Comparison of production parameters, gut histology, organ weights, and portion yields of broilers supplemented with Ateli plus®

by

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DECLARATION

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SUMMARY

Antibiotic growth promoters (AGP's) have been used in feed of production animals to improve their growth performance and disease resistance. However, there has been an increase in the presence of antibiotic residue in animal products, as well as an increase in untreatable illnesses due to antibiotic resistant bacteria. This led to the European Union banning the use of antibiotic growth promoters, in production animals, in 2006. An alternative to AGP's is therefore needed in animal feed in order to maintain performance. Plant extracts and essential oils have gained much attention, due to their natural antimicrobial, antibacterial, anticoccidial and antioxidant properties. This study tested the efficiency of Ateli plus®, an oregano plant extract based product, as a replacement to AGP's on production parameters, carcass characteristics and organ and gut health of broilers.

This study consisted of five treatments fed to broilers from hatch till 33 days of age, fed in three phases; starter, grower and finisher. The treatment diets consisted of a negative control (no AGP), positive control (AGP), Ateli plus® at 1kg/ton (Ateli plus® min), Ateli plus® at 2kg/ton for week one followed by 1kg/ton for the remainder of the period (Ateli plus® max), and AGP plus Ateli plus® max (AGP plus Ateli plus® max).

Results from this study show that there was no difference in performance parameters between treatments. The performance parameters tested included liveability, average daily gain (ADG), average weekly feed intake, average cumulative feed intake, average weekly live weight, average cumulative weight gains, feed conversion ratio (FCR), cumulative FCR and the European production efficiency factor (EPEF). Broiler breast and thigh muscle pH and colour (L*, a* and b*) reading values were measured, showing a trend for improved L* colour reading value and ultimate pH, in broilers supplemented with Ateli plus®. This leads to an increase in water binding capacity and tenderness, therefore resulting in an improvement in meat quality. No differences were shown for dressing percentage and portion percentages relative to carcass weight. No significant differences were seen for tibia bone Ca and P content, or tibia bone fat, moisture or ash percentages. However a significant decrease in tibia bone strength was found in all broilers supplemented with Ateli plus® diets and the negative control diet, compared to AGP supplemented broilers. Gut morphology showed no consistent effect of treatment on villi height or crypt depth of the duodenum, jejunum and ileum. No significant differences between treatments were found for organ pH or organ weights, except the gizzard, which was heavier for Ateli plus® max supplemented broilers.

Ateli plus® shows promise on improving meat quality characteristics of broilers, however the significant decrease in tibia bone strength in Ateli plus® supplemented broilers is a major concern and needs to be researched further. Ateli plus® acts as a good AGP replacement, as broiler performance for the Ateli plus® supplemented broilers was maintained, and not

decreased, when compared to the AGP supplemented broilers. However, the broilers fed the negative control diet had performance parameters statistically equal to both the Ateli plus® and AGP supplemented broilers, as well as no significant differences between organ weights were found. It can therefore be said that the broilers were raised under good management, and their optimal environmental conditions. Therefore conclusive effectiveness of Ateli plus® as a replacement for AGP on broiler performance cannot be reported from this study.

OPSOMMING

Antibiotiese groeistimulante (AGP's) word gereeld as voerbymiddels vir diere gebruik om groei en siekte weerstandbiedenheid te verbeter. Die verbod op die gebruik van antibiotika as 'n groeipromotor in die Europese Unie (sedert 2006) kan toegeskryf word aan die toename in onbehandelbare siektes as gevolg van weerstandbiedende bakterieë, asook die teenwoordigheid van residue in die dierlike produkte. Alternatiewe vir AGP's in dierevoeding word dus benodig om produksie te handhaaf. Plant ekstrakte en essensiële olies het baie aandag gekry as gevolg van hul natuurlike antimikrobiese, antibakteriële, antikoksidiese en antioksidatiewe eienskappe.

'n Studie is gedoen om die doeltreffendheid van Ateli plus® ('n origanum plant ekstrak gebaseerde produk) op groei produksie parameters, karkaseienskappe, orgaan- en dermgesondheid te bepaal wanneer Ateli plus® as 'n AGP plaasvervanger in die dieëte van braaikuikens gebruik word. Gedurende die proef was vyf verskillende diëte/behandelings vir 33 dae vir braaikuikens gevoer. Die behandelings het bestaan uit 'n negatiewe kontrole (geen AGP), positiewe kontrole (AGP), Ateli plus® teen 1kg/ton (Ateli plus min), Ateli plus® teen 2kg/ton vir die eerste week gevolg deur 1kg/ton vir die res van die tydperk (Ateli plus® max), en AGP plus Ateli plus® max (AGP plus Ateli plus® max).

Resultate van hierdie studie toon dat behandelings nie 'n effek op produksie parameters gehad het nie. Die groei produksie parameters wat getoets is sluit in oorlewing, gemiddelde daaglikse toename (GDT), die gemiddelde weeklikse voer-inname, gemiddelde kumulatiewe voerinname, gemiddelde weeklikse lewendige massa, gemiddelde kumulatiewe gewig toename, voeromsetverhouding (VOV) en die Europese produksie doeltreffendheid faktor (EPEF). Die pH en kleur (L*,a* en b* waardes) van die dy- en borsspier is gemeet. Ateli plus® aanvulling in braaikuiken diëte het 'n tendens getoon vir verbeterde L * en finale pH waardes in die spiere, wat lei tot 'n toename in waterhouvermoë en sagtheid en dus verbeterde vleiskwaliteit. Geen verskille tussen behandelings is gevind vir uitslagpersentasie en die massa van porsies (uitgedruk as persentasie relatief tot karkasgewig) nie. Behandelings het nie 'n effek op die vet, vog, as persentasies of Ca- en P-inhoud van die tibia gehad nie.

In vergelyking met AGP aangevulde braaikuikens, is 'n beduidende afname in die tibia breeksterkte van kuikens in die negatiewe kontrole groep en kuikens wat met Ateli plus® aangevul is, gevind. Spysverteringskanaal morfologie het getoon dat behandeling geen konsekwente effek op villi hoogte of krip diepte van die duodenum, jejunum en ileum gehad het nie. Slegs die krop massas van kuikens wat met Ateli plus® max aangevul is, was swaarder in vergelyking met hoenders in die ander behandelings; verder is geen beduidende verskille tussen behandelings gevind vir orgaan pH of orgaan gewigte nie.

Die gebruik van Ateli plus® op die verbetering van die vleiskwaliteit eienskappe van braaikuikens blyk dus belowend te wees, maar die beduidende afname in tibia breeksterkte van hoenders wat aangevul word met Ateli plus® is kommerwekend en moet verder ondersoek word. Die handhawing van produksie in Ateli plus® aangevulde braaikuikens in vergelyking met AGP aangevulde braaikuikens, blyk dat Ateli plus® as 'n goeie AGP plaasvervanger kan dien.

Die produksie parameters van kuikens in die negatiewe kontrole is egter statisties gelyk aan beide die Ateli plus® en AGP aangevulde braaikuikens en daar was geen betekenisvolle verskille tussen orgaan gewigte nie, dus is die gevolgtrekking gemaak dat die hoenders onder goeie bestuur en optimale omstandighede grootgemaak is. Daarom kon die definitiewe doeltreffendheid van Ateli plus® as 'n plaasvervanger vir AGP op braaikuiken prestasie nie uit hierdie studie gerapporteer word nie.

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LIST OF ABBREVIATIONS

A	Amps
ADG	Average daily gain
AGP	Antibiotic growth promoter
ANOVA	Analysis of variance
Ca	Calcium
DM	Dry matter
EOC	Essential oil combination
EPEF	European production efficiency factor
FCR	Feed conversion ratio
FI	Feed intake
g	Grams
kg	Kilogram
km	Kilometre
M	Molar
m^2	Meters squared
max	Maximum
mg	Milligram
min	Minimum
ml	Millilitre
mm	Millimetre
P	Phosphorous
pH_i	Initial pH
pH_u	Ultimate pH
PHS	Pulmonary hypertension syndrome
%	Percent
s	seconds
SD	Standard deviation
SDS	Sudden death syndrome

NOTES

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Meat Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

Contents

Chapter 1

Introduction

The use of in-feed antibiotics in production animals was first discovered in the 1940's, which led to an improvement in growth performance and protected the host animal against subclinical infections. Antibiotics limit the growth of pathogenic and non-pathogenic bacteria in the digestive tract, therefore decreasing the microbial load on the digestive tract. A decreased microbial load leads to a decrease in immune stress on the host animal, which leads to energy that can be used for growth instead. A lower microbial count in the digestive tract also results in a lower demand in nutrients to maintain the digestive tract, as there will be less sloughing of the epithelial cells in the lumen of the host animal, by the microorganisms. This results in more energy available for growth of the host animal, therefore better performance.

Due to the low concentrations at which the antibiotics are added to the diet, the pathogens are able to build up a resistance to the antibiotic and survive. This results in the development of an antibiotic resistant population of pathogens, and therefore untreatable illnesses. There is also concern of antibiotic residues present in the animal products produced for human consumption, leading to public concern on the safety of egg and meat products purchased.

The concern of the safety of in-feed antibiotics, as growth promoters, led to the start of banning in-feed antibiotics in production animals in 1986 in Sweden. By 1998 all but four antibiotics were banned in the European member states. The remaining four antibiotics, avilamycin, bambermycin, monensin and salinomycin, were later banned in 2006 in the European Union (EU). The use of in-feed antibiotics is still permitted in South Africa, however this has limited the countries' potential to export meat and other animal products to the EU for consumption.

This has resulted in the search for alternatives to in-feed antibiotics, which will provide the same protection against pathogenic and non-pathogenic enteric microorganisms, as well as improve animal performance. An alternative to in-feed antibiotics that has gained much interest is plant extracts and essential oils. This is due to the fact that plant extracts and essential oils have been found to naturally have antibacterial, antimicrobial, anticoccidial and antioxidant properties. According to the Food and Drug Administration, plant extracts have been considered safe as a feed additive, by being less toxic, and resulting in residue free animal products for human consumption.

Ateli plus® is a plant extract (Oregano, *Origanum vulgare*) based product, that has been shown to enhance efficiency of feed utilization, increase resistance to disease, as well as improve

overall animal condition. Oregano has strong antimicrobial properties, which makes it a good alternative to AGP's.

The objective of this study was to investigate the efficiency of Ateli plus® as an alternative to in-feed antibiotics. This was achieved by evaluating the performance parameters, carcass characteristics and organ and gut health of broilers supplemented with Ateli plus®. As a replacement to in-feed antibiotics, the expectation for the study was for Ateli plus® supplemented broilers to at least equal the growth performance of the antibiotic supplemented broilers, if not better it. Ateli plus® was tested against the in-feed antibiotic growth promoter, Stafac 500, as well as a negative control with no additives.

Chapter 2

Literature review

2.1 Introduction

Antibiotics have been used in animal feed as growth promoters and also to improve feed conversion over the years, which has made intensive farming possible (Hernandez *et al*., 2004). The use of antibiotics in feed as growth promoters is included at a low rate of about 2.5-5.0 mg/kg (Hashemi & Davoodi, 2011). However, there is concern about the antibiotic residue being present in the animal products, as well as the unintended development of an antibiotic-resistant population of pathogens (Ciftci *et al*., 2005; Jang *et al*., 2007). An antibiotic resistant population would be developed by the presence of low levels of antibiotics in the animals system, and the resistant cells that survive and grow are then the antibiotic resistance bacteria population (Hernandez *et al*., 2004). For this reason, the European Union has restricted the use of antibiotics in broilers, only allowing antibiotics that have no association with human treatment to be used, being avilamycin, bambermycin, monensin and salinomycin (Ferket, 2004). However, on 1 January 2006, the Union banned all antibiotics from being used in animal feed as growth promoters and alternatives to antibiotics had to be investigated to help improve animal production (Jang *et al*., 2007). One such antibiotic growth promoter replacement that has received great attention is essential oils or extracts from herbs and spices (Basmacioglu *et al*., 2004; Hernandez *et al*., 2004). According to Williams & Losa (2001), essential oils are already being added to the diet of production animals to improve growth performance in intensive farming conditions.

The effect of a feed supplement in animal feed is non-nutritive, by acting as a growth or metabolic modifier, or by causing a pH shift, resulting in desirable effects in the animal (Hutjens, 1991). Feed supplements are also fed to animals to improve the quality of product gained from the animal for human consumption (Hashemi & Davoodi, 2011).

Improving growth performance and feed efficiency is possible by manipulating the microorganisms in the animals' digestive tract as well as manipulating the gut function of the domestic animal with the help of feed additives (Collington *et al*., 1990). Essential oils have the ability to manipulate and inhibit microbial growth in the digestive tract as well as enhance nutrient digestibility. According to the Food and Drug Administration (FDA), essential oils are recognised as a safe additive (Ferket, 2004; Jang *et al*., 2007). According to Wang *et al*. (1998), plant derived products are better suited for feed additives in feed for animal production, as they have been proven to be residue free, less toxic, and natural, as opposed to synthetic antibiotics or inorganic chemicals.

Traditionally, aromatic plants have been used in the healing of diseases for many years. The essential oils of the aromatic plants are also used in cosmetics, medicine and food industries (Ciftci *et al*., 2005).

2.2 What are Phytobiotics?

As redefined by Windisch & Kroismayr (2006), phytobiotics are plant-derived products added to the feed in order to improve performance of agricultural livestock, and wellbeing (Vidanarachchi *et al*., 2005). As part of their natural metabolic activities, all plants produce chemical compounds, such as sugars and fats. A small range of plants also produce secondary metabolites, or phytochemicals, that are not necessary for the plant's survival (Hashemi & Davoodi, 2011), but could help them interact with their environment, and also act as protection against herbivores or pathogens, as well as help them against any physiological and environmental stresses (Wenk, 2003). It is these secondary metabolites that are being evaluated as alternatives to in feed antibiotics, as they have been shown to have beneficial effects on the metabolism of the animals, as well as the food products produced by these production animals (Wenk, 2003).

Phytochemicals consist of large range of substances, which can be divided into four groups. Namely: Herbs, which comes from a non-woody flowering plant. Secondly: Botanicals, which are comprised of a part of the plant e.g. roots, leaves and bark. Thirdly: Essential oils, which are the volatile plant compounds. And lastly, oleoresins, which are the plant extracts based on non-aqueous solvents (Windisch & Kroismayr, 2006).

According to Wang *et al*. (1998), phytobiotics have been proven to be natural, contain less toxins, and to have no residue effect on the animal products, making these plant derived products more favourable than their synthetic antibiotic counterparts. Phytobiotics are made up of phytochemicals, and it is these phytochemicals that are believed to have antimicrobial activity (Cowan, 1999), have the ability to manipulate the gut microorganisms (Hashemi & Davoodi, 2011), immune enhancement properties (Guo *et al*., 2004a) as well as have coccidiostatic activities (Allen *et al*., 1997; Youn & Noh, 2001). Phytochemicals have been proven to act as antioxidants (Lopez-Bote *et al*., 1998; Botsoglou *et al*., 2002), hypocholesterolemics (Craig, 1999), as well as to increase the production of digestive enzymes and therefore improve the utilization of digestive products through increased liver function (Hernandez *et al*., 2004).

2.2.1 Essential oil vs. herb vs. active ingredient

2.2.1.1 Essential oils

Essential oils derived from herbal plants are being considered as an alternative to in feed antibiotics, however their effects *in vivo* and *in vitro* are not always corresponding. This could largely be due to the varying content of the essential oils, which consists of aromatic and volatile

substances (Jang *et al*., 2004). It has been found that the quality and quantity of the active chemicals within the essential oils vary, and therefore has an effect on the response by the animal (Cross *et al*., 2007). Wang *et al*. (1998) reported that the age of the plant at harvest, geographical area of the plant, soil composition the herb plant was planted in, and the part of the plant used to extract the product from, all has an effect on the effectiveness of the supplement on the animal. Essential oils are obtained from aromatic plants either by hydro distillation or solvent extraction (Bendahou *et al*., 2008). The method of obtaining the essential oils from the aromatic plants is believed to also play a role in the concentration of the active ingredients and quality of the product (Bendahou *et al*., 2008).

From research done *in vivo* with essential oils, there are studies showing a positive effect of essential oils on growth performance on broilers (Alçiçek *et al*., 2004; Cross *et al*., 2007), and other studies showing no effect of essential oils on broiler growth performance (Botsoglou *et al*., 2002; Lee *et al*., 2003; Botsoglou *et al*., 2004; Hernandez *et al*., 2004; Jang *et al*., 2007).

Cross *et al*. (2007) showed that broilers supplemented with 1 g/kg of thyme essential oil had an improved body weight and weight gain at 28 days age, which was an improvement from the control. This could indicate that the environment the chickens were grown in was not completely pathogen-free, and therefore the essential oils could have a positive impact on the performance of the broilers. Alçiçek *et al*. (2004b) also found an improvement in performance in broilers supplemented with 48 mg/kg essential oil combination. This improvement also exceeded that of the antibiotic supplement, Avilamycin at 10 mg/kg.

In a study by Hernandez *et al*. (2004), neither Labiatae extract (at 5000 mg/kg), or essential oil extract (at 200 mg/kg) had an improvement on performance of broilers grown till 42 days of age. However their control group also did not differ, and therefore it can be concluded that the broilers were grown in an environment that closely resembled that of a pathogen-free environment, and therefore essential oils would not have a beneficial effect on the broilers.

It is thought that the effect of essential oils on broiler performance could be influenced by the basal diet and the environmental conditions, as well as the digestibility of the diet and whether the animals are kept under optimal conditions (Botsoglou *et al*., 2002). Using essential oils as growth promoters will have no effect on the growth rate of healthy animals kept under optimal conditions, as it was documented that even dietary antibiotics have no growth stimulating effects on animals kept in optimal conditions (Visek, 1978). This could be a cause for the contradicting results found when essential oils are tested as growth promoters (Jang *et al*., 2004; Basmacioglu *et al*., 2004; Jang *et al*., 2007). There are also the intrinsic and extrinsic factors to take into consideration, such as the nutritional status of the animal, the composition of the diet and the health status of the animal, which all play a role in how effective the essential oils supplementation will be (Giannenas *et al*., 2003; Lee *et al*., 2004a).

In a study by Jang *et al*. (2004), there is evidence to suggest that the combination of an essential oil and lactic acid supplemented diet fed to broilers could have a synergistic effect, and therefore causes a positive effect on the growth performance of the broilers. In the study, the separate supplementation of lactic acid, essential oil, and antibiotic did not show an increase in weight gain or FCR (Jang *et al*., 2004). In this study the commercial product, CRINA was used, which is a blend of essential oils with the active ingredient thymol. It was reported that the combination of lactic acid and essential oil could work well as the lactic acid is more prone to work in the proximal digestive tract, while the essential oils have greater function in the distal tract. The supplementation of both lactic acid and a commercial essential oil mix together in the diet of the broilers lead to an increase in the activities of the digestive enzymes of the pancreas and intestinal mucosa, which further lead to an increase in the growth performance (Jang *et al*., 2004).

2.2.1.2 Herbs

Halle *et al*. (2004) added oregano essential oils and dried oregano herbal leaves to the diet of broiler chickens, separately. The oregano herb leaves were added at a rate of 2, 4, 10 and 20 g/kg, while the oregano essential oil was added to the broiler diet at a rate of 0.1, 0.2, 0.5, 1.0 g/kg. The supplementation of oregano leaves and its essential oil to the diets of the broilers resulted in a decrease in feed intake compared to the control, and the supplementation of oregano essential oil resulted in an increase in feed efficiency. From the trial it was reported that the essential oil had a more pronounced effect on the animals' production than the dried oregano herbal leaves (Halle *et al*., 2004). Florou-Paneri *et al*. (2005) added oregano herb and its essential oil to the diets of turkeys, and investigated the effect thereof on growth performance. The treatments in the study included the control, oregano herb leaves supplemented at 5 and 10 g/kg, and essential oils supplemented at either 100 or 200 mg/kg. It was reported that neither the oregano herb nor the oregano essential oil had an improving effect on the turkey's performance. However, the control group did not have a lower performance than the oregano supplemented turkeys, suggesting that the turkeys were grown in an environment that closely resembled that of a pathogen-free environment.

2.2.1.3 Plant extracts

The beneficial effects of herbs and some medicinal plants is the work of their bio-active components, which are unique to each species of plant giving them different properties. These bio-active components of plants are mainly the secondary metabolites that the plant produces during its living stages. Secondary plant metabolites include terpenoids (mono- and sesquiterpenes, steroids, etc.) and essential oils, phenolics (tannins) and polyphenols, glycosides and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers and lactones) (Huyghebaert *et al*., 2011). There is speculation that these bio-active components of the plant have synergistic activities (Hashemi & Davoodi, 2011).

There are many factors affecting the composition and amount of bio-active ingredients in the herbal products. Such factors include the time of harvest of the living plant, its geographical position as well as the species type, causing variation in the composition of the herbal products. The method of manufacture also plays a role in the concentration and composition of the herbal products, as well as the storage method used (Huyghebaert *et al*., 2011).

In the case of essential oils, there are synergistic activities between the essential oils, and as such they are never provided as an isolated oil but rather a mixture of oils. There are also reports of synergistic activities between the essential oils and the feed ingredients they are mixed with (Zhang *et al*., 2005).

It has been reported that the phenolic compounds of the herbs and spices are largely responsible for the antioxidant and pharmaceutical properties (Cai *et al*., 2004; Shan *et al*., 2005), as well as responsible for the antimicrobial properties expressed by the herbs and spices (Hara-Kudo *et al*., 2004). However, according to Shan *et al*. (2007), no large specific study has been completed on the relationship of the amount of phenolic compounds present in herbs or spices, and their respective antibacterial ability. In a study completed by Shan *et al*. (2007), where 46 herb and spice extracts were tested on five different foodborne bacteria, it was concluded that there is a strong linear relationship between the concentration of phenolic compounds present in the extracts and the antibacterial activity that they expressed. This study also reported the strong positive relationship between antibacterial activity and antioxidant capacity of the extracts.

Carvacrol and thymol are the phenolic compounds found in many lamiaceae species such as thyme and oregano, as well as others. Cinnamon contains the bio-active ingredient, cinnamaldehyde, which has antimicrobial properties, along with other benefiting properties (Solórzano-Santos & Miranda-Novales, 2012). According to Dorman & Deans (2000), the bio-active components of the essential oils that contained the phenolic structures such as thymol, eugenol, and carvacrol had a more inhibiting effect on the test microorganisms. Cinnamaldehyde, eugenol and carvacrol are the active ingredients in the cinnamon plant, and are responsible for the antimicrobial properties of the plant. This was proven to be correct when cinnamon extract was reported to inhibit *Helicobacter pylori* (Tabak & Neeman, 1999). The bio-active compounds, carvacrol and thymol have considerable antimicrobial and antifungal activity (Basilico & Basilico, 1999). Studies with anise oil in broiler diets, has shown that anise oil can be used as a growth promoter. The bio-active components of anise oil are anothole (85%), eugenol, methylchavicol, anisaldehyde and estragole (Ciftci *et al*., 2005). In an *in vitro* study by Dorman & Deans (2000), it was reported that of the bio-active components, thymol had the widest spectrum against bacteria, followed by carvacrol. Of the essential oils that were tested in

the same study, thyme, oregano and clove had the widest spectrum activity against the bacteria tested, in that order (Dorman & Deans 2000).

In a study by Hernandez *et al*. (2004), the mortality rate of the entire trial (42 days) was lower for the chickens supplemented with essential oils (200 mg/kg) and Labiatae extract (5000 mg/kg), separately, compared to both the control and Avilamycin (10 mg/kg) supplemented broilers. Broiler performance between treatment groups did not differ significantly, showing no difference in the effect of the different forms of the plant supplements (Hernandez *et al*., 2004). However, the performance of the broilers receiving the control diet was not significantly lower than the rest of the treatments, showing that the broilers were grown in an environment that closely resembled that of a pathogen free environment, therefore no positive effect of the plant extract could be expressed.

2.2.2 Mode of action

The role of herbs in animal feeds starts as a flavourant, which can then increase the feed intake of the animal, as well as stimulate the secretion of digestive fluids (Wenk, 2000). However, chickens will not be as affected by this as pigs, as it is known that chickens have less taste buds than pigs (Roura *et al*., 2012), and therefore their taste sensitivity is lower than that of mammals (Kudo *et al*., 2010; Roura *et al*., 2012). The primary site that the herbs have their effect is in the digestive tract, where they have been proven to positively influence the microorganism population (Wenk, 2000). This is achieved by their antimicrobial properties, causing a higher population of beneficial microorganisms, and decreasing the population of pathogenic microorganisms (Wenk, 2000; Ferket, 2004). Their antimicrobial activity along with their immune enhancement properties are the phytobiotics main mechanisms of improving the animals' health as well as their growth performance (Cowan, 1999).

Herbs can stimulate the immune system of the animal, as well as the endocrine system. They are also capable of adding to the nutrient requirements of the animal. The positive effects of herbs that can be expected to be seen are an increase in nutrient utilization and absorption (Wenk, 2003).

Tannins are an example of phytochemicals, present in a wide range of plants, and act to protect the plant from predators. These tannins exert their antimicrobial properties in animals by iron deprivation, hydrogen bounding, or non-specific interactions with enzymes (Scalbert, 1991). The growth of gut bacteria, *Bacteroides fragilis, Clostridium perfringens, Escherichia coli* and *Enterbacter cloacae* were proven to be inhibited by tannic acid (Chung *et al*., 1998).

Saponnins are also part of the phytochemical group, and are present in many plant species, having antimicrobial properties, which they exert by forming complexes with the sterols present

in the microorganism's membrane, damaging the membrane and causing the cells to collapse (Morrissey & Osbourn, 1999).

The mode of action of essential oils is not very well understood, although their antimicrobial efficacy has been proven. Essential oils are lipophilic, which is believed to help in penetrating the pathogens cell membrane, causing the contents of the cell to be released (Helander *et al*., 1998), in which case the cell will die. Carvacrol is the main active ingredient found in the essential oils derived from oregano and thyme, and has strong antimicrobial effects (Ultee *et al*., 1999). In a study by Ultee *et al*. (1999) on *Bacillus cereus*, it was shown that carvacrol's antimicrobial mode of action is achieved by changing the permeability of the microorganisms cell membrane for cations, such as H^+ and K^+. The essential processes of the cell would then be malfunctioning, due to the dissipation of the ion gradients, and this would lead to cell death (Ultee *et al*., 1999). Lambert *et al*. (2001) also showed that carvacrol and thymol are responsible for disrupting the cell membrane's integrity. There is evidence showing that broilers fed a diet supplemented with the commercial blend of essential oils, CRINA® at 25 mg/kg together with 0.1% lactic acid, increased the total trypsin activity, as well as resulted in higher pancreatic α-amylase activities. As a result of the increased digestive enzyme activities of the intestinal mucosa and the pancreas, the respective broilers showed an increase in growth performance (Jang *et al*., 2004). However, in a separate study by Lee *et al*. (2003), broilers also supplemented with the commercial blend of essential oils product, CRINA® at a rate of 100 mg/kg, showed no difference between treatments for the digestive enzyme activities of the pancreas. However, amylose activity in the intestinal digesta was increased for the group supplemented with CRINA®, but only up until 21 days of age. Thereafter, there was no difference between the groups. Growth performance was also not effected by the treatments (Lee *et al*., 2003). In the study by Lee *et al*. (2003), the CRINA® product was supplemented at a much higher rate (100 mg/kg) than in the study by Jang *et al*. (2004), which supplemented the CRINA® product at 25 mg/kg and found results for enzyme activities. This suggests that the inclusion of 0.1% lactic acid supplemented along with the CRINA® had a positive influence on the digestive enzyme activities. However this may not always be the case, as in similar study by the same author, Jang *et al*. (2007), where CRINA® was supplemented in the broiler diets without the inclusion of lactic acid there was still an increase in total and specific pancreatic trypsin activities, as well as an increase in total pancreatic α-amylase and intestinal maltase activities, in the group fed the diet supplemented with CRINA® at a rate of 50 mg/kg. However, there was no improvement in growth performance reported.

The bacteria that could be present in the intestine of the broiler can be of the species that project pathogenic influences on the host, such as *Proteus spp*., *staphylococci*, *veillonellae* and *clostridia*, or which benefits the host such as *lactobacilli* and *bifidobacteria*. *Bifidobacteria* and *lactobacilli* in the intestine of the broiler are known to benefit the host by promoting gut

maturation, immune modulation, improving gut integrity, as well as resistance to pathogenic bacteria (Lan *et al*., 2005). The intestinal microorganisms of the host play an important role in maintaining intestinal immune homeostasis as well as reducing the occurrence of intestinal inflammation. Certain plant extracts, as well as prebiotic oligosaccharides, influence the population of beneficial microorganisms in the digestive tract by supplying the nutrients required for their optimal growth (Lan *et al*., 2005). In further studies, it was shown that extracts from mushrooms and plants have the ability to supply nutrients to the beneficial microorganisms such as *bifidobacteria* and *lactobacilli*, and therefore enhance their growth, and at the same time show reduced numbers for *bacteroides spp*., *enterococci* and *E.coli* , relative to the control and antibiotic groups (Guo *et al*., 2004b). With this, a balanced microorganism ecosystem would exist within the digestive tract, providing optimal conditions for the digestive tract to further fight any pathogenic entrance, and therefore also decrease the chance of any disease in the gut (Wenk, 2003). Disease associated with the digestive tract in chickens would be necrotic enteritis, which is caused by the toxin of the organism *Clostridium perfringens* type C, and causes acute diarrhoea, ending in death if not treated. Coccidiosis is also an intestinal disease, caused by coccidian protozoa in the digestive tract, causing diarrhoea in some animals.

By decreasing the number of pathogenic microorganisms in the intestine there is a decrease in immune stress on the host (Hashemi & Davoodi, 2011). The decrease in pathogenic microorganisms in the digestive tract leads to an increased potential for non-pathogenic microorganisms, therefore improving digestive capacity, and also improve nutrient availability for absorption through the digestive tract, which would then cause an increase in growth (Wenk, 2000; Windisch *et al*., 2008; Hashemi & Davoodi, 2010; Hashemi & Davoodi, 2011).

2.2.3 Oregano

Oregano herb or *Origanum vulgare* L. as it is known scientifically, is from the *Labiatae* family and of the *Origanum* genus, originating from Europe, the Mediterranean and Asia. This is a culinary herb, used to add flavour to foods, which became popular after World War 2 soldiers returned home from Italy with the herb and added it to their pizzas, referring to it as the “pizza herb” (Bertelli *et al*., 2003). It features prominently in Italian and Greek cuisine. Historically, Hippocrates, known as the father of modern medicine, believed that Oregano had antiseptic properties and therefore used it as such, as well as in the treatment of stomach and respiratory ailments.

Traditionally, oregano has been used in the old folk medicine as an antimicrobial (Dorman & Deans, 2000), anticoccidial (Giannenas *et al*., 2003), antifungal (Pina-Vas *et al*., 2004), antispazmolytic (Meister *et al*., 1999), and lastly as an antioxidant (Zheng & Wang, 2001).

This herbaceous plant yields essential oils, which are held in their leaves and stems, located in the glandular trichomes. These trichomes consist of volatile terpenoids, alcohols, esters and various aromatic substances (Baranska *et al*., 2005). The main bio-active components of oregano essential oil are thymol and carvacrol (Bertelli *et al*., 2003; Baranska *et al*., 2005), which are largely responsible for the antimicrobial, antifungal and antioxidant activities of the herb.

Giannenas *et al*. (2003) found that oregano essential oil (at a rate of 300 mg/kg) had an anticoccidial effect when added to the diet of broilers infected with *Eimeria tanella.* There are further reports of the oregano essential oil having inhibiting effect on *Escherichia coli*. *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes* (Aligiannis *et al*., 2001; Friedman *et al*., 2002).

In vitro and *in vivo* studies are sometimes contradicting to one another, with the same results not always being found when the herbal supplements are being tested on the livestock. In a study by Kırkpınar *et al*. (2011), the essential oils of oregano and garlic were tested on broiler chickens. The supplementation of oregano essential oil to the diet reportedly decreased the body weight of the broilers at 42 days age, whereas the garlic essential oil and the combination of garlic and oregano essential oil were not significantly different from the control group (Kırkpınar *et al*., 2011). In a separate study by Botsoglou *et al*. (2002), it was reported that broilers fed a diet supplemented with oregano essential oil showed no positive effect on the growth of the broilers at 50 or 100 mg/kg of feed. In the study by Kırkpınar *et al*. (2011), oregano and garlic essential oil supplemented diets had a decreased effect on feed intake, when compared to the control. This was also reported by Halle *et al*. (2004), when oregano leaves (at a rate of 2/4/10/20 g/kg) or oregano essential oils (at a rate of 0.1/0.2/0.5/1 g/kg) was added to the diet and fed to male broilers, causing a significant decrease in feed intake.

However in a study by Hernandez *et al*. (2004), where broilers were fed a diet supplemented with essential oils (at 200 mg/kg) containing oregano essential oil as well as essential oils from pepper, cinnamon and a labiate extract (at 5000 mg/kg), it was reported that there was no effect of the essential oils on feed intake, nor the feed:gain ratio. However, in a study by Ertas *et al*. (2005), feed intake and live weight gain were improved by the inclusion of an essential oil mixture into the diet of broilers, included at 200 mg/kg, which consisted of essential oils from oregano, clove and anise.

2.3 Gut health

The digestive tract is the major site for digestion and absorption in the body, along with being the first site of protection against exogenous pathogens, making it the body's largest immunological organ (Choct, 2009). By interacting with the nutrients supplied by the diet, digestive tract microorganisms have a significant effect on the host health, nutrition and growth

performance (Hashemi & Davoodi, 2011). Phytogenic feed additives improve the gut health of the animal, by controlling and eliminating pathogenic microorganisms in the digestive tract.

2.3.1 Villi height

The small intestine is the major site for digestion and absorption of nutrients, and is divided into three parts, namely the duodenum, jejunum and ileum. The epithelial layer lining the small intestine has finger like protrusions, protruding into the lumen of the intestine (Shen, 2009; Choct, 2009). These protrusions are called villi and play an important role in the absorption of nutrients in the small intestine. Villi length is longest in the duodenum, and decreases in length towards the ileum (Choct, 2009). An increase in villi length results in an increase in surface area, and therefore more area for absorption of nutrients to take place (Buddle & Bolton, 1992; Parsaie *et al*., 2007; Saeid *et al*., 2013). Alongside the villi are indentations into the *muscularis mucosae*, known as crypts (Choct, 2009), that are responsible for the production of enterocytes and goblet cells (Shen, 2009). As the enterocytes migrate up the villi, where they are eventually sloughed into the lumen, they undergo a functional change, from having a secretory function in the crypts, to an absorptive function as they travel up the villi (Buddle & Bolton, 1992; Uni *et al*., 2000).

The villi height and crypt depth plays an important role in the digestion and absorption of feed in the small intestine, as an increase in crypt depth and a decrease in villi height can lead to increase secretions into the gastrointestinal tract, resulting in diarrhoea, decrease in disease resistance and a decreased animal performance (Nabuurs *et al*., 1993; Parsaie *et al*., 2007; Catalá-Gregori *et al*., 2008). Deep crypts are a sign of a high turnover of cells along the villi, and a high demand on the crypts to produce new cells for villi growth (Xu *et al*., 2003). Enterocytes are damaged by pathogen bacteria in the digestive tract, which leads to an increase in crypt depth (Parsaie *et al*., 2007). A high demand for tissue turnover results in an increase in the energy requirements for maintenance of the digestive tract (Choct, 2009). The longer the villi, the larger the surface area there is available for absorption of nutrients (Shen, 2009; Saeid *et al*., 2013). This is due to the increase in number of enterocytes along the villi in the absorptive phase (Buddle & Bolton, 1992; Choct, 2009). Shallower crypts are associated with a lower tissue turnover, and therefore less demand for new tissue. This also results in less enterocytes in the secretory stage, therefore less secretions, and more villi enterocytes along the longer villi with absorptive functions, resulting in better nutrient absorption (Nabuurs *et al*., 1993; Saeid *et al*., 2013). Therefore, the villi to crypt ratio of the small intestine, plays an important role in the absorptive ability (Buddle & Bolton, 1992), and digestive capacity of the small intestine (Saeid *et al*., 2013).

Along with enterocyte cells, crypts also produce goblet cells (Shen, 2009), which are responsible for producing mucin glycoproteins, which is the main component of the mucus layer lining the entire gastrointestinal tract (Smirnov *et al*., 2006). This mucus layer plays an important role in the absorption of nutrients and protecting the gastrointestinal tract from pathogens (Smirnov *et al*., 2006). Continuous proliferation of the epithelium cells of the digestive tract will lead to a reduction in the age and maturity of the goblet cells. A reduced age of the goblet cells is thought to alter the quality of the mucins produced by the goblet cells, which is then thought to have a negative effect on the absorption of nutrients (Choct, 2009).

A study was completed on chickens fed diets supplemented with either probiotics or a phytogenic blend. The phytogenic blend was a blend of essential oils originating from oregano, anise, citrus and fructo-oligosacharides. Chickens fed the diet supplemented with probiotics reportedly had increased villi height and surface area of the jejunum. The phytogenic supplemented group of chickens showed no signs of an increased villi height or surface area, and was reported to have a reduced villi:crypt ratio. However, there was an increase in animal performance, and it is therefore thought that this increase is not related to gut morphology, but other mechanisms (Perić *et al*., 2010).

2.3.2 Microorganism environment

In monogastric animals the digestive tract contains a high density and selection of microorganisms (Bauer *et al*., 2006), with bacteria being the predominant species (Mackie *et al*., 1999). These microorganisms play an important role in influencing the health and performance of the host, by influencing physiological, nutritional, developmental and immunological processes in the host (Richards *et al*., 2005; Bauer *et al*., 2006). Bacteria that live in a symbiotic relationship with the host's digestive tract have been proven to have an important role in the healthy development of the host's organ, tissue, and immune system (Snel *et al*., 2002), as well as supplying the host with a variety of nutritional compounds (Richards *et al*., 2005). The population of beneficial microorganisms in the gastrointestinal tract also benefits the host by preventing the pathogenic species from colonizing in the gastrointestinal tract. This is attained by a process known as competitive exclusion (Mackie *et al*., 1999; Snel *et al*., 2002), as well as producing volatile fatty acids which reduce the pH of the digestive tract (Ferket, 2004). Pathogenic coliform bacteria and *Clostridia* are important pathogenic microorganisms in production animals, responsible for enteric diseases, and do not survive well in a low pH environment (Ferket, 2004), as produced by the volatile fatty acids the beneficial microorganisms produce. Beneficial bacteria in the digestive tract are those from the lactic acid producing genera's, *Lactobacillus* and *Bifidobacteria* (Snel *et al*., 2002). By having a higher population of favourable microorganisms in the digestive tract, it causes a decreased population

of pathogenic bacteria, which in turn leads to greater feed utilization and digestibility, and therefore increased performance of the animal (Samarasinghe *et al*., 2003).

Older animals with more developed gut microorganisms, are less susceptible to the colonization of enteric pathogens, than that of younger animals with a less diverse and established gut (Snel *et al*., 2002; Ferket, 2004). It is thought that the way in which the resident microorganisms suppress pathogens in the digestive tract is through competition for nutrients, competition for space to attach to the mucosal layer, stimulating intestinal motility, production of volatile fatty acids, production of antimicrobial substrates and stimulating the immune system (Van der Wielen *et al*., 2000; Snel *et al*., 2002).

The microbiota, which consists of the healthy, beneficial microorganisms in the digestive tract, has the ability of stimulating the hosts intestinal defence system. This intestinal defence system involves the mucus layer, the epithelial monolayer, as well as the *lamina propria* (Snel *et al*., 2002). The *lamina propia* is a layer under the epithelium known as immune cells, and contains antibodies, cytotoxic and fighter T cells, as well as phagocytic cells (Richards *et al*., 2005). They are responsible for removing pathogenic bacteria, as well as their toxins, and also the growth or abnormal attachment of healthy microbiota. The mucus layer, produced by goblet cells in the digestive tract, is the first barrier for enteric infection (Ferket, 2004), and is responsible for keeping the pathogenic and non-pathogenic microbes away from the animal's tissue (Richards *et al*., 2005). By serving as an alternative binding site for the pathogens, the glycoproteins of the mucins prevent pathogenic colonization on the enterocyte cells of the villi (Ferket, 2004). If the microbes have penetrated through the mucus layer, the epithelium is the next layer to prevent entry into the animal's tissue. After which it is the immune cells that will then attack the pathogenic microbes (Richards *et al*., 2005).

Beneficial microorganisms in the digestive tract can also have negative effects on the host animal performance. Intestinal microorganisms compete with the host animal for nutrients, produce toxic amino acid catabolites, cause a high turnover of goblet and enterocyte cells, decrease fat digestibility in the small intestine, as well as result in an immune cost to the host animal (Richards *et al*., 2005). This then has a negative effect on the animal's performance and health. The regeneration of epithelial cells requires metabolic energy from the host animal, which then reduces the energy available for muscle and developmental growth (Hashemi & Davoodi, 2011). Because the microbiota signals an immune response, this sends a signal for the body to produce IgA's, which is made up of proteins. This causes metabolic protein to be used for immune response instead of animal growth (Richards *et al*., 2005).

Fat digestion and absorption plays an important role in the animal's performance for production, and takes place in the small intestine with the help of bile, which is secreted into the digestive

tract of the monogastric animal, for the digestion and absorption of fats and fat-soluble vitamins (Bauer *et al*., 2005). The microorganisms in the small intestine jeopardize the fat digestion by disrupting the conjugated double bonds in the bile acid (Hylemon, 1985). Of the bacteria species, the *Lactobacillus* is the main bacteria species responsible for breaking down the bile acids and salts entering the small intestine (Baron & Hylemon, 1997), which results in the production of toxic products that causes a decrease in animal growth, as well as decreased lipid absorption (Eyssen, 1973).

In-feed antibiotics improve performance by reducing the total microorganism load in the digestive tract, which in-turn results in a decrease in energy output for gut maintenance (Ferket, 2004). This extra metabolic energy is then used for growth, resulting in a better performance (Hashemi & Davoodi, 2011). Alternatives to in-feed antibiotics mostly act by manipulating the microorganism ecosystem of the digestive tract to containing more beneficial microorganisms, such as *Lactobacillus* and *Bifidobacteria* (Snel *et al*., 2002), and less potentially harmful microorganisms, such as *Bacteroides* spp. and *E. coli* (Ferket, 2004). Plant-extract based feed additives show promising results as an alternative for in-feed antibiotics, as they have antimicrobial properties. Especially oregano, with the active ingredient carvacrol, has antimicrobial properties (Akgül & Kivanc, 1988), and results in a reduced microbial count, by suppressing bacteria growth, similar to that of antibiotic growth promoters (Ferket, 2004). In a study with mushroom and plant polysaccharides, more specifically *Lentinus edodes* extract, *Tremella fuciformis* extract, and *Astragalus membranaceus Radix* extract, no suppression of bacterial growth was seen (Guo *et al*., 2004b), but rather an increase in the number of potentially beneficial bacteria, and a reduction in the number of potentially harmful bacteria.

Previously exogenous enzymes were added to poultry feed with antibiotic growth promoters, to improve digestibility of the wheat, barley or rye based feed (Ferket, 2004). The combination of feeding antibiotic growth promoters together with exogenous enzymes produces better animal performance results than using the supplements separately (Bedford, 2000). Due to the synergistic effect of antibiotic growth promoters and exogenous enzymes on animal performance, and the similarity between plant-extract based product and antibiotic growth promoter properties, the combination of plant extract based products and exogenous enzymes' effect of animal performance should be researched as a solution to the banning of antibiotic growth promoters, for possible synergistic effects on animal performance.

2.3.3 Viscosity

The viscosity of a substance is described as its resistance to flow, with a substance having a high viscosity being a substance that is more resistant to flow. The viscosity of the digesta is increased in the animal when fed a diet high in non-starch polysaccharides (NSP), which results in the decrease of apparent nutrient digestibility and therefore a decrease in nutrient absorption,

as well as a change in the microorganism ecosystem in the gut (Bedford, 2002). The changes in viscosity and also nutrient absorption could lead to changes in chime mobility and gut development (Svihus, 2006). The mixing of nutrients with pancreatic digestive enzymes and bile acid may also be limited by the increase in viscosity of the digesta (Edwards *et al*., 1988). The increase in viscosity also results in the restriction of the amount of nutrients that get to move towards the digestive tract wall for digestion and absorption, therefore limiting the efficiency of both digestion and absorption (Fengler & Marquardt, 1988).

The feed intake of the chickens in a study by Bedford & Classen (1993) was reported to increase as the viscosity of the digesta in the small intestine increased. The increase in intake therefore results in an increase in transit rate in the intestine. It is proposed that this increase in feed intake is to counter balance the decrease in nutrient absorption due to the increase in viscosity of the small intestine (Bedford & Classen, 1993). Exogenous enzymes are added to the diet with a high concentration of NSP's, to avoid high viscosity in the digesta (Bedford, 2006).

Sufficient mixing of digesta is important for emulsification of fats, as well as fat-soluble vitamins which move with the larger, less digestible fat micelles (Bedford, 2006). Fat digestion is also influenced by bacterial population in the digestive tract, as an increase in the viscosity of the digesta in the small intestine leads to an increase in bacterial growth in the small intestine (Hübener *et al*., 2002). Bacteria are known for producing bile acid hydrolase in order to protect themselves from the bile, which in turn results in a decrease in bile, and therefore a decrease in fat micelle formation, and therefore a negative effect on fat digestion (Hübener *et al*., 2002).

CRINA® is a commercial mix of essential oils that contains the active ingredient thymol, among others, which is found in many herbs. When CRINA® was fed to broilers it reportedly had favourable effects on the viscosity of the digesta, as well as the intestinal concentrations of *clostridium perfringens* (Williams & Losa, 2001). Lee *et al*. (2004b) reported that cinnamaldehyde decreased the viscosity of the digesta of the chicken, as a result of reversing the negative effects of the addition of a non-fermentable viscous fibre, carboxymethyl cellulose, which causes an increase in digesta viscosity. However in the same study, thymol did not improve the viscosity of the digesta, which is contradicting to the previous study by Williams & Losa (2001) using CRINA®. In a separate study by Lee *et al*. (2004a), the viscosity of the digesta was not decreased by the addition of cinnamaldehyde to the diet of chickens when fed rye simultaneously, however the anti-nutritional effects of rye were reversed by the active ingredient.

2.3.4 Knock on effect

A high concentration of microorganisms present within the digestive tract of the host animal results in a high turn-over of enterocyte and goblet cells in the digestive tract epithelium

(Richards *et al*., 2005). This results in shorter villi, which decreases the surface area for absorption, and deeper crypts to keep up with the production of enterocytes along the villi (Xu *et al*., 2003). As a result, there are more enterocytes along the crypt compared to along the villi, as the villi is shorter, which creates more secretions in the lumen of the small intestine and less absorption (Nabuurs *et al*., 1993b; Parsaie *et al*., 2007). As enterocytes along the crypt are secreting type enterocyte cells and once they travel up the villi their function changes to absorptive type enterocyte cells (Buddle & Bolton, 1992b). This imbalance of absorption and secretion in the small intestine, results in less absorption type enterocytes, therefore a decrease in nutrient uptake in the small intestine (Saeid *et al*., 2013a).

Secondly, a high turnover of enterocyte cells in the lumen of the small intestine results in an increase in energy demand of the digestive tract (Choct, 2009). A fast growing, healthy broiler contributes 25% of its metabolic energy to maintenance of the digestive tract (Ferket, 2004), having a high turnover in enterocytes will result in a higher energy expenditure on the digestive tract. Energy that could rather been used for growth, therefore improving production (Hashemi & Davoodi, 2011).

It has been shown that the microbiota of the digestive tract also causes a high rate of turnover of goblet cells and enterocytes in the epithelium of the digestive tract (Imondi & Bird, 1966), which in turn results in the enterocytes and goblet cells to be continuously renewed, and therefore consist mostly of an immature population of cells (Van der Klis & Jansman, 2002). The Goblet cells are responsible for the secretion of mucin glycoproteins, which is the main component of the mucus layer lining the entire digestive tract (Smirnov *et al*., 2006). This mucus layer plays an important role in protecting the gastrointestinal tract from pathogens, as well as aiding the absorption of nutrients (Smirnov *et al*., 2006). An immature population of goblet cells would cause a lower rate of mucus production, and may cause a decrease in absorption efficiency, leading to an overall decrease in performance (Choct, 2009). Continuously renewing the epithelial cells causes a decrease in its ability to act as an efficient barrier, as the tight junctions will be loose due to the cells being immature (Van der Klis & Jansman, 2002).

The microorganisms in the digestive tract are believed to increase the thickness of the digestive tract wall (Saeid *et al*., 2013) which decreases the efficiency of nutrient absorption and utilization of nutrients, due to increasing the distance the nutrients have to travel before reaching the blood system (Visek, 1978).

A decreased concentration of microorganisms in the digestive tract could therefore lead to less sloughing of the enterocytes and goblet cells, leading to longer villi, which increases the surface area for absorption. Due to less sloughing of the enterocytes and goblet cells along the villi, the crypts would be smaller, which would result in less energy for maintenance of the digestive tract (Saeid *et al*., 2013). A lower concentration of microorganism in the digestive tract would lead to

a thinner intestinal wall, which would improve nutrient absorption and utilization (Visek, 1978). Collectively, this would result in an increase in nutrient absorption, a decrease in secretions and an increase in performance (Saeid *et al*., 2013).

2.4 Organ status

2.4.1 Gizzard erosion

Gizzard erosion involves the presence of lesions or extensive sloughing of the lining of the gizzard, as well as a thickening and loosening of this lining (Itakura *et al*., 1982; Fossum *et al*., 1988). This leads to an increase in mortality and a decrease in growth rate (Fossum *et al*., 1988; Tišljar *et al*., 2002). A decrease in feed intake and an increase in proventricular size has also been reported (Shifrine *et al*., 1960).

The exact cause of gizzard erosion is not entirely certain. It was originally thought that gizzard erosion was a hereditary factor, as only two stocks of White Leghorn breeders reported cases of gizzard erosion (Fossum *et al*., 1988). It is currently speculated that it is caused by toxins found in nutrients, in the form of biogenic amines (Dhawale, 2005). Microbial decarboxylation of amino acids produces biogenic amines (Barnes *et al*., 2001; Dhawale, 2005) that include histamine, gizzerosine, cadaverine, serotonine, putrescine, spermine and spermidine (Barnes *et al*., 2001). These biogenic amines are present in many protein sources such as meat and bone meal, poultry meals, fish meal and soya bean meal, as well as vitamin premixes and fats, and are considered toxic to animals (Barnes *et al*., 2001; Dhawale, 2005). A decreased feed efficiency (Brugh & Wilson, 1986; Stuart *et al*., 1986), and enlargement of the proventriculus as a result of dietary biogenic amines has been reported (Shifrine *et al*., 1960). In a study by Itakura *et al*. (1982), proventriculus secretions became hyperactive in response to fishmeal and histamine supplemented diets. It was also reported that the proventriculus had an inflammatory response in the form of mucus lining the inside of the proventriculus and swelling of the epithelial cells (Itakura *et al*., 1982). The biogenic amine, gizzerosine, is formed when fishmeal is overheated, and caesine and histadine interacts (Dhawale, 2005). Gizzerosine has been reported to have a stimulating effect on the proventricular glands, causing an excessive secretion of hydrochloric acid, as well as on the secretion of gastric acids, resulting in a decrease of pH in the gizzard. Damage to the gizzard, as a result of gizzerosine and the high acidic level that results there from, includes ulcers and erosion of the lining (Tišljar *et al*., 2002; Dhawale, 2005).

Biogenic amines become most prominent in raw animal feed products that are stored incorrectly or contaminated (Barnes *et al*., 2001). The formation of the biogenic amine, histamine, is a result of the activity of microbial histidine carboxylase (Dhawale, 2005; Macan *et al*., 2006), as well as the microbial and endogenous proteolytic enzymes and microbial diamine oxidases (Macan *et al*., 2006). Storage conditions of the feedstuff, such as temperature, pH and oxygen

levels, influence the activity of histadine carboxylase activity within the feed, as well as the level of microbial contamination and the amino acid content of the feedstuff (Macan *et al*., 2006).

2.4.2 Heart to breast muscle ratio

The slaughter weight of a broiler chicken is 1.8 kg. In the 1940's it took 90 days to reach this slaughter weight, and by 1980's it took roughly 45 days to reach this weight (Gyles, 1989), and currently the broiler chickens reach a weight of 2.5 kg's in under 40 days (Breeders, 2007). Over the years there has been a process of selection for faster growth, as well as an improvement in the broiler feed formulation, resulting in a faster growing broiler chicken. The heavier bird weight in a shorter period of time is due to the increase in growth during the first two weeks of growth post hatch, according to Ricklefs & Marks (1985), as any increase during this phase has an exponential effect on the chickens growth throughout its life (Konarzewski *et al*., 2000). With this rapid increase in growth rate of the chicken there has since been an increase in metabolic diseases such as "heart failure syndrome", responsible for up to 10% mortality in the flock (Konarzewski *et al*., 2000).

Konarzewski *et al*. (2000) measured the heart size of two different strains of chickens, the broiler and the layer. The broiler strain grew much faster than the layer strain. However there was no difference in heart muscle weight between the two groups. This indicates that the broiler heart is doing more work, due to the higher metabolic rate required during its faster growth. The metabolic cost to the chicken, especially during it fastest growing stage, has been decreased by selection for a faster growing chicken. This means that the overall metabolic cost will be lower because slaughter weight is reached earlier than that of a chicken that has not been selected for growth rate (Konarzewski *et al*., 2000). Due to the fast growth rate of the broiler breed, and the increased body weight of the chicken, there has been an increase in musculoskeletal and cardiovascular diseases (Julian, 1993). Cardiovascular illnesses are responsible for a large portion of the mortality of a broiler flock, while musculoskeletal disorders aren't responsible for as many deaths, but rather decrease the growth rate of the chicken and cause deformities (Julian, 2005). Two cardiovascular diseases, ascites, also known as pulmonary hypertension syndrome (PHS), and sudden death syndrome are prominent in poultry (Konarzewski *et al*., 2000; Julian, 2005).

The fast growth of the broiler chicken leads to the body struggling to supply enough oxygen to all the fast growing tissues and organs. This causes the heart rate to increase and an increase in blood pressure in the arteries of the lungs, known as pulmonary hypertension. A prolonged decrease in oxygen in the blood causes the blood to increase its oxygen carrying capacity by increasing the number of erythrocytes, which then results in an increase in the viscosity of the blood making it more work for the heart to pump blood out. The right ventricle becomes enlarged, resulting in the right atrio-ventricular valve malfunctioning and right ventricle failure

(Julian, 1998; Julian, 2005; Silversides *et al*., 1997). The increase in portal pressure causes the seepage of fluid into the abdominal area of the chicken (Julian, 1998). Ascites forms when the membranes in the abdomen can no longer absorb more fluid, resulting in excess fluid in the pericardial sac and abdomen area due to seepage (Silversides *et al*., 1997; Julian, 2005). The excess liquid in the abdomen results in pressure on the chickens air sacs, leading to respiratory failure and, ultimately, death (Julian *et al*., 1989).

The occurrence of ascites can be increased by factors that would cause an increase in heat production, increase in the metabolic rate of the chicken, or the oxygen requirement (Julian, 1987; Julian *et al*., 1992), as well as factors that would cause an increase in resistance to blood flow (Julian, 1987; Julian, 1993). The red blood cells can become more rigid due to increased sodium concentration in the blood, leading to increased difficulty for the heart to pump the blood, increasing the chance of PH (Mirsalimi & Julian 1991).

In a study by Julian (1989), it was shown that the lung percentage relative to body mass of a broiler is less than that of the leghorn, which is a layer breed, showing that the broiler could have an increased risk of reaching hypoxia, than the leghorn. The broiler breeds susceptibility to PHS or ascites may be heightened as they are bred to carry heavier breast muscle, resulting in an increased pressure on the air sacs from the abdominal organs, and a relatively small lung volume capacity (Julian, 1998). This pressure on the air sacs leads to death due to respiratory failure (Julian *et al*., 1989).

Sudden death syndrome (SDS) is the next common metabolic disease that threatens the poultry industry (Ononiwu *et al*., 1979). Also known as “flip-over”, “acute death syndrome”, “lung oedema”, or “heart attack” (Ononiwu *et al*., 1979), this syndrome, which effects from 60-80% male chickens (Ononiwu *et al*., 1979; Bowes & Julian, 1988), results in the chicken often dying on its back (Moghadam *et al*., 2005), with a sudden burst of wing beating convulsions (Julian, 2005). There is a positive correlation reported between the flock body weight and the incidence of SDS of the flock (Gardiner *et al*., 1988).

It appears to be a ventricular fibrillation that is responsible for the death of the chickens with sudden death syndrome (Moghadam *et al*., 2005; Olkowski *et al*., 2008), as there are no diagnostic lesions that separates a healthy broiler chicken from a chicken that has been humanely euthanized, at autopsy level (Bowes & Julian, 1988; Julian, 2005). Assessment of the chickens that have died from SDS shows that chickens were generally healthy, showing no signs of illness prior to death, which is evident in the presence of a large, normal bursa (Julian, 2005), as well as holding a good size and body weight relative to its age (Bowes & Julian, 1988). Their gall bladders were small or empty, a full intestine was reported, and the gizzard or crop contained recently digested feed, this all showing that the chicken did not have any signs of morbidity prior to death (Bowes & Julian, 1988). The heart had dilated atria, which were filled

with blood, while the ventricles were contracted (Bowes & Julian, 1988; Julian, 2005). From the study by Bowes (1988), which analysed the weight of the organs of broilers that had died from SDS, it was concluded that the weight of the organs could not be a means for diagnosing SDS. In a study by Ononiwu (1979), testing the effect the lighting programme had on the occurrence of SDS, it was reported that continuous lighting produced a higher occurrence of SDS amongst the broilers, than the intermitted lighting programme.

2.4.3 Spleen and bursa weight

The lymphoid system of the chicken differs to that of mammals, in which the latter contain lymph nodes and a more complex lymphoid system (Casteleyn *et al*., 2010), whilst the chicken lacks lymph nodes (Jeurissen *et al*., 1988). The bursa of Fabricius is the primary lymphoid organ of the chicken (Jeurissen *et al*., 1988; Casteleyn *et al*., 2010), and is responsible for B-lymphopoiesis (Jeurissen *et al*., 1988). It is situated on the dorsal wall of the cloaca, connected by a narrow bursal duct (Ekino *et al*., 1985). The immune system of the chicken is also made up of lymphoid tissue associated with the mucosae of the eyes, the respiratory and genital tracts, as well as the thymus, spleen and bone marrow (Jeurissen *et al*., 1988). The bursa of Fabricius and thymus is the site for production of immunologically competent effector cells which then get sent to the spleen, and other secondary lymphoid organs, where immune responses can occur (Jeurissen *et al*., 1988). At the embryonic level, the B-lymphocytes which produces' antibodies, are produced by the liver, yolk sac, and bone marrow, and from there are sent to the bursa of Fabricius (Casteleyn *et al*., 2010). The removal of the bursa of Fabricius leads to the decrease in resistance against infections, showing its relevance in the animal's immunity (Scott, 2004).

The spleen in the chicken is a small soft organ, situated close to the gizzard, and of similar colour to the liver, and is the site where red and white blood cells are formed (John, 1994).

Organ weights give a good indication of the health status of the chicken. In a stressed chicken, in the form of heat stress or increase in stocking densities, it has been reported that the weights of the lymphoid organs, decrease (Pope, 1991; Heckert *et al*., 2002). The bursa of Fabricius weight is reported to be the most accurate representation of the chicken immunity (Heckert *et al*., 2002). Stress can also cause a negative effect on the chicken's immune system, resulting in a decreased ability to overcome viral and bacterial infections (Heckert *et al*., 2002). In a study by Heckert *et al*. (2002) it was reported that with an increase in rearing density of the chickens, there was a significant decrease in the weight of the bursa, as well as the bursa to body weight ratio. In the same study, there was a decrease in spleen weights in relation to an increase in rearing density. However, this decrease was not significant. The spleen to body weight ratios of the chickens did not differ with the increasing rearing densities. This shows that with increasing stress levels on the birds, there is a decrease in lymphoid organ weights (Heckert *et al*., 2002).

Research has focused recently on the function of the immune system of the chicken, more specifically the gut-associated lymphoid tissue (GALT), due to the fact that in-feed antibiotics used as growth enhancers have been banned, and other methods of achieving maximum growth of the chicken needs to be explored (Casteleyn *et al*., 2010).

2.4.4 Liver-toxins

The liver is responsible for supplying bile to the duodenum, which goes via the gall bladder (Jacob, 2010). The bile plays an important role in the digestion of lipids and also the absorption of the fat-soluble vitamins, A, D, E and K (Jacob, 2010). At the embryonic stage of the chickens' life, the liver is also responsible for producing lymphocytes which then gets moved to the bursa of Fabricius (Jacob, 2010), and is an important part of the immune response of the animal. Over all, the liver has many functions in the body which play an important role in the overall health and particularly the productivity of the chicken. These functions include detoxification, protein metabolism, fat metabolism, carbohydrate metabolism, vitamin metabolism, iron metabolism, and is also the site for erythropoiesis, which is the production of red blood cells (Dutta, 2009). The liver is responsible for the synthesis of haemoglobin (Dutta, 2009). Any toxins entering the body via the feed ingested, or toxins produced in the body are detoxified by the liver (Dutta, 2009). Therefore it is clear that the animal's health and productivity relies heavily on the health of it liver. Farm practices are now including the supplementation of herbal liver tonics, which act to protect the liver from toxins, and also improve liver functioning (Dutta, 2009). This results in better production and enhanced growth. Many plant species have been found to have a positive impact on the functioning of the liver, and therefore improve production. *Phyllanthus niruri, Andrographis paniculata, Eclipta alba, Boerhavia diffusa* and *Picrorhiza kurroa* are a few herbal feed additives that can be used to improve feed utilization and protect the liver from liver disorders, causing an improvement in productivity (Dutta, 2009).

Mycotoxins are the secondary metabolites of fungi, produced mainly in cereal grains and forages. The genera of fungi from which the secondary metabolites are produced from, are mainly *Penicillium, Aspergillus* and *Fusarium* fungi (Yiannikouris & Jouany, 2002; Akande *et al*., 2006). These mycotoxins have been shown to have carcinogenic, taratogenic, immunotoxic, neurotoxic, haemorrhagic, oestrogenic and mutagenic properties, depending on their precise form (Yiannikouris & Jouany, 2002; Akande *et al*., 2006). Feed intake, reproductive rate, immunological defence, animal performance, and growth efficiency of the farm animal is negatively affected by mycotoxins, as well as resulting in liver and kidney damage (Akande *et al*., 2006). However, the mycotoxins are also thought to transfer to the animal products, such as eggs, milk, liver, kidney and the muscle of the animal, resulting in harmful effects on humans (Akande *et al*., 2006).

The toxins released from these fungi are metabolized in the kidneys and liver, as well as by microorganisms in the digestive tract. For this reason, mycotoxins do not have such an effect on ruminants, as they have a large biomass of microorganisms in the rumen to metabolize the mycotoxins (Akande *et al*., 2006). Pier & Richard (1992) found that as a result of aflotoxin poisoning the liver becomes covered in lesions, later resulting in bleeding of the liver. Sever cases of mycotoxicosis can also lead to liver necrosis, resulting in death (Yiannikouris & Jouany, 2002).

Antifungal properties of some herbs, spices and their essential oils may act as a protectant against the fungi and their mycotoxins (Akande *et al*., 2006). Mould growth was inhibited by mustard, green garlic and cinnamon bark. However, the production of toxins was inhibited by peppers, cloves, thyme, and green tea (Hitokoto *et al*., 1979). The essential oils and raw products of cinnamon and clove were found to prevent mould growth and therefore also inhibited toxin production (Bullerman *et al*., 1977).

2.4.5 Pancreas

The pancreas is a light pink, yellowish coloured organ, situated between the two loops of the duodenum (Koch *et al*., 1973), and plays an important role in the hormonal and digestive systems. It is responsible for supplying the duodenum with digestive enzymes, which it produces, as well as bicarbonate, which balances the highly acidic pH of the stomach caused by the high levels of HCl from the proventriculus in chickens. Protein digestion is accomplished mainly due to the work of the digestive enzymes the pancreas produces (Jacob, 2010).

The inclusion of an essential oil mixture, including oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil, and citrus peel oil, into the diet of broiler chickens in a study by Cabuk *et al*. (2006) did not affect the weight of the internal organs and showed no difference in the relative weight of the pancreas between treatments. In a study using essential oil extracts from oregano, cinnamon and pepper, as well as a labiate extract from sage also resulted in no significant difference in organ weights between treatments (Hernandez *et al*., 2004). Kırkpınar *et al*. (2011) also confirmed no difference in pancreas weight between treatments, when garlic and oregano essential oils were added to the diets of broilers.

In a study by Jang *et al*. (2007), the commercial product, CRINA®, which is a mixture of essential oils, and contains the active ingredient thymol, among others, was added to the diet of broiler chickens. This study confirmed the reports of no difference in organ weights between treatments. However the pancreas was reported to have an increased activity of pancreatic α-amylase as well as for trypsin, in birds fed the diet supplemented with 50 mg CRINA®/kg diet, when compared to the control and antibiotic supplemented group. In a previous study by Jang *et al*. (2007), it was also reported that the secretion of digestive enzymes from the pancreas was increased with the supplementation of essential oils, extracted from herbs. This increase in

secretion of pancreatic enzymes could therefore lead to better digestion of nutrients in the intestine.

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Chapter 3

Production parameters of broilers fed a diet supplemented with plant extracts

Abstract

A study was conducted to evaluate the effect of a plant extract based product, Ateli plus®, on Cobb 500 broilers' production parameters. Ateli plus® is an oregano based product that has been shown to improve efficiency and resistance to pathogens, and has antimicrobial properties. A total of 2400 broiler chicks were randomly divided into five different dietary treatment groups. Divided between 30 pens, each treatment had six pens, with 80 chickens in each pen that were raised from day 1 to 33. No dietary treatment differences (P>0.05) were observed for liveability, average daily gain (ADG), average weekly feed intake, average cumulative feed intake, average weekly live weight, average cumulative weight gains, feed conversion ratio (FCR), cumulative FCR and the European production efficiency factor (EPEF). Average weekly live weight gains did not differ between dietary treatments for every week, except for the week from day 14 to 21, which had the broilers supplemented with Ateli plus® at a rate of 2kg/ton for the first week, then 1 kg/ton for the remainder of the time period (Ateli plus® max), having the highest weight gain. Broilers supplemented with anti-biotic Stafac 500 (AGP) had the lowest weight gain for this period. The results for this study have shown that plant extract supplemented diets fed to broilers did not improve or worsen their production parameters.

Keywords: Essential oils, Chicken, Performance, Feed additives

3.1 Introduction

Feed additives are added to animal feed to either improve the quality of the feed, quality of the animal products for human consumption, or to improve the animal's health and performance (Hashemi & Davoodi, 2011). Antibiotics are used in animal feed as growth promoters (Hernandez *et al*., 2004; Alçiçek *et al*., 2004a) and to prevent animal disease (Cabuk *et al*., 2006), but are only supplemented at a rate of 2.5-50 mg/kg feed, depending on the type of antibiotic used (Hashemi & Davoodi, 2011). This use for antibiotics as growth promoters in feed of production animals was first discovered in the 1940's (Castanon, 2007), which lead to an improvement in the feed conversion ratio (Hernandez *et al*., 2004). It is suggested by Bedford (2000) that antibiotics' mode of action is related to improving the gut microorganisms, which could lead to the improvement of growth performance in broilers. This is supported by studies showing no improvement in growth performance of germ-free broilers fed antibiotic supplemented diet (Visek, 1978).

Due to the low concentration of the antibiotic in the animal feed, pathogens are able to build a resistance to the antibiotic, and survive, resulting in an antibiotic resistant pathogen population (Hernandez *et al*., 2004). There is also a risk of antibiotic residues in the meat and animal products of animals supplemented with growth promoting antibiotics (Ciftci *et al*., 2005; Jang *et*

al., 2007). This led to the banning of antibiotics being used as growth promoters in production animals in the EU (Ciftci *et al*., 2005; Jang *et al*., 2007; Hashemi & Davoodi, 2011). In 1986, Sweden banned all antibiotics used in animal diets (Wenk, 2003; Dibner & Richards, 2005), followed by Switzerland in 1999 (Wenk, 2003). By the end of 1998, all but four antibiotics were banned in all European member states. These remaining four antibiotics were then banned by the beginning of 2006 (Franz *et al*., 2010).

This has resulted in the search for alternative feed additives for production animals to provide the same growth promoting effects as the antibiotics provided (Humphrey *et al*., 2002; Zhang *et al*., 2005; Jang *et al*., 2007; Yang *et al*., 2009). The alternative to an in-feed antibiotic must be able to maintain and improve animal health, increase nutrient availability and increase animal performance (Wenk, 2003).

Essential oils and plant extracts have received much attention as alternatives to antibiotic growth promoters (Revington, 2002; Ferket, 2004; Brenes & Roura, 2010; Hashemi & Davoodi, 2011). This is due to the fact that essential oils and plant extracts have been found to have antibacterial, anticoccidial (Giannenas *et al*., 2003; Basmacioglu *et al*., 2004), antimicrobial (Cowan, 1999; Dorman & Deans, 2000), and antioxidant properties (Dorman & Deans, 2000; Botsoglou *et al*., 2002), and are considered a safe feed additive by the Food and Drug Administration (Alçiçek *et al*., 2004a; Jang *et al*., 2007). These extracts are also reported as having an appetite and digestion stimulating effect (Hernandez *et al*., 2004; Zhang *et al*., 2005). Plant extracts and essential oils are a good alternative to in-feed antibiotics, as they have been proven to be less toxic, more natural and produce residue free products for human consumption (Wang *et al*., 1998; Hashemi *et al*., 2008).

The rate at which essential oils are supplemented (Williams & Losa, 2001; Alçiçek *et al*., 2004b), as well as the quantity and quality of the active ingredients within the essential oil and plant extracts (Cross *et al*., 2007) influences the effectiveness of the feed additive. The type of diet to which the essential oil is supplemented also influences the effectiveness of the essential oil on the animals' performance (Williams & Losa, 2001; Giannenas *et al*., 2003). The effectiveness of plants and their extracts is determined largely by which plant parts are used, where they are sourced, the time they were harvested and their compatibility with other ingredients in the feed (Wang *et al*., 1998).

Studies with plant extracts and essential oils on broiler chickens yield conflicting results, with some studies showing improvement in animal performance (Giannenas *et al*., 2003; Alçiçek *et al*., 2004b), and other studies showing no effect on animal performance (Botsoglou *et al*., 2002; Papageorgiou *et al*., 2003; Hernandez *et al*., 2004; Jang *et al*., 2007). Antibiotics are known to only be effective when broilers are raised in suboptimal environmental conditions and act by decreasing the pathogenic microorganisms of the digestive tract, resulting in better nutrient

utilization and therefore an increase in performance (Ferket, 2004). In the study by Giannenas *et al*. (2003), broilers were infected with *Eimeria tenella* and reported that essential oils could have a positive impact on the microorganisms of the digestive tract, which lead to an improvement in performance. The control broiler group of the study by Alçiçek *et al*. (2004b) had a significantly lower performance compared to the essential oil (48 mg/kg) supplemented broilers, therefore giving strong indications that if the environment in which broilers were raised was not optimal, essential oils could have a positive effect. On the other hand, the control groups from the studies by Botsoglou *et al*. (2002), Papageorgiou *et al*. (2003), Hernandez *et al*. (2004) and Jang *et al*. (2007) were all statistically equal to the supplemented groups, showing that birds raised in a near optimal environment, with possibly a low pathogen count, could result in no effect of plant extract supplementation on performance.

In this study the effect of supplementing different levels of Ateli plus® in broiler diets on production parameters i.e. average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), liveability and European production efficiency factor (EPEF) were compared.

3.2 Materials and methods

3.2.1 Birds, housing and management

The research was conducted at the Mariendahl experimental farm of Stellenbosch University, located near Stellenbosch, Western Cape Province, South Africa. A total of 2400 first grade, day old vaccinated, as hatched, Cobb 500 chicks were obtained from a commercial hatchery and transported 50km to the farm where they were housed in a positive pressure and temperature controlled house. The house was divided into 32 pens of which 30 were used. On arrival chicks were checked, counted and placed randomly at a rate of 80 per pen. The design rendered five dietary treatments with six pens per treatment. Feed and water were supplied *ad libitum* and temperature and lighting were managed according to the guidelines of the Cobb International ^{TM} (2008).

Each pen was equipped with two tube feeders as well as a bell drinker, totalling a space of 0.221 m^2. Each pen had a floor space of 4.01 m^2, resulting in a space of 3.67 m^2 left for the chicks. With 80 chicks placed per pen, this resulted in a stocking density of 21.8 chicks /m^2. Chicks were placed on clean sawdust bedding, which was turned three times a week. All methods within this study were practiced according to the ethical requirements that have been approved by Subcommittee B of the University of Stellenbosch.

3.2.2 Treatment diets

Broilers were fed a diet supplemented with a plant extract based product, Ateli plus®, at different rates resulting in five different dietary treatments, as described in Table 3.1. Table 3.2

describes the antibiotic and coccidiostat used in the respective treatment diets. Starter and grower diets contained the anticoccidial Salinocox 12%, and the finisher diet contained the anticoccidial, Avatec. Ateli plus® is an oregano based product, that has been shown to improve efficiency and resistance to pathogens, and has antimicrobial properties associated with the main active ingredient carvacrol.

Table 3.1 Treatment number and description of five experimental treatment diets for broilers comparing different levels of Ateli plus® with a positive and negative control diet

Treatment	Description
1	Positive control with antibiotic Stafac 500 (AGP)
2	Negative control without antibiotic (no AGP)
3	Ateli plus® at a rate of 1 kg/ton (Ateli plus® min)
4	Ateli plus® at a rate of 2 kg/ton week 1 and then 1 kg/ton for the remainder of the period (Ateli plus® max)
5	Positive control and same rate as treatment 4 (AGP and Ateli plus® max)

Table 3.2 Antibiotic and coccidiostat description of the diet fed to broilers

		Starter and Grower		Finisher	
Treatment	Description	Coccidiostat	Antibiotic growth promoter	Coccidiostat	Antibiotic growth promoter
1	AGP	Salinocox 12%	Stafac 500	Avatec	Stafac 500
2	No AGP	Salinocox 12%	None	Avatec	None
3	Ateli plus® min	Salinocox 12%	None	Avatec	None
4	Ateli plus® max	Salinocox 12%	None	Avatec	None
5	AGP and Ateli plus®	Salinocox 12%	Stafac 500	Avatec	Stafac 500

The starter feed was crumbed and fed at a rate of 900 g/chick, from day 1 to ~16 days of age by when all the feed had been consumed. The grower and finisher diets were pelleted using a 2mm die and supplied at a rate of 1200 g/chick, and fed from day ~17 till day ~25 days of age and from day ~26 till day 33 days of age, respectively.

3.2.3 Measurements and sampling

Chick weights were measured at placement (day 0) and weekly thereafter, until slaughter at 33 days of age using an ae ADAM® (Adam Equipment Co Ltd, Kempton Park, Johannesburg) scale. Individual weights were calculated as an average. Weekly feed intake was determined by subtracting the feed remaining at the end of the week period (7 days), from the initial feed supplied to the chicks.

Mortalities and morbidities were recorded twice daily and all the dead chicks were weighed and necropsies conducted. From the data recorded, feed conversion efficiency (FCR), average daily gain (ADG), European production efficiency factor (EPEF) and feed intake, were calculated.

At 33 days of age two chickens per pen were selected for slaughter from around the mean weight per group. Each chicken was stunned using an electrical stunner set at 50-70 volts, with a current of 2 A applied for 5 s. Exsanguinations of the broilers took place within 10 seconds of stunning. Slaughter took place at an abattoir on site.

At slaughter the chickens' live weight and carcass weight was measured, using an ae ADAM® (Adam Equipment Co Ltd, Kempton Park, Johannesburg) scale and a Mettler PC 4400 scale (Mettler –Toledo, Switzerland), respectively.

3.2.4 Statistical analyses

The data obtained from this trial was analysed using PROC GLM of SAS software, version 9.3 of the SAS system for Windows, and was subjected to a one-way ANOVA, where treatment (diet) was the main effect (SAS, 2009). Tests were done on the 95% confidence level, where a P-value less than 0.05 indicates that there is a difference between dietary treatments, and a P-value larger than 0.05 indicates there are no differences between dietary treatments. When a P-value less than 0.05 were reported, Bonferonni post hoc test was performed on the data to determine where the differences were.
EPEF was calculated using the following equation (Perić *et al*., 2009):

Equation 1

$$\text{EPEF} = \frac{\text{Live weight(g) x Liveability (\%)}}{\text{(age(d) x FCR)}} \text{ x } 100$$

Equation 2

$$\text{FCR} = \frac{\text{Cummulative feed intake per chick weight(g) x Liveability (\%)}}{\text{(Average live weight gain per chick)}}$$

Liveability is a representative of the percentage chickens surviving until slaughter.

3.3 Results and discussion

The production parameters measured in this trial included feed intake, weight gain, feed conversion ratio (FCR), mortality and European production efficiency factor (EPEF), which are shown in Table 3.3 to Table 3.10.

From Table 3.3 it is evident that there are no difference between dietary treatments for EPEF, liveability and ADG. As mentioned in Equation 1, EPEF takes into consideration the age, liveability (%), live weight and FCR of the broilers, and the higher the EPEF value, the better the technical performance (ROSS, 2007). All the EPEF values for this trial were higher than 300, which are considered as excellent production efficiency values for a broiler flock (Basson, 2011).

Liveability is a representative of the percentage chickens surviving until slaughter compared to the number of chickens placed on day one. Liveability rate of a commercial flock is approximately 97 %, which is slightly higher than the results in the current study which ranged from 94.4 % for the Ateli plus®min supplemented broilers to 95.5 % for the Ateli plus®max supplemented broilers. The AGP supplemented broilers had a liveability of 97.3 %. Results from the current study, showing no difference between treatments for liveability, agree with that of other researchers (Botsoglou *et al*., 2002; Alçiçek *et al*., 2004b; Zhang *et al*., 2005). Botsoglou *et al*. (2002) fed broilers a diet supplemented with oregano essential oil at 50 or 100 mg/kg, and reported no significant differences for mortality between treatments. Results from a study by Zhang *et al*. (2005) also showed no positive or negative effect on mortality when broilers were fed a diet supplemented with a commercial product, consisting of essential oils from oregano, cinnamon, thyme and capsicum. Alçiçek *et al*. (2004b), supplementing broilers with an essential oil combination, also reported no significant differences between treatment groups for mortality in the broiler flock. Although no significant differences between dietary treatments were reported for liveability for the current trial, there is a visible increase in liveability (%) (Table 3.3) for the broilers fed the AGP treatment (97.3 %), compared to the other dietary treatment groups (94.4 – 95.5 %). A reduced mortality is a known benefit of in-feed antibiotics (Ferket, 2004). Hernandez *et al*. (2004) however found an increase in mortality in broilers fed the antibiotic and control diet groups, when compared to broilers fed the Labiatae or essential oil extract diet groups, showing a positive effect of plant extracts on the flock mortality rate. Jang *et al*. (2007) studied the effects of feeding broilers a diet supplemented with different levels of the commercial blend of essential oils, CRINA®. From this study there were no mortalities from the broilers fed a diet supplemented with CRINA® and one mortality from the antibiotic supplemented group. The flock mortality of the current trial was low (< 6%) for all dietary treatment groups, showing that the flock had minimal pathogenic challenges. In-feed antibiotics are known to only improve flock performance under suboptimal conditions, i.e. when the flock is challenged with pathogens or

under bad management conditions. The low flock mortality (average of 4.5%) in this trial indicates minimal pathogen challenge on the birds, and could explain why the EPEF and ADG values did not differ between dietary treatments.

Table 3.3 Means ± standard deviations (SD) of European production efficiency factor (EPEF), liveability (%) and average daily gain (ADG) from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatments[1]	EPEF[2]	Liveability (%)	ADG[3] (g/day)
1	358.32 ± 19.32	97.3 ± 3.46	61.01 ± 0.79
2	353.46 ± 16.41	95.4 ± 2.48	61.45 ± 1.56
3	345.60 ± 5.35	94.4 ± 1.03	61.44 ± 1.0
4	347.44 ± 8.18	95.5 ± 0.96	61.21 ± 1.04
5	343.95 ± 17.9	94.8 ± 3.26	60.51 ± 1.03
P Value	0.4181	0.3347	0.8169

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter
[2] EPEF = Liveability (%) x live weight (kg) x age (d)/FCR x 100
[3] ADG = Average daily gain

Average cumulative weekly feed intake was calculated as the sum of the weekly intake per pen divided by the number of chickens in the pen during that week (after adjusting for mortalities). Results obtained from the feed intake data is shown in Table 3.4 and Table 3.5, with no differences (P<0.05) for average weekly feed-intake and average cumulative feed intake, respectively. Results from this study agree with Hernandez *et al*. (2004) and Jang *et al*. (2007). Jang *et al*. (2007) showed no significant differences in feed intake in broilers supplemented with a commercial essential oil blend (CRINA®), at either 25 mg/kg or 50 mg/kg, from day 3 to 35. Hernandez *et al*. (2004) fed broilers a diet supplemented with either Labiatae extract (5000 mg/kg) or an essential oil extract (200 mg/kg), both giving the same feed intake levels of that of the antibiotic supplemented and control group of broilers. However, in a study by Alçiçek *et al*. (2004b), feed intake, up until 21 days age, was negatively affected by the addition of an essential oil combination to the diet of broilers. The broiler treatment diets included the basal diet, and the basal diet supplemented with either antibiotic or 24, 48 or 72 mg/kg of an essential oil combination containing six different essential oils. This essential oil combination (EOC) included oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil, as well as citrus peel oil. The broilers supplemented with 72 mg/kg EOC had the least feed intake, while the broilers supplemented with the antibiotic had the highest feed intake. As the concentration of

EOC in the broiler diets decreased, so the feed intake increased in their study. At 42 days age, there was no difference in feed intake between treatments (Alçiçek *et al*., 2004b).

Table 3.4 Average weekly feed intake (g) ± standard deviation (SD) of broilers grown from hatch up to 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 33
1	165.29 ± 0.00	414.03 ± 0.01	771.25 ± 0.02	1028.62 ± 0.05	977.35 ± 0.04
2	159.06 ± 0.01	405.66 ± 0.01	780.95 ± 0.02	1041.95 ± 0.04	974.64 ± 0.04
3	163.72 ± 0.00	406.31 ± 0.01	781.49 ± 0.02	1066.32 ± 0.06	975.71 ± 0.04
4	166.22 ± 0.01	407.17 ± 0.01	790.48 ± 0.02	1047.34 ± 0.03	967.84 ± 0.05
5	162.89 ± 0.00	403.31 ± 0.01	765.53 ± 0.02	1032.6 ± 0.03	962.95 ± 0.03
P Value	0.3101	0.1683	0.2367	0.5908	0.971

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 3.5 Average cumulative feed intake (g) ± standard deviations (SD) of broilers grown from hatch up to 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 33
1	165.29 ± 0.00	579.32 ± 0.01	1350.57 ± 0.02	2379.2 ± 0.06	3356.55 ± 0.08
2	159.06 ± 0.01	564.72 ± 0.01	1345.67 ± 0.03	2387.62 ± 0.06	3362.26 ± 0.09
3	163.72 ± 0.00	570.03 ± 0.01	1351.52 ± 0.02	2417.84 ± 0.07	3393.55 ± 0.11
4	166.22 ± 0.01	573.39 ± 0.01	1363.86 ± 0.02	2411.21 ± 0.04	3379.05 ± 0.09
5	162.89 ± 0.00	566.2 ± 0.01	1331.73 ± 0.02	2364.33 ± 0.04	3327.28 ± 0.06
P Value	0.3101	0.1022	0.1915	0.4534	0.7562

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Average weekly live weights of chickens were determined by weighing all the chickens in the cage and dividing this weight by the number of chickens in the pen. The results obtained from this data are shown in Table 3.6, and show no differences ($P<0.05$) between dietary treatments. These results agree with Jang *et al*. (2007) when supplementing broilers with a blend of essential oils, as well as with Botsoglou *et al*. (2002) when supplementing broilers with oregano essential oils at either 50 or 100 mg/kg. Hernandez *et al*. (2004) fed broilers a diet supplemented with either an essential oil extract (200 mg/kg) or a Labiatae extract (5000 mg/kg), and reported no differences in body weight at 42 days age. However, at 35 days age the broilers supplemented the Labiatae extract was found to be significantly heavier than that of the control group, and at a closer weight to the antibiotic supplemented broilers than the essential oil supplemented broiler group. The broiler body weight at 35 days old, for this study by Hernandez *et al*. (2004), was closer to the recommended broiler abattoir weight (1.8 kg).

Average weekly live weight gains per chicken were calculated as the difference between the previous weeks' average live weight and the current weeks' average live weight of the chickens. As mentioned earlier, live weight was determined by weighing all the chickens in the pen and dividing the weight by the number of chickens in the pen. The results for this data are shown in Table 3.7, which shows no differences ($P>0.05$) between dietary treatments for average live weight gains for the following time periods: Day 0-7, day 7-14; day 21-28 and day 28-33. However, differences ($P<0.05$) between dietary treatments were found for average live weight gain from day 14-21. The Ateli plus® max treatment had significantly higher weight gain than the AGP treatment, with all other dietary treatments being intermediary and statistically equal ($P>0.05$). Although, with no significant difference, this balanced out towards the end of the growth trial, with the AGP treatment ending with the highest average weekly live weight gain, and Ateli plus® max with the lowest average weekly live weight gains (Table 3.7). No differences were found between dietary treatments for ADG for the overall growth period, as shown in Table 3.3. Hernandez *et al*. (2004) fed broilers a diet supplemented with either an essential oil extract (200 mg/kg) or a Labiatae extract (5000 mg/kg), and also reported the same increase in growth rate from day 14 to 21. Hernandez *et al*. (2004) reported that the broilers supplemented with the Labiatae extract grew faster than the broilers supplemented with essential oil extract and the control group, and was statistically similar to the antibiotic supplemented group. As in the current trial, the weight gain from Hernandez *et al*. (2004) study also balanced out over the whole growth period, with no significant differences in overall weight gain.

Average cumulative live weight gain was calculated as the difference between the average live weight on the day of weighing and the average live weight on day 0. Results obtained for this data are shown in Table 3.8. No significant differences were reported between dietary treatments for

average cumulative live weight gain, for the current trial. Jang *et al*. (2007) also reported no difference in weight gain in broilers supplemented with different levels of an essential oil blend. However, Alçiçek *et al*. (2004b), who supplemented broilers with an essential oil combination (EOC) at 24, 48 or 72 mg/kg feed, reported a significant increase in body weight in broilers for all EOC treatment groups at 21 days old, when compared to the antibiotic and control treatment group. At 42 days age, only broilers supplemented with 48 mg/kg EOC showed a significant increase in body weight (Alçiçek *et al*., 2004b). The reason for this weight increase is uncertain as there are many factors that could play a role on the effect of the essential oils on the broilers and the optimal inclusion rate of essential oils is not known.

Table 3.6 Average weekly live weight (g) ± standard deviation (SD) of broilers grown from hatch up to 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment1	Day 0	Day 7	Day 14	Day 21	Day 28	Day 33
1	45.33 ± 0.00	180.76 ± 0.00	471.51 ± 0.01	932.41 ± 0.01	1518.63 ± 0.02	2042.22 ± 0.04
2	44.54 ± 0.00	180.59 ± 0.00	465.44 ± 0.00	941.90 ± 0.02	1532.94 ± 0.04	2049.83 ± 0.06
3	44.42 ± 0.00	181.60 ± 0.00	468.34 ± 0.01	938.09 ± 0.01	1538.63 ± 0.03	2047.21 ± 0.04
4	44.33 ± 0.00	181.97 ± 0.00	464.48 ± 0.00	945.48 ± 0.01	1535.08 ± 0.02	2035.85 ± 0.04
5	45.17 ± 0.00	181.55 ± 0.00	461.69 ± 0.01	930.26 ± 0.03	1514.49 ± 0.03	2018.56 ± 0.03
P Value	0.1373	0.9361	0.3987	0.4794	0.461	0.7055

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 3.7 Average weekly live weight gains (g) ± standard deviations (SD) of broilers grown form hatch up to 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 33
1	135.42 ± 0.00	290.75 ± 0.01	460.89[b] ± 0.01	586.23 ± 0.02	523.59 ± 0.04
2	136.05 ± 0.00	284.85 ± 0.00	476.46[ab] ± 0.02	591.03 ± 0.03	516.89 ± 0.03
3	137.18 ± 0.00	286.74 ± 0.01	469.76[ab] ± 0.01	600.54 ± 0.04	508.58 ± 0.04
4	137.64 ± 0.00	282.51 ± 0.00	481.00[a] ± 0.01	589.6 ± 0.02	500.77 ± 0.03
5	136.39 ± 0.00	280.14 ± 0.01	468.57[ab] ± 0.01	584.23 ± 0.02	504.07 ± 0.02
P Value	0.8208	0.1401	0.0318	0.8072	0.7034

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 3.8 Average cumulative live weight gains (g) ± standard deviations (SD) of broilers grown from hatch up to 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 33
1	135.42 ± 0.00	426.18 ± 0.01	887.07 ± 0.01	1473.3 ± 0.02	1996.89 ± 0.04
2	136.05 ± 0.00	420.9 ± 0.00	897.36 ± 0.02	1488.39 ± 0.04	2005.29 ± 0.05
3	137.18 ± 0.00	423.92 ± 0.01	893.68 ± 0.01	1494.21 ± 0.03	2002.79 ± 0.04
4	137.64 ± 0.00	420.15 ± 0.00	901.15 ± 0.01	1490.74 ± 0.02	1991.52 ± 0.04
5	136.39 ± 0.00	416.53 ± 0.01	885.1 ± 0.03	1469.32 ± 0.03	1973.39 ± 0.03
P Value	0.8208	0.4214	0.4202	0.4194	0.6968

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Feed conversion ratio was determined as the ratio of feed consumed per unit of body weight gain. This was done for each week individually (Table 3.9), as well as cumulatively (Table 3.10). No differences (P>0.05) between dietary treatments were observed for individual FCR or cumulative FCR. Results from this study agree with Jang *et al*. (2007), who supplemented broilers with different levels of an essential oil blend. The essential oil blend was supplemented to broilers at 25 mg/kg and 50 mg/kg, from 3 days old to 35 days. Hernandez *et al*. (2004) also reported no difference between treatments for the FCR for broilers fed a diet supplemented with either essential oil extract or a Labiatae extract, when compared to the antibiotic supplemented broilers and control broiler group. Botsoglou *et al*. (2002) also reported no difference in FCR in broilers fed a diet supplemented with oregano essential oil at a rate of either 50 or 100 mg/kg. However, in a study by Alçiçek *et al*. (2004b), broiler FCR at 21 and 42 days age, was reported to have been improved by the supplementation of a blend of essential oils, at a rate of 48 and 72 mg/kg diet. This essential oil blend included oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seeds oil, and citrus peel oil (Alçiçek *et al*., 2004b).

It is well known that antibiotics used as growth promoters in broiler diets have not resulted in any growth promoting effect on broilers when the broilers were kept under their optimal environment conditions (Coates *et al*., 1963). The lack of improvement in performance for the broilers supplemented with Ateli plus® in the current study could be due to the broilers being kept in optimal conditions, such as being fed highly digestible and nutritious diets, as well as being reared in a well-managed environment.

In a study by Guo *et al*. (2004), broilers were challenged with *Mycoplasma gallisepticum*, and supplemented with antibiotics, or either of two mushroom extracts, or a herbal extract *Astragalus membranaceus Radix* (Astragalus), at 2 g/kg feed. The antibiotic supplemented broilers had a significantly higher body weight gain than the non-supplemented broilers. Overall, the mean body weight gain of the extract supplemented broilers was significantly lower than the antibiotic supplemented broilers, so no growth promoting effect of the extracts can be concluded. However, when evaluating the results, the broilers supplemented with the herb, *Astragalus membranaceus Radix,* had the highest body weight gain of the supplemented broilers, with 21.6 g/day, and was closest to the antibiotic supplemented broiler group, which had a weight gain of 25.6 g/day (Guo *et al*., 2004).

Giannenas *et al*. (2003) completed a study on broiler chickens infected with *E. tenella*, and fed a diet supplemented with either an oregano essential oil at 300 mg/kg, or anticoccidial lasalocid at 75 mg/kg. Results for broilers at 28 and 42 days show that the oregano supplemented, *E. tenella* infected broilers had a higher body weight gain than that of the infected, non-supplemented

broilers, and were statistically the same as the uninfected broiler group. However, the weight gain of the oregano supplemented, *E. tanella* infected broilers was still lower than that of the lasalocid supplemented broiler group (Giannenas *et al*., 2003). Therefore oregano essential oil does have anticoccidial properties, and resulted in an increase in growth in broilers infected with *E. tenella*, however it is not as effective as lasalocid.

Table 3.9 Means ± standard deviations (SD) for the weekly feed conversion ratios (FCR) of broilers grown from hatch up to day 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Day7	Day 14	Day 21	Day 28	Day 33
1	1.22 ± 0.02	1.43 ± 0.03	1.67 ± 0.04	1.75 ± 0.04	1.87 ± 0.08
2	1.17 ± 0.1	1.42 ± 0.03	1.64 ± 0.03	1.76 ± 0.04	1.89 ± 0.05
3	1.19 ± 0.02	1.42 ± 0.02	1.66 ± 0.04	1.78 ± 0.11	1.93 ± 0.15
4	1.21 ± 0.04	1.44 ± 0.03	1.64 ± 0.02	1.78 ± 0.02	1.94 ± 0.12
5	1.2 ± 0.03	1.44 ± 0.03	1.63 ± 0.03	1.77 ± 0.02	1.91 ± 0.06
P value	0.4884	0.4609	0.2239	0.9419	0.7667

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 3.10 Mean cumulative feed conversion ratios (FCR) ± standard deviations (SD) of broilers grown from hatch up to day 33 of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment1	Day 7	Day 14	Day 21	Day 28	Day 33
1	1.22 ± 0.02	1.36 ± 0.02	1.52 ± 0.03	1.61 ± 0.03	1.68 ± 0.03
2	1.17 ± 0.10	1.34 ± 0.03	1.5 ± 0.02	1.60 ± 0.01	1.68 ± 0.01
3	1.19 ± 0.02	1.34 ± 0.01	1.51± 0.03	1.62 ± 0.05	1.69 ± 0.03
4	1.21 ± 0.04	1.36 ± 0.01	1.51 ± 0.01	1.62 ± 0.01	1.70 ± 0.03
5	1.20 ± 0.03	1.36 ± 0.03	1.51 ± 0.03	1.61 ± 0.02	1.69 ± 0.03
P Value	0.4884	0.3415	0.5237	0.9033	0.6593

[a] Means within columns with the same superscript do not differ significantly (P>0.05)
[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

3.4 Conclusion

The current study shows no additional growth promoting effect of a plant extract based product, Ateli plus®, on broiler performance, although there was no decrease in growth performance found between the Ateli plus® supplemented broiler groups and the antibiotic supplemented broiler group. However, the negative control broiler group also had the same growth performance as the antibiotic supplemented broiler group. This could be due to the broilers being kept under their ideal environmental conditions, causing no effect of the antibiotic or Ateli plus® supplementation.

There are many conflicting results when assessing herbal extracts and essential oils as a replacement for in-feed antibiotics, as a growth promoter. Many different factors could play a role in the variation in results such as the form of the extract, the interaction with feed ingredients, and the combination of extracts. Therefore growth promoting effects of herbal extracts cannot be totally excluded, and further research in exact herbal supplement, form and combination should be conducted.

One of the major concerns surrounding using in-feed antibiotics is their effect (residues) on the meat composition and quality of the broilers' meat. The effect that natural herbal extracts and essential oils have on the meat quality and gastrointestinal tract of the broilers is poorly reported on and needs to be researched further.

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Chapter 4

Carcass and meat quality characteristics of broiler chickens fed a diet supplemented with plant extracts

Abstract

This study evaluated the effect of a plant extract based product, Ateli plus®, on Cobb 500 broiler meat and carcass characteristics. A total of 2400 broiler chicks were randomly divided into five different dietary treatment groups, with six repetitions per treatment, and raised from day 1 to 33. No significant differences were seen between dietary treatments for carcass portion percentage, dressing percentage, skin and fat percentage of the breast portion, tibia bone length, tibia bone moisture, ash or fat percentages. Dietary treatment also had no effect on tibia bone Ca and P content. Antibiotic growth promoter (AGP) supplemented broilers had significantly less breast meat, while broilers receiving the Ateli plus® at a rate of 1 kg/ton (Ateli plus® min), and AGP plus Ateli plus® in the treatment had the highest breast meat portion. Breast bone percentage was highest in AGP supplemented broilers, and lowest in the AGP plus Ateli plus® dietary supplemented broilers. AGP plus Ateli plus® dietary supplemented broilers had the highest ultimate pH (pH_u) for breast and thigh muscles, with the negative control having the lowest pH_u. Initial pH (pH_i) in the thigh did not differ significantly. Breast pH_i was lowest in broilers supplemented with Ateli plus® min treatment, and highest in the AGP supplemented broilers. Breast and thigh colour L, a* and b* values differed significantly between dietary treatments, aside from the breast a* value, which did not differ significantly. Tibia bone breaking strength was significantly lower in all Ateli plus® supplemented broilers, when compared to the AGP supplemented broilers. As mortalities due to leg abnormalities are a huge loss in the broiler industry, it is suggested that further research be conducted on the effect of plant extracts on bone strength.*

***Keywords*:** Essential oils, Plant extracts, Chicken, Meat, Carcass, Feed additives

4.1 Introduction

Improving growth rate and carcass quality has been the main focus for broiler breeding in the recent years (Le Bihan-Duval *et al*., 1999). The carcass quality desired by the consumer is a healthier meat, which would involve selection for less abdominal fat. Due to the high demand of specific portions of the broiler and increased demand in processed products, an increase in breast meat yield is selected for (Barton, 1994). The selection for higher breast meat yield and lower abdominal fat content would increase the profitability margin of the broiler carcass, as well as produce a more desired product to the consumer (Le Bihan-Duval *et al*., 1999). Through selection, and the high heritability of the respective traits, it has been possible to produce a carcass with a higher breast meat yield (Le Bihan-Duval *et al*., 1998).

The appearance of meat, more specifically colour, is an important factor affecting the sale of the meat product to the consumer (Swatland, 2004; Huff-Lonergan & Lonergan, 2005), and is largely affected by the meat pH (Huff-Lonergan & Lonergan, 2005). Once the animal has been slaughtered, the metabolism in the body changes from aerobic to anaerobic, converting glycogen to lactic acid, which dissociates into H^+ and lactate in the meat. This in turn decreases the pH of the meat from near neutral to 5.4-5.8 (Huff-Lonergan & Lonergan, 2005). The meat

pH plays an important role in the colour, water holding capacity and tenderness of the meat (Van Laack *et al*., 2000; Huff-Lonergan & Lonergan, 2005), as well as cooking yield (Van Laack *et al*., 2000).

Van Laack *et al*. (2000) reported that paler breast meat had a lower pH value, a higher L* reading value and a lower a* reading value, when compared to normal coloured breast meat. In a separate study it was found that ultimate pH (pH_u) is inversely proportional to the L* value, meaning as the L* value decreases (meat becomes darker) the pH_u increases (Le Bihan-Duval *et al*., 1999). Low pH_u (i.e. paler meat) is associated with a low water holding capacity of the meat, which results in an increase in drip loss and is undesirable to the consumer (Le Bihan-Duval *et al*., 1999). Van Laack *et al*. (2000) also reported an increase in drip loss in broiler meat that had a higher L* colour reading value. An increase in drip loss results in a decrease in the tenderness of the meat, due to moisture loss (Huff-Lonergan & Lonergan, 2005). Lightness of meat appears to be highly heritable, therefore selecting for lower L* values may in turn result in high pH_u values. Therefore, it is proposed, that darker meat is going to have a higher pH_u, and therefore a higher water holding capacity, resulting in more tender meat (Le Bihan-Duval *et al*., 1999).

In previous studies it has been reported that plant extract additives have caused an increase in *post mortem* pH in different meats. Dietary oregano essential oil, supplemented at a rate of 1 ml/kg, to female lambs resulted in an increase in meat pH (Simitzis *et al*., 2008). Likewise, dietary garlic powder, supplemented at a rate of 1 g/kg, has been reported to increase the pH of pork meat, as well as the water binding capacity and result in lower colour (L*, a* and b*) reading values (Chen *et al*., 2008). However, broilers fed a diet supplemented with 3 g/kg Turkish oregano showed no improvement on meat pH. Although, these meat pH measurements were only taken up until 4 hours *post mortem* (Young *et al*., 2003).

Broilers have been selected for fast growth over the last 50 years, which has in-avertedly resulted in skeletal problems in the broiler (Thorp & Waddington, 1997). According to a study in 1990, it was reported that approximately 2 to 6% of all broilers show signs of skeletal problems (Day, 1990). This includes leg abnormalities, resulting in an increase in culling, mortalities, reduced growth and feed efficiency, as well as causing a downgrade in the processing plant. This, in turn results in leg abnormalities being the major single cause for economic loss in the broiler house (Shim *et al*., 2012). Leg abnormalities also reduce the welfare of the animal, as it is subjected to less movement and more pain (Shim *et al*., 2012).

The fast growing chicken has more porous cortical bones, which could lead to bone deformities (Thorp & Waddington, 1997). The selection for a broiler to reach slaughter weight in a shorter period of time has resulted in the increase in bone characteristics, such as the tibia bone length, weight, density, mineral composition and ash content, as well as the breaking strength (Shim *et*

al., 2012). However in relation to the body weight of the fast growing broiler that the skeletal structure has to carry, the tibia and shank bone qualities of the fast growing broilers were in fact of a lessor quality compared to that of the slow growing broilers. This results in a higher risk of bone breakage for the fast growing broilers (Shim *et al*., 2012).

The aim of this experiment was to compare the carcass characteristics of broilers fed a diet supplemented with different levels of Ateli plus®, to broilers fed a diet supplemented with either a commercial antibiotic growth promoter or neither. The aim in feeding an alternative to AGP's, such as plant based products, is to improve carcass weight and over-all production efficiency, however this needs to be accomplished without compromising the carcass characteristics. Therefore meat quality and bone strength tests were conducted.

4.2 Materials and methods

4.2.1 Birds, housing and management

From the experimental trial described in Chapter 3, where 2400 one day old Cobb 500 chicks were placed in a commercial type broiler house at Mariendahl experimental farm, broilers were selected and slaughtered for assessing carcass characteristics. Broilers were fed a diet supplemented with a plant extract based product, Ateli plus®, at different rates resulting in five different dietary treatments, as described in Table 4.1. Table 4.2 describes the antibiotic and coccidiostat used in the respective diets.

The broiler house was divided into 32 pens, of which 30 were used, with six pens allocated per treatment. Each pen was equipped with two tube feeders as well as a bell drinker, totalling a space of 0.221 m^2. Each pen had a floor space of 4.01 m^2, resulting in a space of 3.67 m^2 left for the chicks. With 80 chicks placed per pen, this resulted in a stocking density of 21.8 chicks /m^2.

At 33 days of age two chickens per pen were selected from around the mean weight per group. This results in a repetition of 12 birds per treatment being assessed for carcass characteristics. Each chicken was stunned using an electrical stunner set at 50-70 volts, with a current of 2 A applied for 5 s. Exsanguinations of the broilers took place within 10 seconds of stunning.

Ateli plus® is an oregano based product, that has been shown to improve efficiency and resistance to pathogens, and has antimicrobial properties. The main active ingredient of Ateli plus is carvacrol.

Table 4.1 Treatment number and description of five experimental treatment diets for broilers comparing different levels of Ateli plus® with a positive and negative control diet

Treatment	Description
1	Positive control with anti-biotic (AGP)
2	Negative control without anti-biotic (no AGP)
3	Ateli plus® at a rate of 1kg/ton (Ateli plus® min)
4	Ateli plus® at a rate of 2 kg/ton week 1 and then 1kg/ton for the remainder of the period (Ateli plus® max)
5	Positive control and same rate as treatment 4 (AGP plus Ateli plus® max)

T**able 4.2** Antibiotic and coccidiostat description of the diet fed to broilers

		Starter and Grower		Finisher	
Treatment	**Description**	**Coccidiostat**	**Antibiotic growth promoter**	**Coccidiostat**	**Antibiotic growth promoter**
1	AGP	Salinocox 12%	Stafac 500	Avatec	Stafac 500
2	No AGP	Salinocox 12%	None	Avatec	None
3	Ateli plus® min	Salinocox 12%	None	Avatec	None
4	Ateli plus® max	Salinocox 12%	None	Avatec	None
5	AGP and Ateli plus® max	Salinocox 12%	Stafac 500	Avatec	Stafac 500

4.2.2 Meat quality characteristics

The pH of the thigh and breast muscle of each chicken slaughtered was measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25°C) portable Crison 25 pH-meter (Alella, Barcelona). The initial pH (pH_i) was measured 15 min *post mortem* and the ultimate pH (pH_u) measured 24 hours *post mortem*. For the pH_i reading the electrode was placed in an incision that was made in the centre of the breast muscle, one third from the top, as well as in the centre of the thigh muscle. For the pH_u readings, similar incisions into the respective muscles were done on the opposite side of the carcass. Following the pH_i measurements, the carcasses were hung in cold storage at 4°C for 24 hours where after the remaining measurements were taken.

Once both pH measurements were completed, the carcass weight was recorded, using a Mettler PC 4400 scale (Mettler-Toledo, Switzerland), accurate to 0.01g. The chilled carcass weight was used to determine the dressing percentage, which is calculated as the weight of the chilled carcass relative to the live weight of the chicken, expressed as a percentage.

The carcasses were then portioned into commercial parts, using a portion cutter, first cutting the carcass in half. The thigh and drum stick were removed from the one half of the carcass, by cutting above the thigh towards the acetabulum and behind the pubic bone. Cutting perpendicularly to the joint between the thigh and drumstick, the thigh and drumstick were separated from each other. By cutting the joint between the scapula and the coracoid, the wing was removed. These portions were weighed using a Mettler PC 4400 (Mettler – Toledo, Switzerland) scale. Portion weight was calculated as the percentage of the chilled carcass weight. The breast portions were separated into the meat, bone, skin and fat portions, and weighed. These portions were expressed as a percentage relative to the total breast weight.

The thigh portion had its skin removed, and along with the breast muscle weighed previously, was left to bloom for 15 min (Warris, 2000). A CIELab colour meter (BYK-Gardner GmbH, Gerestried, Germany) was used to measure the meat colour spectrophotometrically. The L* measurements represents the lightness of the meat, a* represents the red-green range, while b* represents the blue-yellow range. Negative a* values represent green colour, while positive a* values would represent redness in the meat. Negative b* values would represent blueness in the meat colour, while positive values would represent yellow colour in the meat. A minimum of three readings were taken for each portion of meat. The average of the colour readings per portion was calculated, and used for statistical analyses.

4.2.3 Bone strength

The broilers that were slaughtered for the assessment of gut and organ health discussed in Chapter 5, were also used for assessment of bone strength and mineral content. One broiler

per pen was slaughtered, giving six repetitions per treatment. At slaughter both their tibia's were removed and frozen.

Upon assessment the tibia bones were defrosted overnight in a 4°C walk-in cooler, and the meat and cartilage removed. The bones were weighed, using a Mettler PC 4400 scale (Mettler –Toledo, Switzerland). The length and width of the tibia bones were recorded using a Vernier Caliper (0.1 mm). Tibia bone strength was measured using the three-point destructive bending test, as done by Fleming *et al*. (1998). This test uses an Instron 3345 (Instron 3345/J8415; Model 2519-107. Capacity: 5000N) materials testing machine, which uses a crosshead probe of a diameter of 18mm to come down at 30 mm/min, on the tibia bone place in the centre of two 14mm retaining bars, set 38 mm apart. The force needed to break the bone was measured in newton's (N), and recorded. The breaking distance from the proximal joint was recorded using a Vernier Caliper.

The tibia bones were further used for proximal analyses to determine dry matter, fat, ash and mineral content of the bone.

4.2.4 Analytical and mathematical methodologies

Analytical methodologies were performed at the Department of Animal Science, Stellenbosch University, Stellenbosch, South Africa. Mineral analyses of the tibia bones were performed at the Institute of Animal Production, Department of Agriculture, Western Cape Government, South Africa.

4.2.4.1 Dry matter determination

Dry matter (DM) of the tibia bone of the broilers was determined according to the Association of Official Analytical Chemists International (AOAC, 2002), Official Method 934.01. All weights measured during the proximal analysis, were done using a Mettler AE 200 scale (Mettler-Toledo, Switzerland).

Bones were defrosted, weighed and placed in a crucible, which was placed in an oven at 100°C for 24 hours. Thereafter, the dry samples were weighed and the DM calculated using Equation 3:

Equation 3

$$\% \text{ Moisture} = \frac{(A+B)-C}{B} \times \frac{100}{1}$$

% Dry Matter = 100 - % Moisture

Where:

A = Weight of empty and dry crucible

B = Weight of air dried test sample

C = Weight of crucible and moisture few test sample

4.2.4.2 Fat determination

The samples remaining from the DM analyses were placed in a glass flask, submerged in diethyl-ether for 48 hours, according to Rama Rao *et al*. (2009). Once 48 hours had passed, bones were removed from the diethyl-ether and placed in an oven set at 100°C for 24 hours, to dry. The dry, fat free bone weight was recorded, and the fat percentage was calculated using Equation 4.

Equation 4

$$\% \text{ Fat} = \frac{A-B}{A} x \frac{100}{1}$$

Where:

A = Weight of moisture free bone

B = Weight of moisture and fat free bone

4.2.4.3 Ash determination

The bone samples remaining from the moisture and fat determination analyses were used for the ash determination. The bones were placed into individual crucibles and placed into a 600°C incinerator oven for 24 hours, allowed to cool, and their weights recorded (Boling-Frankenbach *et al*., 2001; Hall *et al*., 2003). Ash content was calculated using Equation 5.

Equation 5

$$\% \text{ Ash} = \frac{D-A}{B} \times 100$$

% Organic material = 100 - % Ash

Where:

A = dry crucible mass

B = weight of moisture and fat free bone

D = weight of bone sample and crucible

4.2.4.4 Mineral analysis

After the ash process, the tibia bone residues were ground down to a powder form, from which Ca and P composition was determined. The ground tibia bone samples had 5 ml of 6 M hydrochloric acid added to each individual sample. The sample was then placed in an oven for 30 minutes at 50°C, after which 35 ml distilled water was added and the solution filtered into a bottle and made up to a final volume of 50 ml with distilled water.

The minerals, P and Ca were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler.

4.2.5 Statistical analyses

The data obtained from this trial was analysed using PROC GLM of SAS software, Version 9.3 of the SAS system for Windows, and was subjected to a one-way ANOVA, where treatment was the main effect (SAS, 2009). Tests were done on the 95% confidence level, where a P-value less than 0.05 indicates that there is a difference between dietary treatments, and likewise a P-value larger than 0.05 would indicate no differences between dietary treatments. When the P-value was less than 0.05, indicating there is a difference between treatments, the Bonferonni post hoc test was used to determine where the differences were. In a few instances the Bonferonni post hoc test did not show a difference between dietary treatments, while the P-value of the ANOVA was significant. In these cases the Tukey's pairwise comparisons test was used to determine where the differences were. This was done as the Tukey's pairwise comparisons test is more sensitive than the Bonferonni post hoc test.

4.3 Results and discussion

The results obtained from the carcass data for the portion percentage of the broilers, as well as dressing percentage, are shown in Table 4.3. In this study, no differences (P>0.05) occurred between dietary treatments for the various portion sizes of the broiler carcass. This shows a constant and equal growth of different muscles in the broiler between the dietary treatments tested.

There are many conflicting findings regarding the effect of plant related supplements on carcass portion yield, however results from this trial are in accordance with results by Simsek *et al*. (2007), Zhang *et al*. (2005), Garcia *et al*. (2007), Taylan & Bozkurt, (2009), Dieumou *et al*. (2009) and Scheuermann *et al*. (2009). Zhang *et al*., (2005), found that where RepaXol™ was supplemented in the broiler diets, no significant effect of dietary treatment on breast meat (skinless and boneless) and wing portions were observed. RepaXol™ is a mixture of essential oils, including oregano, cinnamon, thyme and capsicum. However, RepaXol™, at a rate of 100 g/ton resulted in a significant (P<0.10) decrease in the leg quarter (thigh and drumstick

combined) portion of the broiler carcass. However, higher (150 g/ton decreasing to 75 g/ton from day 35 – 42) and lower (100 g/ton decreasing to 50 g/ton from day 35-42) levels of RepaXol™ resulted in no significant differences in leg quarter portions (Zhang *et al*., 2005).

In a study supplementing broiler diets with anise oil, at 100 mg/kg, 200 mg/kg and 400 mg/kg, no significant differences were reported for portion percentage of the leg, breast or back and neck combined (Simsek *et al*., 2007). A significant difference was observed for the wing percentage of the carcass, with anise oil at a rate of 100 mg/kg showing significantly higher wing portion percentage than the treatment group supplemented with anise oil at 400 mg/kg (Simsek *et al*., 2007). Scheuermann *et al*. (2009) tested the phytogenic additive, Biostrong 510®, a mixture of microencapsulated essential oils, capsaicins, and saponins, and reported no significant differences between treatments for the portion of breast, thigh, drumstick and wings as a percentage relative to broiler carcass weight. Similarly, no significant difference was found for leg percentage by Dieumou *et al*. (2009), who supplemented garlic and ginger essential oil to the broiler diets at a rate of 10 mg/kg/day, 20 mg/kg/day or 40 mg/kg/day.

No significant differences between treatments for breast and thigh portions were also reported by Garcia *et al*. (2007) when broilers were fed a diet supplemented with plant extracts. The supplementation of plant extracts was in the form of two treatments, one being a blend of oregano, cinnamon and pepper essential oils, collectively rich in cinnamaldehyde, carvacrol, and capsaicin, supplemented at a rate of 200 mg/kg. The second treatment was a blend of hydroalcoholic plant extract from sage, thyme and rosemary leaves, collectively rich in rosmarinic acid, apigenin-7-glucoside, and isocutellarein-7glucoside, supplemented at a rate of 5000 mg/kg (Garcia *et al*., 2007).

Another study on broiler chickens reported no significant differences between treatments for wing, breast, thigh and neck portions when fed a commercial blend of EO, Fitococci™ at a rate of 1000 mg/kg. Fitococci™ is a commercial blend of essential oils containing the active ingredients thymol and carvacrol from thyme, origanium, garlic, anise and fennel oil (Taylan & Bozkurt, 2009).

Jamroz *et al*. (2005) fed broilers either a maize based diet, or a wheat and barley based diet. Both supplemented with plant extracts composed of carvacrol, cinnamaldehyde and capsaicin, at a rate of 100 mg/kg, or no supplement (control). The wheat barley based diet supplemented with plant extracts resulted in an increase in breast meat by 1.2%. However no increase was reported within the maize based diet. The wheat barley control diet had a higher portion of breast muscle than both the maize control diet and the maize diet supplemented with plant extract (Jamroz *et al*., 2005). This suggests an interaction between the plant extracts and diet ingredients of the wheat and barley diet, and no interaction between the maize base diets. Further research is needed to explore the extent of this interaction.

Results for the current trial show no differences (P>0.05) between dietary treatments for dressing percentage of carcasses. Similarly, in two separate studies supplementing various plant based essential oils, no significant differences were found in dressing percentage (Tekeli *et al*., 2006; Cabuk *et al*., 2006), or when plant extracts, consisting of capsaicin, cinnamaldehyde and carvacrol, were supplemented at a rate of 100 mg/kg (Jamroz *et al*., 2005). The study by Cabuk *et al*. (2006) involved a commercial essential oil mixture™ consisting six different essential oils derived from Turkey, including oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil, and citrus peel oil, at 24 mg/kg and 48 mg/kg. Tekeli *et al*. (2006) used various essential oils including *Oreganum vulgare* essential oil, *Thymus vulgaris* essential oil, *Syzygium aromaticum* essential oil and *Zingiber officinale* essential oil, at a rate of 120 mg/kg. Nor were any significant differences in dressing percentage reported when anise seeds were supplemented to broiler diets at rates ranging from 0.25 g/kg to 1.5 g/kg (Soltan *et al*., 2008). However, an improvement in dressing percentage was reported when a mixture of essential oils were supplemented to the broilers diet (Alçiçek *et al*., 2004a; Alçiçek *et al*., 2004b). Both these trials used the same commercial essential oil mixture used by Cabuk *et al*. (2006) mentioned earlier. Alçiçek *et al*. (2004a) and Alçiçek *et al*. (2004b) both found an increase in dressing % for broilers fed a diet supplemented with 48 mg/kg essential oil mixture. However, the same was not found by Cabuk *et al*. (2006), and all three trials used a maize and soya bean based diet, and grew the chickens to 42 days age. In a separate study by Al-Kassie (2009), broilers were supplemented with essential oils derived from thyme and cinnamon at 100 mg/kg and 200 mg/kg for each essential oil, with yellow maize and soya bean meal based diet. Al-Kassie (2009) reported an increase in dressing % for broilers supplemented with 200 mg/kg thyme essential oil and cinnamon essential oil, separately.

Dressing percentage is calculated from the weight of the chilled carcass as a percentage of the live weight and therefore a higher dressing percentage is desired as to give a higher market value to the carcass.

Table 4.3 Mean percentage ± standard deviation (SD) of the breast, thigh, leg, back, and wing of chilled carcasses obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	% Breast	% Thigh	% Leg	% Back	%Wing	Dressing %
1	19.0 ± 2.23	14.7 ± 1.06	6.5 ± 1.01	4.6 ± 0.72	6.6 ± 0.73	66.8 ± 1.97
2	18.8 ± 1.39	13.7 ± 1.12	6.9 ± 0.84	4.6 ± 0.86	6.6 ± 0.66	66.1 ± 1.91
3	17.6 ± 2.16	14.2 ± 0.88	6.9 ± 0.83	4.5 ± 0.73	7.2 ± 0.76	64.7 ± 1.63
4	18.4 ± 2.03	14.4 ± 1.39	6.8 ± 0.75	4.3 ± 0.86	7.2 ± 1.02	65.6 ± 2.44
5	18.6 ± 1.74	13.9 ± 0.82	7.1 ± 0.83	4.4 ± 0.64	6.6 ± 0.77	64.6 ± 2.35
P value	0.4822	0.1404	0.5109	0.8822	0.0567	0.0982

[a,b] Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

The results obtained from the carcass data regarding the different portions of the breast tissue, i.e. the fat and skin, bone and muscle, is shown in Table 4.4. No significant differences were observed for the fat and skin percentage, while differences (P<0.05) were observed for percentage bone and muscle. The AGP plus Ateli plus® treatment had significantly less bone than the AGP treatment, with the remaining dietary treatments being intermediary and statistically equal to both, AGP and the AGP plus Ateli plus® treatments. Broilers receiving treatments containing AGP plus Ateli plus® and Ateli plus® min had significantly higher breast meat portions than that of broilers receiving the AGP treatment. Broilers receiving all other dietary treatments were intermediary and statistically equal to the AGP plus Ateli plus®, AGP and Ateli plus® min treatments. No scientific literature could be found comparing the effects of plant extracts on the different portions of the breast tissue.

Table 4.4 Mean percentage ± standard deviation (SD) of skin, fat and skin, and bone of the breast obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	% Muscle	% Fat and skin	% Bone
1	64.6[b] ± 6.4	8.5 ± 2.4	25.6[a] ± 7.81
2	68.6[ab] ± 3.77	7.6 ± 2.15	21.6[ab] ± 3.72
3	71.0[a] ± 5.21	7.5 ± 0.86	20.7[ab] ± 5.38
4	69.4[ab] ± 4.05	7.6 ± 1.14	20.5[ab] ± 4.33
5	73.9[a] ± 5.98	7.6 ± 1.63	17.8[b] ± 6.29
P Value	0.001	0.614	0.029

[a,b] Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

The pH recording results of the breast and thigh muscles are depicted in Table 4.5. No differences (P>0.05) were observed for pH_i of the thigh muscle, while differences (P<0.05) were observed for pH_i in the breast muscle, pH_u in the breast muscle and pH_u in the thigh muscle. Data for pH_i breast recordings were analysed using Tukey's pairwise comparisons test, as Bonferonni's post hoc test failed to show difference between dietary treatments. Tukey's pairwise comparisons test is more sensitive than Bonferonni's post hoc test. The rest of the data in Table 4.5 was tested using Bonferonni's post hoc test.

Broilers receiving the AGP treatment showed a higher pH_i (P<0.05), of 5.88 for the breast muscle compared to the broilers receiving the Ateli plus® min treatment, with a pH_i of 5.71. All other dietary treatments for breast pH_i were intermediary and statistically equal (P>0.05) to both the AGP and Ateli plus® min treatments.

Broilers receiving the AGP plus Ateli plus® treatment had a significantly higher pH_u for both breast and thigh muscles, compared to broilers receiving the no AGP treatment. All other treatments for pH_u of the breasts and thighs were intermediary and statistically equal (P>0.05) to the no AGP treatment and the AGP plus Ateli plus® treatment. A higher pH_u is associated with a darker meat (Van Laack *et al*., 2000) as well as a higher water binding capacity, which results in a higher water holding capacity (Le Bihan-Duval *et al*., 1999), and more tender meat (Huff-Lonergan & Lonergan, 2005). From Table 4.5 it can be seen that broilers receiving the

Ateli plus® max treatment, showed the next highest muscle pH_u values for both the breast and thigh, compared to the AGP plus Ateli plus® treatment. This pH_u value for breast and thigh muscles for the Ateli plus® max treatment is higher than that of the value for the AGP treatment, although not significantly. This provides evidence of a plant extract diet marginally improving the pH_u of the meat, thereby improving the water binding capacity of the broiler meat (Le Bihan-Duval *et al*., 1999), and improving the meat tenderness.

Young *et al*. (2003), who supplemented oregano at a rate of 3 g/kg to the diet of broiler chickens, reported no significant effect of oregano on meat pH. The pH measurements were only recorded up until 4 hours *post mortem* in their study (Young *et al*., 2003), unlike in the current study, where pH was measured up until 24 hours *post mortem*. This could be the reason for the conflicting results.

Table 4.5 Mean initial and ultimate pH ± standard deviation (SD) of breast and thigh muscle obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	pH_i Breast muscle	pH_i Thigh muscle	pH_u Breast muscle	pH_u Thigh muscle
1	5.88^{a} ± 0.13	5.68 ± 0.23	5.55^{ab} ± 0.13	5.60^{ab} ± 0.18
2	5.84^{ab} ± 0.21	5.64 ± 0.18	5.50^{b} ± 0.13	5.56^{b} ± 0.18
3	5.71^{b} ± 0.12	5.80 ± 0.76	5.56^{ab} ± 0.10	5.68^{ab} ± 0.06
4	5.74^{ab} ± 0.13	5.54 ± 0.19	5.61^{ab} ± 0.06	5.69^{ab} ± 0.13
5	5.74^{ab} ± 0.11	5.65 ± 0.12	5.66^{a} ± 0.12	5.74^{a} ± 0.11
P Value	0.027	0.5683	0.0128	0.021

^{a,b} Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

The results obtained from the carcass data for the colour readings of the breast and thigh are shown in Table 4.6 and Table 4.7, respectively. Significant differences occurred between dietary treatments for the L* and b* breast colour reading values, while no significant differences occurred for the breast a* colour reading value. The AGP and Ateli plus® treatment had a significantly lower L* colour reading value than broilers from treatments with AGP, no AGP, and the treatment containing Ateli plus® max. While Ateli plus® min was statistically equal (P>0.05) to all other treatments. All three treatments containing Ateli plus® had lower breast L* colour reading values than both the AGP and no AGP treatments, although not always significantly so.

Lower L* colour reading value occurs in darker meat. The data in Table 4.5 shows that all the treatments containing Ateli plus® have higher breast pH_u values, which correlates back to what Le Bihan-Duval *et al*. (1999) reported, with L* colour reading value being inversely proportional to the pH_u value of the meat. A low pH_u would result in a lower water binding capacity, resulting in higher drip loss (Le Bihan-Duval *et al*., 1999). This therefore shows that the meat from broilers supplemented with Ateli plus®, having a higher pH_u will result in meat with a higher water binding capacity and therefore a more tender meat.

The breast b* value was significantly higher in the Ateli plus® max dietary treatment, compared to the no AGP treatment. All other dietary treatments were intermediary and statistically equal ($P>0.05$) to both the Ateli plus® max treatment and the treatment receiving no AGP for the breast b* value.

According to Van Laack *et al*. (2000), normal broiler breast meat would have an L* colour reading value of 55.1, a* colour reading value of 2.2, and b* colour reading value of 9.6. However, results from Table 4.6 show higher L* colour reading values, as well as substantially higher a* and b* colour reading values. Higher L* colour reading values are signs of a pale meat, also sometimes referred to as pale soft exudative (PSE) meat, a phenomenon that frequently occurs with ante mortem stress in chickens; it could be that the chickens from this investigation were more stressed than normal due to the per mortem handling procedure followed in the small scale abattoir used. The a* colour reading values from pale breast meat, according to Van Laack *et al*. (2000), is supposed to be lower than the normal range of 2.2, with paler breast meat showing a value of 1.2. However, the breast meat from the current trial showed a* colour reading values of > 4.2. Rathgeber *et al*., (1999) reported that in turkey meat, a delay in carcass chilling resulted in a lighter, redder, and more yellow meat. Higher L*, a* and b* values, as is seen in the current trial. Again, it is possible that the manual slaughter line in the current study may have delayed the initiation of the chilling regime with the resultant colour disparity noted.

In a study using rosemary leaf meal essential oil, the meat colour of the broilers fed a diet supplemented with 0.5 g/kg and 1 g/kg rosemary essential oil, were positively affected (Ghazalah & Ali, 2008). However this article failed to describe how they measured colour, and what they prescribe as positive effects.

In a separate study, using dried rosemary leaves, rosemary volatile oil and vitamin E, no significant differences were reported for the L*, a* or b* colour reading values of the breast meat of broilers (Yesilbag *et al*., 2011). In their study, rosemary plant was supplemented at a rate of 5.7 g/kg, 8.6 g/kg, 11.5 g/kg, and rosemary essential oil was supplemented at 100 mg/kg, 150 mg/kg, and 200 mg/kg. Young *et al*. (2003) reported an increase in the b* colour reading value for both breast and thigh broiler meat, when broilers were supplemented with oregano at a rate

of 3 g/kg. This resulted in a more yellow colour in the meat (Young *et al*., 2003), which agrees with the results from the current trial, where Ateli plus®, also containing oregano, supplemented at its maximum rate resulted in a significantly higher b* colour reading value for both breast and thigh muscle. This could be attributed to the carotenoid content of the oregano. Although, the differences in the breast b* colour reading value is minimal, and therefore hold no biological difference. Oregano may have a potential for changing broiler meat colour, and requires further research.

Table 4.6 Mean L*, a* and b* colour values ± standard deviation (SD) of the breast muscle obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	L* value Breast muscle	a* value Breast muscle	b* value muscle
1	60.54^{a} ± 2.44	4.22 ± 1.87	12.22^{ab} ± 1.39
2	59.06^{a} ± 2.85	5.41 ± 2.14	10.47^{b} ± 1.29
3	57.75^{ab} ± 2.60	5.71 ± 1.69	12.05^{ab} ± 1.97
4	58.73^{a} ± 1.98	4.35 ± 0.81	12.46^{a} ± 1.13
5	54.78^{b} ± 2.70	5.26 ± 1.99	12.29^{ab} ± 2.22
P Value	<0.0001	0.162	0.031

^{a,b} Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 4.7 shows the results for thigh colour recording values. Significant differences were observed between dietary treatments for L*, a* and b* colour values for the thigh muscle. L* values were significantly higher for the AGP and the no AGP treatment, compared to the AGP plus Ateli plus® treatment. A lower L* colour reading value represents a darker meat. All other treatments were intermediary and equal for the L* thigh value. The Ateli plus® min and Ateli plus® max treatments showed a lower L* colour reading value than AGP treatment, although not statistically significant. This resulted in a darker meat colour in the Ateli plus® supplemented broilers compared to the AGP supplemented broilers, which may be beneficial to the tenderness of the meat, as darker meat is reported to having a higher water binding capacity, resulting in a more tender meat (Le Bihan-Duval *et al*., 1999).

The Ateli plus® max treatment had a significantly higher a* colour reading value for the thigh, compared to the AGP treatment, no AGP treatment, and the Ateli plus® min treatment, while

the treatment containing AGP plus Ateli plus® was intermediary and statistically equal (P>0.05). A higher a* colour reading value is an indication of a more red meat.

The Ateli plus® max treatment had a significantly higher b* colour reading value for the thigh, compared to the AGP treatment. The remaining treatments were all intermediary and statistically equal (P>0.05) to both the AGP and the Ateli plus® max treatment. Tukey's pairwise comparisons test was used to analyse the data for the b* colour reading value as Bonferonni's post hoc test, which was used for the remaining colour data, did not show a difference between treatments. Young *et al*. (2003) also reported a higher b* colour reading value in the thigh and breast muscle of broilers supplemented with oregano essential oil. Higher b* colour reading values is an indication of a more yellow meat.

From the current study, both breast and thigh had significantly higher b* colour reading values for the Ateli plus® max supplemented dietary treatments. As oregano is included in the Ateli plus® additive, and the oregano supplementation in the study by Young *et al*. (2003) also resulted in an increase in b* colour reading value, oregano appears to have meat colour changing capabilities. Therefore further research needs to be conducted to evaluate the effect of oregano on broiler meat colour.

Table 4.7 Mean L* a* b* colour values ± standard deviation (SD) of the thigh muscle obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	L* value thigh muscle	a* value thigh muscle	b* value thigh muscle
1	$57.06^{a} \pm 2.47$	$5.31^{b} \pm 1.60$	$9.73^{b} \pm 1.61$
2	$57.31^{a} \pm 2.29$	$5.13^{b} \pm 1.30$	$11.08^{ab} \pm 2.21$
3	$55.37^{ab} \pm 1.95$	$5.06^{b} \pm 0.96$	$12.20^{ab} \pm 1.86$
4	$54.99^{ab} \pm 1.88$	$8.09^{a} \pm 2.62$	$12.85^{a} \pm 3.362$
5	$53.74^{b} \pm 1.89$	$7.25^{ab} \pm 2.39$	$12.29^{ab} \pm 3.55$
P Value	0.0004	0.0002	0.043

[a,b] Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Results obtained from the tibia bone data are shown in Table 4.8. No significant differences were observed for tibia bone length, while significant differences were observed for tibia bone weight, width, breaking distance and breaking force. The tibia bone weight for the treatment receiving only AGP was significantly heavier (12.06 g) than that of the group receiving Ateli plus® max treatment (10.22 g). All other dietary treatments were intermediary and statistically equal ($P>0.05$) to both the AGP treatment and the Ateli plus® max treatment.

Bone breaking force results show a significantly lower breaking force for treatments receiving no AGP, and both Ateli plus® min and Ateli plus® max treatments, compared to the treatment receiving AGP, which had a higher ($P<0.05$) breaking force. The AGP and Ateli plus® treatment was intermediary and statistically equal ($P>0.05$) to all other treatments. This indicates that the treatment receiving AGP had a significantly stronger bone than that of the no AGP treatment and the two treatments receiving Ateli plus®. From Table 4.8 it is clear that both treatments supplemented with only Ateli plus® had the lowest bone breaking strength, lower than the negative control, giving reason to believe that broilers supplemented with plant extracts will have weaker bones. Broilers' skeletal structure are under pressure with the fast rate of growth, causing the largest economic loss in the broiler house to be due to leg abnormalities, which results in an increase in mortalities and culling, a decrease in growth and FCR as well as down grading of the chicken in the slaughter house (Shim *et al*., 2012). Chickens suffering with leg abnormalities have a reduced mobility also making this a welfare concern (Shim *et al*., 2012). Contrary to the current study, a study on broiler chickens that tested alternatives to AGP's, namely organic acids, a commercial herbal blend, a probiotic, and a prebiotic, found an increase in tibia bone characteristics in all the broilers fed the alternative AGP additives, when compared to the control (Ziaie *et al*., 2011). In a separate study with rats, bone resorption was inhibited by 1 g of powdered dry sage, rosemary or thyme leaves, as well as by the essential oils extracted from sage and rosemary (Mühlbauer *et al*., 2003). A decrease in bone resorption has a beneficial effect on the skeleton of the animal (Putnam *et al*., 2007). Bone strength plays an important role in the poultry industry for production and welfare of the chicken, and further compromise needs to be avoided, therefore further research needs to be conducted to evaluate the effects of plant extracts on bone strength.

Broilers receiving no AGP, Ateli plus® max, and AGP plus Ateli plus® treatments had significantly narrower tibia bone widths than broilers receiving AGP alone. The Ateli plus® min treatment was intermediary and statistically equal ($P>0.05$) to all other treatments. The distance from the proximal joint to the point of break was shorter ($P<0.05$) for broilers receiving the AGP plus Ateli plus® treatment, compared to the broilers receiving the AGP treatment. All other dietary treatments were intermediary and equal ($P>0.05$) to the AGP and the AGP plus Ateli plus® treatments. Bonferonni's post hoc test did not show a difference between dietary treatments for tibia bone breaking distance, therefore Tukey's pairwise comparisons test was

used to analyse the breaking distance data. Bonferonni's post hoc test was used on the remaining data in Table 4.8.

Table 4.8 Mean values ± standard deviations (SD) of bone strength (N), weight (g) and lengths (cm) of the tibia bone obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	Bone weight (g)	Bone length (cm)	Bone width (cm)	Bone breaking distance (cm)	Bone break force (N)
1	12.06^{a} ± 0.75	8.95 ± 0.20	0.73^{a} ± 0.04	4.03^{a} ± 0.53	315.82^{a} ± 69.80
2	11.06^{ab} ± 1.35	9.07 ± 0.30	0.66^{b} ± 0.05	4.02^{ab} ± 0.53	246.16^{b} ± 47.73
3	11.36^{ab} ± 1.11	9.22 ± 0.17	0.68^{ab} ± 0.05	3.98^{ab} ± 0.27	233.9^{b} ± 46.72
4	10.22^{b} ± 1.19	8.94 ± 0.35	0.63^{b} ± 0.04	3.85^{ab} ± 0.30	239.74^{b} ± 29.54
5	11.01^{ab} ± 1.10	8.95 ± 0.29	0.65^{b} ± 0.06	3.48^{b} ± 0.54	262.49^{ab} ± 64.70
P Value	0.0113	0.0703	0.0011	0.0308	0.006

^{a,b} Means within columns with different superscripts differ significantly (P<0.05)

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

The results for the proximal analyses of the tibia bones are shown in Table 4.9. No significant differences between dietary treatments were observed for percentage of the fat, moisture, dry matter or ash content of the tibia bones. The ash percentage of the bone gives a good indication of how mineralized the bone is, and also shows the susceptibility of bone disorders, as chickens with bone disorders usually have a lower bone ash percentage than that of healthy chickens (Shim *et al*., 2012). Fast growing broiler lines have been shown to generally have a lower bone mineral content than that of the slow growing broiler lines (Venäläinen *et al*., 2006). It is postulated that the broiler diet has not been adjusted to keep up with this increased demand on mineral requirements needed for the increased growth rate (Thorp & Waddington, 1997).

Results for phosphorous and calcium content of the tibia bones are shown in Table 4.10. No significant differences are observed between dietary treatments.

Table 4.9 Proximal analysis values (mean) ± standard deviation (SD) of the tibia bone obtained from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatment[1]	% Fat	% Moisture	% Dry matter	%Ash
1	14.0 ± 3.87	52.5 ± 1.41	47.5 ± 1.41	21.8± 1.21
2	15.8 ± 3.14	52.7 ± 2.33	47.4 ± 2.33	21.1 ± 1.22
3	16.7 ± 2.43	53.6 ± 1.16	46.5 ± 1.16	20.1 ± 0.83
4	14.1 ± 2.76	51.8 ± 2.50	48.2 ± 2.50	22.2 ± 1.51
5	16.4 ± 5.27	51.6 ± 1.28	48.4 ± 1.28	21.7 ± 1.57
P-value	0.591	0.3953	0.3953	0.0892

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Table 4.10 Mean ± standard Deviations (SD) of phosphorous and calcium content of tibia bones extracted from broilers slaughtered at 33 days of age fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (Negative control)

Treatments[1]	[2]Phosphorus %	Calcium %
1	20.76 ± 1.71	41.84 ± 3.03
2	20.34 ± 3.09	39.54 ± 6.47
3	20.45 ± 2.12	39.43 ± 5.21
4	20.45 ± 1.57	40.38 ± 4.32
5	18.59 ± 0.82	37.12 ± 2.63
P- value	0.4552	0.5685

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

[2]Phosphorus and Calcium as a percentage of bone ash

4.4 Conclusion

The current trial indicated that supplementing broiler diets with different rates of a plant extract based product, Ateli plus®, had no significant effect on the portion percentage of the carcass as well as the dressing percentage. However, an improvement in muscle ultimate pH and muscle colour reading value, L*, of the broiler meat was seen in broilers supplemented with Ateli plus®. Ultimate pH in the breasts and thighs was seen to be higher in all Ateli plus® dietary supplemented broilers, but only significantly higher in AGP plus Ateli plus® supplemented diets, which is related to an increase in water binding capacity and tenderness of the muscles. This will result in an improvement in the meat quality of the broiler. The L* colour reading value of the Ateli plus® supplemented broilers also gave lower L* values than the AGP and no AGP supplemented broilers, although not always significantly lower, resulting in darker meat, which relates back to having a higher water binding capacity and improved tenderness, and therefore improved meat quality.

In the current study, the results showed how the plant extract based product reduced the bone breaking strength of the broilers, even lower than that of the negative control supplemented broilers. Further compromise of the broiler skeletal structure cannot be entertained on a welfare level or a production level, and further research will need to be conducted to confirm this finding.

There are various intrinsic and extrinsic factors playing a role in the activity of plant based products, leaving room for variation and conflicting results. Further research needs to be conducted on the ideal form and composition of the plant based products, to conclude on the efficacy of the product as an alternative to AGP's.

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Chapter 5

Evaluation of gut and organ health of broilers fed a diet supplemented with plant extracts

Abstract

This study evaluated the effect of a plant extract based product, Ateli plus®, on Cobb 500 gut and organ health. A total of 2400 broiler chicks were randomly divided into five different treatment groups, with six repetitions per treatment, and raised from day 1 to 33. No significant differences were seen between dietary treatments for heart, liver, spleen and bursa weights, as well as gizzard erosion. Gizzard weight for the Ateli plus® max supplemented broilers was significantly higher than the negative control broilers. The pH of the duodenum, jejunum, ileum, proventriculus and caecum were measured within 15 minutes post-mortem, with no significant differences between dietary treatments. Duodenum, jejunum and ileum villi height and crypt depths were measured. Duodenum villi height was longest for broilers supplemented with antibiotic diet (AGP), and shortest for broilers supplemented with Ateli plus® min and AGP plus Ateli plus® treatment, with crypt depth being the longest for Ateli plus® min supplemented broilers. Broilers fed the basal treatment (negative control) had the shortest jejunum villi height and crypt depth. Ateli plus® max supplemented broilers had the longest jejunum villi length, while crypt depth was longest in AGP supplement broilers. Ileum villi height and crypt depth was longest for AGP supplemented broilers, and statistically shorter and equal for the remaining dietary treatments. Broilers fed plant extract supplemented diets showed no significant improvement in villi height to crypt depth ratio. No significant effect of plant extracts on gut morphology or organ health can be concluded from this study.

***Keywords*:** Plant extracts, Chicken, Villi, Organ weight, pH, Gizzard erosion, small intestine

5.1 Introduction

The digestive tract is the main site for digestion and absorption of nutrients in the animal. It also acts as the largest immunological organ in the body, as it is the first point of protection against exogenous pathogens that enter the body, preventing the pathogens from colonisation and entering the host cells and tissues (Choct, 2009). A balanced gut microorganism population, consisting of less pathogenic bacteria, and an increase in beneficial bacteria, may result in an increase in the availability of nutrients (Hashemi & Davoodi, 2010). Previously, antibiotics were added to the diets of production animals, and improved the growth rate of these animals by improving their gut health (Bedford, 2000). All in-feed antibiotics have been banned in the European Union since 2006 (Dibner & Richards, 2005), due to the risk of residue transfer to animal products as well as the production of an antibiotic resistant bacterial population (Hernandez *et al*., 2004). This has led to the search for an alternative and safe replacement for in-feed antibiotics.

Herbal plants, their extracts and essential oils are gaining attention as an alternative for in-feed antibiotics, due to their antimicrobial effects and their stimulating effect on the animals' digestive

system (Ciftci *et al*., 2005). The response in the animal due to supplementation of plant extracts has been reported to be influenced by the quality and quantity of the active chemicals within the plant extract (Cross *et al*., 2007).

Phytogenic feed additives act by improving the balance of the microorganism population in the digestive tract, and by doing so, decreasing the quantity of pathogenic bacteria within the digestive tract (Wenk, 2000; Hashemi & Davoodi, 2011). This leads to a decrease in sub-clinical immune stress experienced by the host animal, so the animal can spend its energy on growth, and grow to its full genetic potential, instead of fighting infection (Hashemi & Davoodi, 2011). The reduction in pathogenic bacteria also leads to an increase in nutrients becoming available for absorption in the digestive tract (Windisch *et al*., 2008; Hashemi & Davoodi, 2010), further improving growth of the animal within its genetic potential, as well as reducing the production of growth depressing toxins produced by the pathogenic microorganisms (Hashemi & Davoodi, 2010). An increase in pathogenic bacteria in the digestive tract causes damage to the enterocytes along the villi, resulting in deeper crypts (Parsaie *et al*., 2007). Deeper crypts are associated with a high cell turnover, and a higher demand for new tissue (Xu *et al*., 2003).

Crypts are indentations into the mucosal layer, which lie next to the villi, and are responsible for the production of enterocytes and goblet cells (Shen, 2009). As the enterocytes migrate up the villi, where they are eventually sloughed into the lumen, they undergo different functional changes, from having a secretory function in the crypts, to an absorptive function as they travel up the villi (Buddle & Bolton, 1992; Uni *et al*., 2000). The villi height and crypt depth plays an important role in the digestion and absorption of feed in the small intestine, as an increase in crypt depth and a decrease in villi height can lead to increase secretions into the gastrointestinal tract, resulting in diarrhoea, decrease in disease resistance and decreased animal performance (Parsaie *et al*., 2007; Catalá-Gregori *et al*., 2008). The longer the villi, the larger the surface area there is available for absorption of nutrients (Shen, 2009; Saeid *et al*., 2013), due to the increase in number of enterocytes along the villi in the absorptive phase (Buddle & Bolton, 1992; Choct, 2009). Shallower crypts are associated with a lower tissue turnover, and therefore less demand for new tissue. This also results in less enterocytes in the secretory stage, therefore less secretions, and more villi enterocytes along the longer villi with absorptive functions, resulting in better nutrient absorption (Nabuurs *et al*., 1993; Saeid *et al*., 2013). Therefore, the villi to crypt ratio of the small intestine, plays an important role in the absorptive ability (Buddle & Bolton, 1992), and digestive capacity of the small intestine (Saeid *et al*., 2013). According to Van der Klis & Jansman (2002), the ideal villi height and crypt depth of the jejunum for healthy poultry, is 612 µm and 188 µm, respectively. Therefore the optimal healthy villi height to crypt depth ratio for the jejunum is 3.26.

Organ weights give a good indication of the health status of the chicken. In a stressed chicken, as typically found in the form of heat stress or increase in stocking densities, it has been

reported that the weights of the primary and secondary lymphoid organs, decrease (Pope, 1991; Heckert *et al*., 2002). The bursa of Fabricius weight is reported to be the most accurate representation of the chicken immunity (Heckert *et al*., 2002).

The objective for the following study was to evaluate whether plant extracts improve the intestinal health of broilers by evaluating their effect on gizzard erosion, gut morphology and organ health.

5.2 Materials and methods

5.2.1 Birds, housing and management

The research trial was conducted at Mariendahl experimental farm of Stellenbosch University, located near Stellenbosch, Western Cape Province, South Africa, where 2400 one day old Cobb 500 chicks were placed in a commercial type broiler house, and five treatments tested. The commercial broiler house was divided into 32 pens, of which 30 were used, with six pens per dietary treatment. Each pen was equipped with two tube feeders as well as a bell drinker, totalling a space of 0.221 m^2. Each pen had a floor space of 4.01 m^2, resulting in a space of 3.67 m2 left for the chicks. With 80 chicks placed per pen, this resulted in a stocking density of 21.8 chicks /m^2.

The broilers were fed a diet supplemented with a plant extract based product, Ateli plus®, at different rates resulting in five treatments, as described in Table 5.1. The antibiotic and coccidiostat used in the diets are shown in Table 5.2. A crumble starter feed was supplied at a rate of 900 g/chick from day 1 to ~16 days of age by when all the feed had been consumed. The grower and finisher feeds were pelleted using a 2mm die and supplied at a rate of 1200 g/chick from day ~17 to ~25, and day ~26 to 33 days of age, respectively. Feed and water were supplied *ad libitum* throughout the trial.

Ateli plus® is an oregano based product, that has been shown to improve efficiency and resistance to pathogens, and has antimicrobial properties associated with the main active ingredient, carvacrol.

Table 5.1 Treatment number and description of five experimental treatment diets for broilers comparing different levels of Ateli plus® with a positive and negative control diet

Treatment	Description
1	Positive control with anti-biotic Stafac 500 (AGP)
2	Negative control without anti-biotic (no AGP)
3	Ateli plus® at a rate of 1kg/ton (Ateli plus® min)
4	Ateli plus® at a rate of 2 kg/ton week 1 and then 1kg/ton for the remainder of the period (Ateli plus® max)
5	Positive control and same rate as treatment 4 (AGP plus Ateli plus® max)

Table 5.2 Antibiotic and coccidiostat description of the diet fed to broilers

Treatment	Description	Starter and Grower		Finisher	
		Coccidiostat	Antibiotic growth promoter	Coccidiostat	Antibiotic growth promoter
1	AGP	Salinocox 12%	Stafac 500	Avatec	Stafac 500
2	No AGP	Salinocox 12%	None	Avatec	None
3	Ateli plus® min	Salinocox 12%	None	Avatec	None
4	Ateli plus® max	Salinocox 12%	None	Avatec	None
5	AGP plus Ateli plus®	Salinocox 12%	Stafac 500	Avatec	Stafac 500

5.2.2 Sampling Procedure

At 33 days of age one chicken per pen were selected from around the mean weight per group. This results in a repetition of six birds per treatment being assessed for organ, gut health and bone strength. Each chicken was stunned using an electrical stunner set at 50-70 volts, with a

current of 2 A applied for 5 s. Exsanguinations of the broilers took place within 10 seconds of stunning.

5.2.2.1 Organ weights

The heart, liver, gizzard, spleen and bursa of Fabricius were removed from the fresh carcass and weighed using a Mettler PC 4400 scale (Mettler – Toledo, Switzerland), accurate to 0.01 g. The gizzard was cut open from the cranial to the caudal end of the gizzard, and rinsed before being weighed and assessed for gizzard erosion.

5.2.2.2 Digestive tract pH

Once the organs were removed from the fresh carcass, samples of the digestive tract were taken for histological analyses. The incision for removal of the duodenum was made at the gizzard side of the duodenum, at the start of the pancreas. The jejunum was cut out at approximately the centre of the small intestine and the ileum was cut out 5mm from the ileocaecal junction. The pH was measured at these three incision points of the small intestine, as well as at the proventriculus and the caecum. The pH was measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25°C) portable Crison 506 pH-meter (Alella, Barcelona), which was inserted into the digestive tract area to measure the respective pH readings. Between each reading, the probe was rinsed with distilled water and maintained in a crysolite (5% KCl) solution.

5.2.2.3 Gizzard erosion

The gizzard of each chicken was extracted from the fresh carcass, cut open from the cranial end to the caudal end of the gizzard, and rinsed of its contents with tap water. The gizzard was then assessed for gizzard erosion. Scoring for gizzard erosion was done on an ordinal scale, ranging from 1 to 5, as described in Table 5.3.

Table 5.3 Gizzard erosion scoring description

Score	Description
1	No erosion
2	Light erosion (roughness of epithelia)
3	Modest erosion (roughness and gaps)
4	Severe erosion (roughness, gaps and ulcers on stomach wall showing slight haemorrhaging)
5	Extreme erosion (roughness, gaps and haemorrhagic ulcers on stomach wall and separation of epithelia from stomach wall)

5.2.2.4 Villi height and crypt depth

Duodenum, jejunum and ileum samples (1-2 cm in length) were taken from the small intestine of the broilers slaughtered a 33 days of age. Samples were rinsed with 9% saline solution and fixed in 10% buffered formalin, before being sent for histopathogenic examination at the Stellenbosch University School of Medicine Histology laboratory, Tygerberg Campus, Tygerberg, Cape Town, South Africa. Tissue species were trimmed, dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections of 3-4µm thickness were cut and stained with haematoxylin and eosin (Humason, 1974).

Slides were analysed using a Zeiss Axio light microscope, with 2.5X magnification objective lens. Images from the built in digital camera were analysed using Aviovision image-analysis software (version 4.7.2, Carl Zeiss microscopy). Ten consecutive villi were analysed, measuring their height and crypt depths. Villi height was measured from the base of the villi at the villus crypt junction, to the tip of the villi, as shown in Plate 5.1.

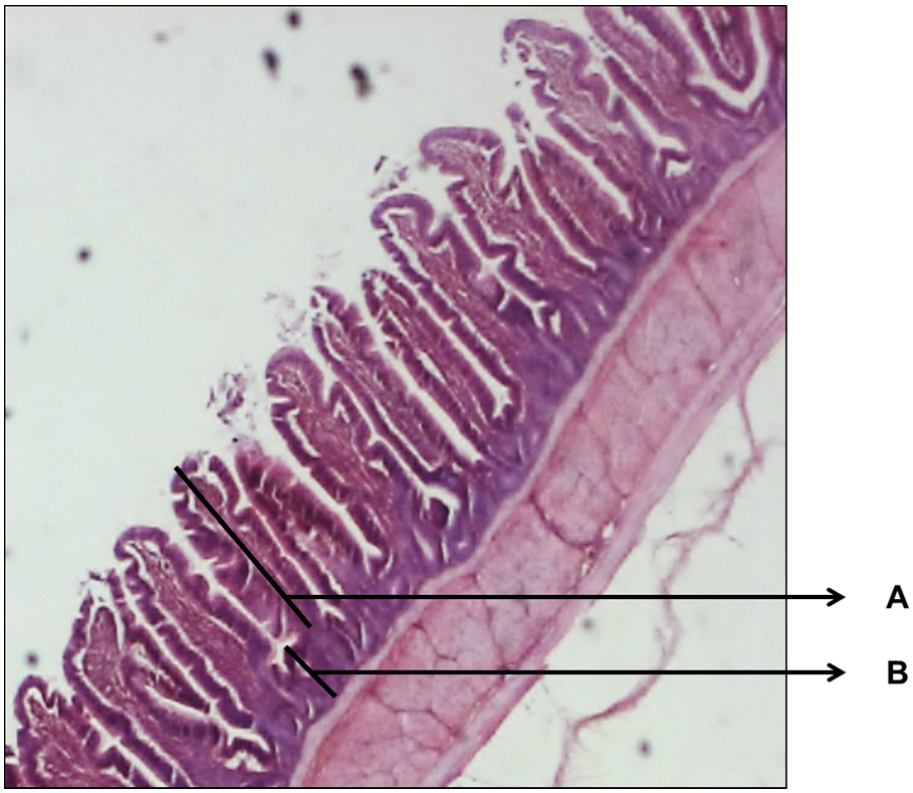

Plate 5.1 A representation of the histological measures taken from the digestive tract of broilers slaughtered at 33 days of age, fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control) A – Villi height; B – Crypt depth

5.2.3 Statistical analyses

The data obtained from this trial was analysed using PROC GLM of SAS software, Version 9.3 of the SAS system for Windows, and was subjected to a one-way ANOVA, where treatment

(diet) was the main effect (SAS, 2009). Tests were done on the 95% confidence level, where a p-value less than 0.05 indicates that there is a difference between dietary treatments, and likewise a p-value larger than 0.05 would indicate no differences between dietary treatments. When the p-value was less than 0.05, indicating there is a difference between treatments, the Bonferonni post hoc test was used to separate means. Gizzard erosion scores were analysed using Chi-squared test (SAS, 2009).

5.3 Results and Discussion

5.3.1 Organ weights and Gizzard erosion

The results from the data for broilers slaughtered at 33 days of age from the current study, is shown in Table 5.4. No differences (P>0.05) between dietary treatments were observed for the heart, liver, spleen or bursa weights of broilers slaughtered at 33 days of age. Gizzard weights did differ significantly between dietary treatments. Broilers supplemented with Ateli plus® max had the heaviest gizzard weight, while broilers supplemented with no AGP had the lightest gizzard weight. The results from the current study are in agreement with results of Hernandez *et al*. (2004), Cabuk *et al*. (2006), Tekeli *et al*. (2006), and Kirkpinar *et al*. (2011), except for the gizzard weights.

With an increase in stress for the chicken, in the form of heat or stocking densities, or other forms of stress, the lymphoid organs decrease in weight (Heckert *et al*., 2002). Measuring the lymphoid organ weights is an accurate method in assessing the immunity of the chicken (Pope, 1991), with the weight of the bursa being the most accurate representation (Heckert *et al*., 2002). Decrease in bursa weight can be due to viral infection or stress (Pope, 1991).

Hernandez *et al*. (2004) supplemented broilers with 200 mg/kg essential oil mixture (oregano, cinnamon, and pepper), or 5000 mg/kg Labiatae extract from sage, thyme, and rosemary, and found no differences between treatments for the liver, proventriculus and pancreas weight. However, contrary to the current study, Hernandez *et al*. (2004) also found no difference between treatments for gizzard weight. Cabuk *et al*. (2006) supplemented 24 mg/kg or 48 mg/kg of an essential oil mixture, containing oregano, laurel leaf, sage leaf, myrtle leaf, fennel seed and citrus peel oil, to broilers. No significant differences were reported between dietary treatments for liver, pancreas, proventriculus and gizzard weights. Tekeli *et al*. (2006) supplemented broilers with 120 mg/kg of *Yucca schidigera (*Mojave Yucca)*,Oreganum vulgare* (Oregano)*, Thymus vulgaris* (Thyme)*, Syzygium aromaticum (*Clove) or *Zingiber officinale* (Ginger) essential oil, and reported no significant difference between treatments for the heart, liver, proventriculus or gizzard weights. In a study supplementing oregano essential oil (300 mg/kg) or garlic essential oil (300 mg/kg), or combined (150 mg/kg + 150 mg/kg), no effect on heart, liver, gizzard, spleen, proventriculus, and bursa weights were reported (Kırkpınar *et al*., 2011).

An increase in gizzard weight, as seen in the Ateli plus® max supplemented broilers, is correlated to an increase in gizzard function, which in turn results in a stimulated secretion of hydrochloric acid by the proventriculus and results in a decrease in the gizzard pH (Engberg *et al*., 2002; Engberg *et al*., 2004). A lower pH in the gizzard acts as a barrier for many of the pathogenic bacteria ingested with the food, which is prevented from entering the small intestine (Engberg *et al*., 2002; Engberg *et al*., 2004). A heavier gizzard is also correlated to an improvement in nutrient utilization (Choct, 2009). Therefore the report of a heavier gizzard in the Ateli plus® max supplemented broilers could result in fewer pathogenic bacteria entering the digestive tract, and therefore less immune stress on the broiler. Microbial population, however, was not evaluated in the current study.

Table 5.4 Weights (mean (g)) ± standard deviation (SD) of organs obtained from broilers slaughtered at 33 days of age, fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

	Organ Weights				
Treatment[1]	**Gizzard weight**	**Heart weight**	**Liver weight**	**Spleen weight**	**Bursa Weight**
1	24.01[ab] ± 4.38	11.5 ± 1.53	66.45 ± 14.21	2.88 ± 1.46	4.10 ± 0.94
2	19.42[b] ± 3.74	10.69 ± 1.68	60.11 ± 9.88	2.89 ± 0.47	4.62 ± 1.02
3	24.17[ab] ± 2.36	10.66 ± 0.73	55.82 ± 7.11	2.28 ± 0.25	3.68 ± 1.10
4	26.54[a] ± 4.15	10.86 ± 1.83	52.34 ± 11.68	2.05 ± 0.42	3.86 ± 1.61
5	22.64[ab] ± 2.72	10.95 ± 1.49	58.03 ± 9.25	2.34 ± 0.45	4.16 ± 2.20
P Value	0.029	0.83	0.245	0.221	0.853

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

Gizzard erosion score results are shown in Table 5.5, where it can be seen that no gizzard were scored above 2. Only one broiler from dietary treatment groups AGP and AGP plus Ateli plus® max, was scored 2. One broiler from each dietary treatment group scored a gizzard erosion score of 1. Although the Ateli plus® max treatment had two broilers receiving a gizzard erosion score of 1.

Gizzard erosion involves the presence of lesions or extensive sloughing of the koilin lining of the gizzard, as well as thickening and loosening (Itakura *et al*., 1982; Fossum *et al*., 1988). This results in a decrease in feed intake, and therefore a decrease in growth rate. Mortality can also

be increased due to gizzard erosion (Fossum *et al*., 1988; Tišljar *et al*., 2002). No literature on the effect of plant extract or essential oils on gizzard erosion could be sourced.

Table 5.5 Number of specimens per gizzard erosion score for each treatment group from broilers slaughtered at 33 days of age, fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

GE* score	Treatments[1]				
	1	2	3	4	5
1	1	1	1	2	1
2	1	0	0	0	1
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0

(*) GE – Gizzard Erosion

[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

5.3.2 Organ pH

The results from the pH data, of the respective organs from broilers slaughtered at 33 days of age, are shown in Table 5.6. From the table it can be seen that no difference between dietary treatments were seen for the pH of the duodenum, jejunum, ileum, proventriculus and the caecum. Similar results were reported by Khalaji *et al*. (2011), where no effect on duodenum, jejunum or ileum pH was reported when black cumin seed (1 g/kg), *Artemisia* leaves (*Artemisia sieberi*)(1 g/kg), and *Camellia* L. plant extract (0.5 g/kg) were supplemented to broilers as phytogenic products. However, this study reported an increase in gizzard and proventriculus pH in broilers supplemented with *Camellia* L. plant extract (Khalaji *et al*., 2011). Similarly, no significant differences were reported for caecal pH in broilers supplemented with mushroom and herb polysaccharide extracts, *Lentinus edodes* extract, *Tremella fuciformis* extract, and *Astragalus membranaceus Radix* extract, at a rate of 2 g/kg (Guo *et al*., 2004). No difference in ileum pH was reported in pigs, slaughtered 14 days post weaning, fed a diet supplemented with either, a control, 0.75 g/kg herbal extracts containing cinnamon, thyme and oregano, or an acid supplemented diet (Namkung *et al*., 2004).

Table 5.6 Mean pH ± standard deviations (SD) of various areas of the digestive tract obtained from broilers slaughtered at 33 days of age, fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

	pH of digestive tract				
Treatment1	**Duodenum**	**Jejunum**	**Ileum**	**Proventriculus**	**Caecum**
1	5.53 ± 0.38	5.73 ± 0.46	6.07 ± 0.80	3.98 ± 0.46	6.24 ± 0.19
2	5.33 ± 0.34	5.67 ± 0.72	5.97 ± 0.69	4.48 ± 0.52	5.92 ± 0.48
3	5.13 ± 0.57	5.83 ± 0.21	5.55 ± 0.39	3.78 ± 0.90	5.94 ± 0.20
4	5.15 ± 0.58	5.56 ± 0.35	6.57 ± 0.56	3.91 ± 0.54	5.93 ± 0.42
5	5.39 ± 0.33	5.73 ± 0.23	6.11 ± 1.03	3.82 ± 0.57	6.04 ± 0.45
P Value	0.531	0.863	0.229	0.315	0.554

[ab] Means within columns with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promoter

5.3.3 Villi height and Crypt depth

Small intestinal morphology results for broilers slaughtered at 33 days of age, is shown in Table 5.7, reporting villi height and crypt depth. Significant differences between dietary treatments were measured for the duodenal villi height and crypts depths. Villi height was longest for the broilers supplemented with the AGP treatment, and shortest for the broilers supplemented with Ateli plus® min and AGP plus Ateli plus® treatments. Villi height for broilers receiving diets supplemented with either Ateli plus® max or no AGP, were statistically equal to the previously mentioned dietary treatments. Duodenum crypt depths were significantly deeper in broilers supplemented with Ateli plus® min, and shallower and statistically equal for broilers supplemented with the AGP, no AGP, Ateli plus® max, and AGP plus Ateli plus® treatments. Duodenum villi height (VH):crypt depth (CD) ratio was significantly higher in broilers supplemented with Ateli plus® min, and statistically lower and equal in the broilers receiving the AGP, no AGP, Ateli plus® max, and AGP plus Ateli plus® treatments.

Jejunum villi height was significantly longer for broilers supplemented with Ateli plus® max, while broilers receiving the no AGP treatment (negative control) had the shortest jejunum villi. Jejunum crypt depths were deepest in the broilers supplemented with AGP, and shallowest in broilers receiving the negative control diet. Broilers receiving the remaining Ateli plus® supplemented treatments were statistically equal to both the AGP and no AGP supplemented

broilers concerning jejunum crypt depth. No significant differences were seen in jejunum VH:CD ratio.

Ileum morphology results show that the broilers supplemented with the AGP treatment had the tallest villi height and crypt depth, with broilers receiving the remaining dietary treatments having statistically shorter and equal villi heights and crypt depths in the ileum. The Ileum VH:CD ratio was highest for the AGP supplemented broilers, and lowest for the broilers receiving the AGP plus Ateli plus® treatment. From the table it shows that as the Ateli plus® concentration increases, the ileum VH:CD ratio decreases. A decrease in VH:CD ratio is associated with a decrease in nutrient absorption (Catalá-Gregori *et al*., 2008), due to there being a higher proportion of secretory cells as a result of longer crypts, and fewer cells in the absorptive phase due to the shorter villi (Buddle & Bolton, 1992).

Besides the significant decrease in VH:CD ratio found in the ileum as a result of the AGP plus Ateli plus® supplementation, no conclusions can be drawn for the effect of Ateli plus® on broiler gut morphology. Similar results were found in a study by Perić *et al*. (2010), where jejunum gut morphology of broilers, supplemented with a phytogenic blend consisting of essential oils from oregano, anise, citrus and fructo-oligosaccharides, showed no significant effect on villi height, crypt depth or surface area, however it did show a reduced jejunum VH:CD ratio. A reduced VH:CD ratio can be associated with the presence of toxins, a decrease in nutrient absorption, decrease in disease resistance, an increase in secretions in the gastrointestinal tract, diarrhoea and also a lower performance of the animal (Catalá-Gregori *et al*., 2008). Perić *et al*. (2010) suggested that the improvement in the phytogenic supplemented broilers performance was not directly connected to the gut morphology of the broiler, but rather through other mechanisms. Similar results were found in a study by Garcia *et al*. (2007), where broilers supplemented with 200 mg/kg plant extract, based on oregano, cinnamon, and pepper essential oil, showed improved FCR and body weight gain (BWG). However, these broilers with improved performance had the shortest villi and crypt depths. This confirms speculations by Perić *et al*. (2010) that improvement in broiler performance due to the use of phytogenic additives may not be due to gut morphology.

A study by Catalá-Gregori *et al*. (2008), reported that broilers supplemented with a plant extract blend (XTRACT™), consisting of carvacrol, cinnamaldehyde, and capsicum oleoresin, showed no effect of treatments on ileum villi height. However, this study showed a tendency for the plant extract supplemented broilers to have a deeper crypt depth, and the broilers supplemented with the prebiotic (PROFEED®) and plant extract (XTRACT™), combined, at 600 and 100 mg/kg, respectively, to have a shorter crypt depth. However, no significant differences were reported between treatments for the VH:CD ratio of the ileums (Catalá-Gregori *et al*., 2008).

In a separate study, supplementing broilers with either 50, 100 or 150 mg/kg of *Mentha piperita, Thymus vulgaris, Citrus lemon* and *Carum copticum* essential oils, it was reported that there was no effect on duodenum villi height and crypt depth (Samadian *et al*., 2013). However, an increase in ileum villi height was reported in broilers receiving the *C. copticum* (100 mg/kg) supplemented diet, but no effect on crypt depth was reported (Samadian *et al*., 2013). Broilers from this trial also showed no significant effect of essential oil supplementation on VH:CD ratio for the duodenum and ileum (Samadian *et al*., 2013).

A recent study, however, found a significant increase in villi height, crypt depth, and VH:CD ratio in broilers supplemented with garlic powder, at a rate of 0.5 g/kg, or the combination of garlic powder and black seed, at a rate of 0.5 g/kg combined (Saeid *et al*., 2013). The broilers with improved gut morphology, supplemented with garlic powder and the combination of garlic powder and black seed, also reported having a significantly improved BW, BWG and increased feed intake (Saeid *et al*., 2013).

Villi height to crypt depth ratio plays an important role in the absorptive capacity of the small intestine (Buddle & Bolton, 1992), with longer villi having more enterocyte cells for absorption (Parsaie *et al*., 2007), and deeper crypts showing a higher demand for new tissue due to proliferation in the lumen (Xu *et al*., 2003). A higher turnover of cells, therefore a deeper crypt, would lead to a higher energy demand for maintenance of the digestive tract of the broiler (Choct, 2009); energy that could rather be used for growth and therefore improved performance. Pathogenic bacteria present in the digestive tract are believed to damage the enterocyte cells, which leads to less absorption, as well as deeper crypts (Parsaie *et al*., 2007). Phytogenic feed additives are believed to reduce the pathogenic bacteria count in the digestive tract (Wenk, 2000; Hashemi & Davoodi, 2011), and would therefore lead to less damage on the enterocytes and improved absorption, as well as better villi height to crypt depth ratio. However, many studies show no effect of plant extracts on the gut morphology, making it difficult to link improved gut morphology to improved animal performance.

Table 5.7 Small intestine morphology mean (µm) ± Standard deviation (SD) results obtained from broilers slaughtered at 33 days of age, fed a diet supplemented with either Ateli plus®, antibiotic (positive control) or none (negative control)

Treatment[1]	Villi	Crypt	Villi height: Crypt depth ratio
		Duodenum	
1	1365.51^{a} ± 414.68	253.87^{b} ± 76.47	5.33^{a} ± 2.65
2	1310.34^{ab} ± 309.93	238.61^{b} ± 45.33	5.70^{a} ± 1.85
3	1115.34^{b} ± 331.64	295.37^{a} ± 77.60	4.08^{b} ± 1.77
4	1253.96^{ab} ± 328.62	239.85^{b} ± 57.66	5.55^{a} ± 2.06
5	1162.07^{b} ± 476.10	222.32^{b} ± 54.24	5.20^{a} ± 1.82
P value	0.0033	<0.0001	<0.0001
		Jejunum	
1	1120.72^{ab} ± 308.03	283.65^{a} ± 79.77	4.19 ± 1.36
2	886.59^{c} ± 224.70	214.97^{c} ± 29.51	4.19 ± 1.19
3	994.6b^{c} ± 288.02	257.37^{ab} ± 68.63	4.01 ± 1.13
4	1159.26^{a} ± 258.66	269.50^{ab} ± 83.50	4.61 ± 1.44
5	1093.11^{ab} ± 193.55	245.66^{bc} ± 40.61	4.53 ± 0.98
P value	<0.0001	<0.0001	0.057
		Ileum	
1	686.39^{a} ± 395.55	193.94^{a} ± 93.84	3.55^{a} ± 0.93
2	485.13^{b} ± 84.18	154.06^{b} ± 22.02	3.22^{ab} ± 0.73
3	443.14^{b} ± 110.28	146.51^{b} ± 34.10	3.11^{ab} ± 0.85
4	507.36^{b} ± 92.31	173.53^{b} ± 38.14	3.01^{bc} ± 0.65
5	433.17^{b} ± 127.43	165.77^{b} ± 27.48	2.63^{c} ± 0.70
P value	<0.0001	<0.0001	<0.0001

^{ab} Means within columns per organ with different superscripts differ significantly (P<0.05)
[1]Treatments 1 - Antibiotic growth promoter (Positive control); 2 - No additive (Negative control); 3 - Ateli plus (1kg/ton); 4 - Ateli plus 2kg/ton week 1 then 1kg/ton; 5 – Treatment 4 plus antibiotic growth promote

5.4 Conclusion

The results from the current study showed no significant differences in organ pH or organ weights of broilers supplemented with the plant extract based product, Ateli plus®. Except for gizzard weight, which was heavier for the Ateli plus® max supplemented broilers, which could have resulted in fewer pathogens entering the digestive tract, due to increased secretion of hydrochloric acid. As organ weights are a good indication of health status of the broilers, and there were no significant differences between the negative and positive control treatment broilers, this shows that there was no significant immune stress on the broilers throughout the trial. Therefore no positive results on organ weights could be seen by the inclusion of the plant extract based product, or the antibiotic growth promoters. Neither was any consistent trend of improvement in gut morphology seen in Ateli plus® or AGP supplemented broilers, with respect to villi height, crypt depth and VH:CD ratio.

Due to antibiotic supplementation having no effect on broiler performance when broilers are raised in optimal conditions, and Chapter 3 showing no difference in growth performance between dietary treatments, as well as organ weights showing no differences between dietary treatments, it can be said that broilers were raised in near optimal conditions for this trial. Therefore no improvement on gut health from the current study is shown. To fully evaluate the potential of plant extracts as a supplement to improve gut and organ health, future studies should be conducted on broilers raised in inferior environmental conditions.

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Chapter 6

General Conclusion

Due to consumers demands for healthier meat (i.e. no antibiotic residues) and the increase in untreatable illnesses in humans due to antibiotic resistance, alternatives to in-feed antibiotics are important to the poultry industry. Using an alternative to AGP's in poultry feed in South Africa may also open doors to export our poultry meat to other countries.

No effect on production parameters were shown in this study, although the Ateli plus® supplemented broilers had the same performance as the AGP supplemented broilers, therefore showing no negative effect on broiler performance. As a replacement to in-feed antibiotics, the expectation for the study was for Ateli plus® supplemented broilers to at least equal the growth of the antibiotic supplemented broilers if not, better it. However, due to the broilers from the negative control diet being statistically equal to broilers from the Ateli plus® and AGP treatments, for performance as well as organ weights, gives reason to believe the broilers for this trial were raised under their optimal environmental conditions and good management. Under these conditions, even antibiotic supplementation will have no positive effect on growth performance therefore no additional growth promoting effects can be expected to be seen from the Ateli plus® supplemented broilers.

No consistent pattern was seen for the effect of Ateli plus® or AGP on gut morphology, which is consistent with broilers being raised under their optimal conditions, and therefore low pathogenic interference in the digestive tract.

A trend for improvement in carcass characteristics was seen in broilers supplemented with Ateli plus®, with improved ultimate pH and lower L* colour reading values for the breast and thigh. This leads to a higher water binding capacity, and therefore more tender meat resulting in an improvement in meat quality. However, bone strength in the broilers supplemented with Ateli plus® was significantly decreased. Leg abnormalities in the broiler flock is a major cause for mortalities, and further decrease in bone strength of the broilers will result in further production losses, as well as a concern for the welfare of the broilers. Further studies need to be conducted to evaluate the severity of bone strength loss due to plant essential oil and extracts on broilers, as further compromise on bone strength cannot be entertained for the welfare of the animal, as well as production losses due to mortalities.

As a replacement for AGP's, Ateli plus® shows promising results in broiler production, maintaining the same performance parameters as AGP supplemented broilers, as well as improving the meat quality compared to the AGP supplemented broilers. An improvement in

meat quality as well as a residue free product, due to no AGP supplementation, will lead to a happier consumer, and healthier product.

On a performance level, Ateli plus® should be tested on broilers kept in sub-optimal conditions to evaluate the true growth performance enhancing capabilities of the product.