] - supplemented with 41.2g of DL-methionine

Table 4.3 *Nutritional analysis of Kick Start.*

Moisture	<5
Gross Energy, MJ/kg	16.32
Fat g/kg	0.18
Carbohydrates g /kg	0.38
Protein g/kg	0.28
Fibres g/kg	0.13
Phytosterols mg/kg	1.9
Minerals (mg/kg)	
Ca	2.0
Mg	2.4
K	14.8
P	5.4
Zn	0.22
Cu	0.05
Mn	0.28
Vitamins (per kg)	
Carotene IU	0.32
Vitamin C mg	0.05
Vitamin B6 mg	0.01
Vitamin E IU	0.12
Riboflavin ppm	1.8mcg
Thiamin ppm	2.8mcg
Niacin ppm	13.6mcg

4.3 Results

Mean body weight gain (g/d), food intake (g/d) and feed conversion efficiency (FCE, g gain/ kg food consumed) of broilers over the two-week trial period are shown in Table 4.4. As the trial reported here dealt only with the evaluation of the feed supplement 'Kick-Start', and as there was no interaction ($P>0.05$) between the methionine contents of the feed and the level of Kick-Start used, only the means for the two levels of Kick-Start over all seven methionine levels will be discussed further.

The supplementation of feeds with the organic feed supplement, Kick-Start, had no influence ($P>0.05$) on any of the performance parameters measured during the trial period.

4.4 Discussion

The objective of this experiment was to determine whether supplementation of diets with Kick Start would have an effect on growth and/or feed efficiency of broiler chickens. Due to lack of reports on work previously done on this product, this discussion is based on some of the ingredients from which the product is derived e.g. fenugreek and lupin. Fenugreek is known to be rich in vitamins and minerals, and because it is a seed and a legume, it is high in protein (Altuntaş *et al.*, 2004). Fenugreek has been known to aid digestion, and to improve metabolism and health (Basch *et al.*, 2003). Lupins (*Lupinus angustifolius*) have a high concentration of protein and can potentially be substituted for soya bean meal as a protein source in poultry diets (Steenfeldt *et al.*, 2003).

Table 4.4 *The effect of organic feed supplement (Kick Start) with or without methionine on mean body weight gain, feed intake and feed conversion efficiency (FCE) of broilers to 21d of age*[1].

	Weight gain (g/d)				Feed intake (g/d)				FCE (g gain/kg feed consumed)			
	0-14		0-21		0-14		0-21		0-14		0-21	
Methionine	-[2]	+[2]	-	+	-	+	-	+	-	+	-	+
5.40	17.4	16.5	45.1	41.9	39.7	36.2	64.0	58.1	321	329	438	455
4.80	18.8	17.9	49.8	49.3	35.8	37.9	66.5	66.8	367	326	538	468
4.30	19.7	20.9	49.8	51.4	40.7	41.9	65.4	67.6	327	318	485	491
3.80	17.3	20.5	46.0	51.6	38.7	42.4	63.8	70.7	313	322	448	484
3.30	17.1	17.5	44.6	44.1	40.3	39.4	66.5	64.5	308	310	423	439
2.80	13.1	15.4	38.0	37.9	35.9	40.1	62.7	63.1	327	319	384	381
2.80	15.5	17.8	42.9	44.2	38.3	41.3	68.1	65.4	309	317	404	430
Mean	17.0	18.1	45.2	45.8	38.5	39.9	65.3	65.2	325	320	446	450
SEM[3]	**1.286**	**1.818**	**2.328**	**3.293**	**2.033**	**2.875**	**2.428**	**3.434**	**19.07**	**26.96**	**23.98**	**33.92**

[1] There was no significant effect on performance parameters with the inclusion of feed supplement (Kick Start)

[2]- Without feed supplement + With feed supplement

[3] SEM Standard Error of means

El-Ghamry (2004) reported that the inclusion of fenugreek in a Muscovy duckling diet significantly improved final body weight, feed intake and feed conversion, but this was not the case in the trial reported here. It is possible that the supply of vitamins and trace minerals in the basal feeds used by El-Ghamry (2004) was inadequate, with the result that the birds responded to the addition of further quantities of these nutrients derived from fenugreek. Presumably the basal feed in the present trial was adequately supplied with these nutrients.

The non-significant effect ($P>0.05$) observed on growth performance agrees with findings by Tarasewicz *et al.* (1995) who reported no significant effect when lupin extracts were added to a basal diet given to male broiler chickens. The important point to bear in mind in such studies is that the supplements used would only be of value if the basal feeds were limiting or deficient in those nutrients supplied by the supplements. It appears that this was not the case in the trial reported here.

It could be argued that when the feed consists of higher-than-necessary amounts of protein or amino acids, as was the case with the highest protein feeds used here, the birds could respond to some nutrients that only show up to be limiting when all other limiting factors have been supplied in adequate amounts. Had this been the case in the present trial there would have been a significant interaction between the methionine content and the amount of supplement used. That there was no interaction demonstrates that the nutrients supplied by Kick-Start were not limiting at any of the methionine levels used in the trial.

4.5 Conclusion

Supplementing broiler diets with the organic supplement 'Kick Start' demonstrated that the basal feeds used in the trial were not deficient in the nutrients contained in this supplement. Whether the methionine contents of the feed were excessive or deficient, the addition of Kick-Start had no effect on the performance of the broilers used here.

Chapter 5

Effect of All-Lac XCL 5x, Acid-Pak 2x, Bio-Mos and Zinc bacitracin on performance of Broiler chickens challenged with *Clostridium perfringens*

5.1 Introduction

Disease is one of the major factors that contribute to loss of productivity in the poultry industry. Necrotic enteritis (NE) caused by the bacterium *Clostridium perfringens* (C. *perfringens*) is a common and financially devastating bacterial disease of poultry (Hofacre *et al.* 2003) that can be effectively treated or prevented with antibiotic growth promoters (AGPs) such as virginiamycin, bacitracin, penicillin, tylosin or flavomycin (Kocher *et al.*, 2004). Although AGPs are highly effective, their use has been limited or banned in many countries, and where still in use, they are under scrutiny by consumers and medical authorities due to the potential development of antibiotic-resistant bacteria. The focus of the poultry industry, certainly in Europe, is therefore to find alternative products that will replace AGPs in controlling diseases such as NE as well as enhancing the performance of broilers.

In addressing the issues concerning necrotic enteritis, Alltech designed an antibiotic-free production program– the Alltech Necrotic Enteritis (ANE) program. The main objective of the program is to reduce the degree of antibiotic resistant bacteria comprising both the gut and house flora (Stephen Collett, Alltech, Inc Nicholasville, Pers. Com. 2006). Protecting flock health by establishing and maintaining a stable normal flora as soon as possible after hatch is again the basis of the ANE program.

Some of the products used in the program include All-Lac XCL, Acid-Pak 4-way and Bio-Mos. All-Lac XCL^{TM} is a direct-fed microbial product made up of culture of selected strains of *Lactobacillus acidofilus, Enterococcus faecium, Lactobacillus plantarum* and *Pediococcus acidilactici* (Alltech, Inc Nicholasville, KY). The All-Lac XCL organisms have been used after hatch to help develop beneficial microflora in the gut of broilers (Sun, 2004). It has been shown to be an alternative to AGPs in preventing necrotic enteritis (Hofacre *et al.* 2003).

Acid-Pak 4-way is an organic-acid-based product containing lactic acid bacteria, organic acids, enzymes and electrolytes (Kocher *et al.*, 2004). The organic acids (citric and ascorbic) contained in Acid-Pak 4-Way, have the ability to drop the pH of the drinking water to less than 5 and maintain this low pH through to the point of consumption, which increases water intake and enhances the profile of the bacterial flora that colonise the gut (Stephen Collett, Alltech, Inc Nicholasville, Pers. Com. 2006). The organic acids are able to inhibit growth of bacteria unfavourable to the normal flora (Patten and Waldroup, 1988).

Bio-Mos blocks mannose-sensitive type 1 fimbriae lectin binding sites thus preventing pathogenic organisms from attaching to the gut wall and colonizing the gastrointestinal tract (Ferket *et al.,* 2002). Bio-Mos has also been found to decrease the colonization of enteric pathogens such as *Salmonella, E. coli* or *Campylobacter* when supplemented in broiler diets (Spring *et al.*, 2000). Bio-Mos may be effective in reducing NE lesions in broiler chickens (Kaldhusdal, 2000). Diets supplemented with Bio-Mos significantly impact the chicken's intestinal microflora and reduce susceptibility to *S. enteritidis* colonization (Fernandez *et al.*, 2002).

Altering the bacterial population in the intestines of broilers by using feed supplements such as prebiotics (oligosaccharides), probiotics or organic acids have some effect in controlling necrotic enteritis (Kocher *et al.*, 2004). The oligosaccharides have been found to improve weight gain, efficiency of feed conversion and health status of the animal (Monsan and Paul, 1995) when included in their diets. According to Sun (2004), organic acids are claimed to suppress growth and multiplication of pathogenic bacteria in the gastrointestinal tract by creating an unfavorable acidic environment for pathogenic bacteria although favourable for the survival of beneficial bacteria.

This experiment was designed to evaluate and compare the effectiveness of three Alltech products, namely, All-Lac XCL 5x, Acid-pak 2x and Bio-Mos with Zinc bacitracin, the most commonly used antibiotic growth promoter, on growth performance of broiler chickens challenged with *C. perfringens*.

5.2 Materials and Methods

5.2.1 Birds and housing

Two thousand eight hundred and eighty (2880) day-old Ross 1 broilers, (1440 males and 1440 females) used in this study were purchased from National Chicks Farms. Sixty sexed chicks were randomly assigned to each of 48 deep-litter pens in a broiler house at Ukulinga research farm.

Each pen was equipped with three chick feeders and two water founts for the first seven days, in addition to nipple drinkers. Three tube feeders were introduced at day 7 when the chick

founts and feeders were removed. During the entire experimental period of 42 d water and feed were offered *ad libitum.* Temperature was reduced stepwise from 31^0C at day-old to 21^0C at day 21, and maintained at this level for the rest of the experiment. Gas brooders were used for the first two weeks to maintain temperature, during which time minimum ventilation was ensured with the use of cross-ventilation fans. Thereafter the house was longitudinally ventilated and cooled using four large extractor fans and curtains. The humidity in the house was dependent on exchange of air provided by the tunnel ventilation system. Evaporative cooling was used when necessary.

5.3 Experimental design

A completely randomised block design was used. Six dietary treatments were used (Table 5.1), each replicated eight times (four replicates being males and four being females) in three blocks, with each pen of 60 birds representing a replicate. Three blocks of 16 pens were allocated down the length of the house to account for variation introduced as a result of the tunnel ventilation system used.

Table 5.1 *Dietary treatments and their description.*

Treatment	Description
1	Broiler mash without additives (Control)
2	Control + All-Lac XCL 5x
3	Control + Acid-Pak 2x
4	Control + Bio-Mos
5	Control + Zinc Bacitracin
6	Control + combination of treatments 2, 3 and 4

5.4 Feeds and treatments

Three phases of feeding (starter, grower and finisher) were used in this trial and each phase lasted for a period of 21d, 14d and 7d respectively. The feeds were obtained from Meadow Feed Mills (P.O. Box 426, Pietermaritzburg, South Africa) in a mash form without any antibiotics. The ingredient and nutrient compositions of the feed are shown in Table 5.2. The feed was fed as such, with the addition of Zinc bacitracin (0.333g/kg) and Bio-Mos (2g/kg in starter phase, 1g/kg in grower phase and 0.5g/kg in finisher phase) as shown in Table 5.1. The chicks allocated to Treatments 2 and 6 were sprayed with All-Lac 5x immediately after being removed from the hatcher. Birds on treatment 3 and 6 were given Acid-Pak 2x in drinking water.

5.5 *Clostridium perfringens* challenge

Birds were challenged with *C. perfringens* twice a day (at 09H30 and at 14H00), for three consecutive days (21, 22 and 23 d of age) via the feed. The inoculum was prepared at the

Allerton Regional Laboratories (Pietermaritzburg). Each day a 20mL inoculation of *C. perfringens* culture, containing 1.1 x 10^{10} bacteria per mL, was diluted in an isotonic saline solution (0.9% Na) to bring it to 4800 mL. Feed was withdrawn from the pens one hour before inoculation, so that all the chickens would eat simultaneously when feed was next provided. Each of the 48 pens was inoculated with 100mL of the solution, supplied in 20kg of feed, this being mixed thoroughly in a bucket prior to being given to the birds. Once all birds had eaten the inoculated feed, the experimental feed was restored.

Table 5.2 *Ingredient and nutrient composition (g/kg) of the three basal feeds.*

Ingredient	Starter	Grower	Finisher
Maize	542	540	604
Soya bean full fat	171	296	260
Soya bean oil cake	105	24.0	0.0
Sunflower oil	78.5	98.5	66
Fish meal	65	0.0	41
Gluten	0.0	2.7	0.0
Limestone	18.0	16.8	12.1
Dical Phos	8.8	10.2	9.4
Sodium bicarbonate	3.1	0.5	0.0
Vit + Min premix	4.0	4.0	2.2
Salt	1.3	2.7	1.6
DL Methionine	2.0	2.0	1.8
Lysine-HCl	0.8	2.2	1.4
Choline Chloride	0.6	0.3	0.5
Threonine	0.0	0.2	0.01
Nutrients			
Crude Protein	230	207	202
AMEn (MJ/kg)	12.1	12.6	12.9
Calcium	12.0	10.2	8.5
Avail Phosphorus	3.8	3.1	4.1
Methionine	6.2	5.4	5.4
TSAA	10.0	9.1	9.0
Lysine	13.2	12.1	11.7
Crude Fat	615	773	762
Crude Fibre	433	477	394
Sodium	2.2	1.4	1.4
Potassium	7.9	8.1	7.2

5.6 Measurements

To determine mean body weight per pen and mean daily gain per bird day, birds were group-weighed weekly by pen (32 birds were chosen at random per pen). Feed consumption was calculated at the end of each week by weighing back the unconsumed feed and subtracting this from the total food allocated. Mortality was recorded as it occurred during the trial.

At the end of the trial (42 d) three birds per treatment were randomly selected and sacrificed through asphyxiation with CO_2. The duodenum, ileum and caeca were examined for the level of NE intestinal lesions.

5.7 Statistical analysis

Body weight, feed intake and feed conversion efficiency (FCE) were the variables tested. Data were processed in MS-Excel and then transferred to Genstat (2002) statistical software where an analysis of variance was used to calculate the treatment means and standard errors.

5.8 Results

Mean body weight gain, feed intake and FCE of male and female broilers to 42 d are presented in Tables 5.3, 5.4 and 5.5.

5.8.1 Body weight gain

Body weight gain was not affected ($P>0.05$) by any of the dietary treatments during the experimental period (42d) (Table 5.3). There were differences between sexes during the grower (21-35d) and finisher phases (25-42d) and over the entire trial period (0-42d), with males gaining more weight than the females.

5.8.2 Feed intake

Differences were noted in feed intake during the starter and grower phases as well as over the entire period (Table 5.4). Birds on Zinc bacitracin, Bio-Mos and on Treatment 6 (Sprayed with All-Lac XCL 5x, drinking water with Acid-pak 2x and consuming feed supplemented with Bio-Mos) consumed less feed ($P<0.05$) during the starter and grower phases compared to those on the control diet. There were also differences in feed intake between sexes ($P<0.05$) during the grower and finisher phases and over the full period, with males having consumed more than females.

5.8.3 Feed conversion efficiency

Significant differences ($P<0.05$) were noted in FCE during the 42d experimental period. Birds given Zinc bacitracin and Bio-Mos had higher FCE's than those that were on the control diet. There were no differences in FCE between sexes (Table 5.5).

5.8.4 Challenge performance

The birds did not show any clinical signs of necrotic enteritis after being challenged with *C. perfringens*. There was also no evidence of necrotic enteritis lesions in the small intestines observed in the birds sampled.

Table 5.3 *The effect of Bio-Mos, Zinc-bacitracin, All-LAC and Acid-Pak on mean body weight gain of broilers to 42d of age*

Treatment	Weight gain (g/bd^{-1})							
	0-21d		21-35d		35-42d		0-42d	
	M	F	M	F	M	F	M	F
1	25.7	26.0	54.8	51.7	68.1	58.3	47.1	43.2
2	25.0	25.4	55.8	50.0	66.0	57.7	48.2	42.9
3	25.0	24.6	52.0	51.6	63.5	58.1	46.7	41.8
4	25.9	25.6	54.8	51.6	65.0	61.1	47.0	43.2
5	26.1	26.2	55.8	51.8	67.4	59.1	48.0	42.9
6	25.5	25.0	54.9	48.9	63.8	58.7	47.3	42.9

Source of variation	Probability of greater *F* value in analysis of variance[1]			
LSD	1.5	3.1	4.5	3.0
Treatment	NS	NS	NS	NS
Sex	NS	***	***	***
Trt * Sex	NS	NS	NS	NS

[1] * P<0.05, ** P<0.01, *** P<0.001

Table 5.4 *The effect of Bio-Mos, Zinc-bacitracin, All-LAC and Acid-Pak on feed intake of broilers to 42d of age*

	Feed intake (g/bd^{-1})							
	0-21d		21-35d		35-42d		0-42d	
Treatment	M	F	M	F	M	F	M	F
1	68.8	65.9	241.9	211.6	173.3	148.3	119.5	106.0
2	69.1	65.4	240.6	212.9	163.5	150.9	116.7	105.5
3	69.1	68.4	235.9	219.2	165.7	147.0	116.2	106.6
4	59.9	62.3	212.2	186.7	178.4	150.7	107.8	102.3
5	60.1	59.0	209.9	188.1	168.0	145.5	108.3	97.7
6	56.2	60.2	200.8	195.5	164.3	148.2	104.4	100.2
Source of variation	Probability of greater *F* value in analysis of variance[1]							
LSD	5.3		25.1		18.8		10.4	
Treatment	***		***		NS		*	
Sex	NS		***		***		***	
Trt * Sex	NS		NS		NS		NS	

[1] * P<0.05, ** P<0.01, *** P<0.001

Table 5.5 *The effect of Bio-Mos, Zinc-bacitracin, All-LAC and Acid-Pak on feed conversion efficiency of broilers to 42d of age*

	Feed conversion efficiency (g/kg)							
	0-21d		21-35d		35-42d		0-42d	
Treatment	M	F	M	F	M	F	M	F
1	374	396	227	245	350	364	393	410
2	363	390	233	236	352	359	414	408
3	363	361	222	238	345	359	403	393
4	432	412	259	281	372	383	437	423
5	434	446	266	278	382	391	444	439
6	454	417	275	256	373	375	454	429
Source of variation	Probability of greater *F* value in analysis of variance[1]							
LSD	36.9		30.7		35.0		29.1	
Treatment	***		***		*		***	
Sex	NS		NS		NS		NS	
Trt * Sex	NS		NS		NS		NS	

[1] * P<0.05, ** P<0.01, *** P<0.001

5.9 Discussion

This experiment was designed to examine the effects of All-Lac XCL 5x, Acid-pak 2x and Bio-Mos on growth performance of broiler chickens and to compare the efficacy of these Alltech products against the well-known antibiotic growth promoter, Zinc bacitracin. The results showed that none of the feed additives had any significant effect on body weight gain at any age compared with the control treatment that was devoid of any additives. There have been other reports indicating the non-significant effect ($P>0.05$) on body weight gain of these additives. For example, Eren *et al.* (1999) supplemented broiler diets with Zinc bacitracin, a probiotic and Mannan oligosaccharide and found no significant differences in body weight gain. Sarica *et al.* (2005) reported no significant influence in body weight gain from 7 to 42 d in broilers where feeds were supplemented with AGP, Mannan oligosaccharides and an organic acid mixture. Similarly, Kocher *et al.* (2004) reported no significant differences among treatments for body weight gain when broiler chickens challenged with *C. perfringens* were supplemented with Bio-Mos and Acid-Pak 4-Way. In contrast, Kramomtong *et al.* (2001) reported a greater ($P<0.05$) weight gain of birds treated with Bio-Mos and Acid-Pak compared to untreated birds, when challenged with *Salmonella enteritidis*.

The absence of a response of the treatments in body weight gain may suggest either that challenging the birds with *C. perfringens* did not expose them to any stress, or that the inoculum used was insufficient to cause pathogenic symptoms in the birds. Hooge (2004) indicated that increased disease or crowding stress generally results in greater improvements by beneficial additives. Gürbüz (2004) also stated that Bio-Mos improved growth performance mainly when birds were challenged with enteric pathogens.

The significant differences observed among treatments in FCE are consistent with findings of Kocher *et al.* (2004) and Hofacre *et al.* (2003) who challenged broilers with *C. perfringens* and found a significant improvement in feed efficiency. The results also indicate that supplementation with Bio-Mos improved FCE. Previous studies have demonstrated that Bio-Mos has the ability to bind pathogenic bacteria expressing type-1 fimbriae such as *Salmonella* species and *E. coli* (Spring *et al.*, 2000; Finucane *et al.*, 1999b). Although *Clostridia* do not express type 1 fimbriae, Hofacre *et al.* (2003) reported that the addition of Bio-Mos alone or in combination with Acid-Pak in diets fed to birds challenged with *C. perfringens* had some influence in reducing mortality and sub-clinical effects of *C. perfringens* on feed efficiency. Sun *et al.* (2004) also found that feeding broilers without growth promoters resulted in greater mortality and decreased performance compared to using an antibiotic, while Bio-Mos in combination with All-Lac XCL helped to reduce the negative effects when birds were challenged with coccidia.

The lack of necrotic enteritis lesions in the small intestines of birds examined may suggest that the challenge was not effective. This is not unusual: Kocher *et al.* (2004) reported that birds did not die from, or develop a clinical form of necrotic enteritis when challenged artificially, while Sun (2004) reported that the lesion scores of challenged birds were not significantly affected by different feed additives. However, the highest NE lesion scores observed by Hofacre *et al.* (2003) were among birds given fructose oligosaccharides, whilst there was little practical difference among the other treatments, which included Bacitracin, Bio-Mos, All-Lac and Acid-Zap.

The extent to which antibiotic growth promoters and their alternatives will improve gut health and hence broiler performance depends on several factors, such as the severity of the necrotic enteritis challenge, the prevailing management and environmental conditions, the vaccination program used, and the clean-out program used at the end of each cycle. Clearly, there will be situations where it is unnecessary to use these feed additives, whilst in high-risk situations considerable intervention would be needed.

5.10 Conclusion

Similar improvements in FCE were observed with the addition to broiler diets of Bio-Mos and Zinc bacitracin when broilers were challenged with *C. perfringens* but no significant differences were observed in weight gain or gut health. It appears from the results of this trial that, in spite of the NE challenge, the conditions under which the broilers used in the trial were kept were unfavourable for the spread of diseases, hence negating the need for feed additives.

General discussion

The major objective of this thesis was to evaluate the effect of various feed supplements as potential alternatives to AGPs on performance of broiler chickens. The concern that the unrestricted use of sub-therapeutic levels of feed antibiotics may be associated with the development of antibiotic-resistant human pathogens has become a major issue that requires immediate attention. Consequently the EU phased out the use of all AGP's from livestock and poultry diets from January 2006. The poultry industries worldwide are now faced with the challenge of finding possible alternatives to AGP's in order to maintain efficient poultry production.

Supplementing broiler diets with feed additives demonstrated interesting results in all the four experiments conducted. In Chapter 2, the results showed that supplementing the feeds for broiler breeder males with Selplex and Bio-Mos significantly increased the number of normal spermatozoa but had no influence on sperm concentration or motility. Bio-Mos marginally reduced the *E. coli* count in faecal samples. Further research may be warranted to determine the effect of higher inclusion levels of the additives.

The findings in Chapter 3 indicated that the addition of Bio-Mos alone or in the presence of Zinc bacitracin had no influence on body weight gain. Both feed additives depressed food intake in the early growing period without influencing growth rate, resulting in an enhanced efficiency of feed utilization. However, the overall performance of the birds was not improved by either of these feed additives.

The result in Chapter 4 showed that the organic supplement 'Kick Start' had no effect on the performance of broilers. This confirms that the basal feeds were not limiting or deficient in nutrients supplied by the supplements.

In Chapter 5, where broilers were challenged with *C. perfringens,* supplementation of their feed with All-Lac XCL 5x, Acid-Pak 2x, Bio-Mos or Zinc bacitracin had no effect on body weight gain when compared to the control diet. This non-significant response suggests either that challenging the birds with *C. perfringens* did not expose them to any stress, or that the inoculum used was insufficient to cause pathogenic symptoms in the birds.

The health status of the birds and the hygienic level in a house can greatly influence response to the supplements (Talay *et al.*, 2004). The non-significant differences observed in the overall performance of the broilers in each of the experiments may suggest that infection rates at the farm were low, thereby reducing the impact of additives on performance. The value of many of the feed additives evaluated in this project may be greater where conditions in broiler houses are unhygienic and hostile, such as exist in most commercial operations, suggesting that trials in which these additives are evaluated should not be conducted in research facilities such as those at Ukulinga, but instead under commercial conditions.

Appendix A

Formula used to calculate the number of live sperm per mL of sample.

$$B \times \frac{1000^*}{0.004} \times \frac{1}{A} \times \frac{C}{1} \times \frac{D}{100}$$

$$= \frac{B \times C \times D}{A} \times 2\,500$$

Total number of blocks counted (e.g. 10)

A- Total number of blocks counted (e.g. 10)

B- Total number of sperms counted (e.g. 64 in 10 blocks)

C- Dilution factor (e.g. 0.1mL in 8.0 mL gives a factor of 80)

D- Motility of the sperms (e.g. 65/5/30 gives 65+5=70)

Therefore count the % progressive motile normal sperm and the % motile abnormal sperm together to get D)

$^*\frac{1000}{0.004}$ = factor to convert volume of blocks in haemocytometer to mL.

Example:

$$\frac{64 \times 80 \times 70}{10} \times 2\,500$$

= 89.6 million live sperm/mL semen

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