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GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND MEAT QUALITY IN BROILERS SUPPLEMENTED WITH FEVER TEA *(LIPPIA JAVANICA)* LEAF POWDER

Dissertation submitted in fulfillment of the requirements for the Master of Science degree in Animal Science

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DECLARATION

I, **David Advice Mpofu**, declare that this dissertation has not been submitted to any University. This is my original work which was conducted under the supervision of Dr U. Marume and Prof. V. Mlambo. All assistance towards the production of this work and all the references contained herein have been duly credited.

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2015

ABSTRACT

The present study was conducted to determine the effect of supplementing *Lippia javanica* (fever tea) leaf powder in broiler diets on growth performance, carcass characteristics and meat quality. One hundred and eighty Cobb 500 broiler chickens were allocated to four diets with three replicates of 15 chicks per pen. Fresh leaves of *L. javanica* were collected, dried and ground before addition to broiler diets. Two levels *L. javanica* were tested: 5 g/kg and 12 g/kg of *L. javanica* leaf powder. The other treatments were negative control group (without supplementation) and positive control group (with antibiotics). The experimental study lasted for 42 days. Body weight (BWT), feed intake (FI) were recorded weekly and used to calculate feed conversion ratio (FCR) at 42 days. At the end of the trial (42 days) three chickens (one chicken per replicate) were randomly selected for sampling blood before being slaughtered for assessment of internal organs, fatty acids profiles and sensory evaluation of meat. The results indicated that supplementing broiler diets with 5 g/kg of *L. javanica* leaf powder significantly increase body weight ($P < 0.05$) among the treated groups. With regards to survival rates, only one bird in the 12 g/kg of *L. javanica* treatment group died. Broilers fed were supplemented with 12 grams of *L. javanica* meal in their diet had the highest ($P < 0.05$) weights for the proventriculus, gizzard and small intestines and also had the longest small intestines. On the other hand, broilers were supplemented with 12 grams of *L. javanica* meal in their diet also had the lowest weights for liver and pancreas had the highest weight in positive control treatment group. There was no difference ($P >0.05$) observed across the treatments in terms of heart and spleen sizes. The inclusion of *L. javanica* in broilers diet had no effect on the breast and thigh weights ($P>0.05$). Differences were observed on the weight of abdominal fat pad, carcass weight and dressing percentages. Broilers that were receiving *L. javanica* in their dietary treatment had significantly higher abdominal fat weight compared with the other two groups ($P<0.05$). High haematological values were also obtained in broilers fed *L .javanica.*

The fatty acids (FA) composition of the subcutaneous adipose was also determined. Dietary treatments significantly *(P* < 0.05*)* affected subcutaneous fat (SCF %). The broilers that were supplemented with 12 grams *L. javanica* meal in diet had the lowest proportion of SCF while positive control had the highest proportion of subcutaneous fat (SCF). Diet, however, had no effect on fat free dry matter (FFDM) and moisture. Sensory evaluation was done on boiled and fried meat using 30 panellists. Aroma intensity and atypical flavour intensity were not significantly affected by diet (*P*>0.05). However, diet had an effect on other sensory attributes with the meat from broilers fed *L. javanica* being rated more superior than from the control groups. The results from this experiment indicated that *L. javanica* can be used as a growth promotant in broiler diets.

Keywords: *Lippia javanica*, broiler chickens, growth performance, fatty acids, sensory evaluation

DEDICATION

This thesis is dedicated to my family, especially my mother, my late father, my sisters and brother, for believing in me and for their support since I started my project. Without them I could not have done this.

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LIST OF ABBREVIATIONS

ADF – Acid Detergent Fiber

ADL – Acid Detergent Lignin

AOAC –Association of Official Analytical chemist

BWG – Body Weight Gain

BWT – Body Weight

CRD – Completely Randomized Design

DM – Dry Matter

FA – Fatty Acid

FCR – Feed Conversion Ratio

FDA – Food and Drug Administration

FI – Feed Intake

GLM –General Linear Model

LJ – *Lippia javanica*

NDF – Neutral Detergent Fiber

NRC – National Research Council

NWU –North West University

OM – Organic Matter

REP – Replication

SA – South Africa

SANBI – South African National Biodiversity Institute

SAS – Statistical Analysis System

SLW– Slaughter weight

TRT – Treatment

VFA – Voluntary Fatty Acid

WHC – Water Holding Capacity

1 INTRODUCTION

1.1 Background

The use of antibiotics as growth promotants in poultry production and other meat types is becoming topical among the increasingly health conscious consumers. Although antibiotics have been used successfully in poultry to increase growth rates due to improved gut health and better nutrient utilization (Engberg *et al.,* 2000; Landy *et al.,* 2011), there is increasing public concern that continuous use of antibiotics in poultry feeds can induce bacterial resistance increasing the associated health risks in humans. There is, therefore, an increasing need to explore alternatives to antibiotics that can be used to improve animal growth and disease prevention. Natural alternatives that have received increased attention is the use of phytogens and herbal products (Toghyani *et al.,* 2010, ; Landy *et al* 2011; Ghalamkari *et al.,* 2012; Hong et al., 2012;). Phytogenic plants and herbal products have been traditionally used, in South African households and across the world, against some animal diseases and can play a critical role in maintaining human health (South African National Biodiversity institute, 2004; Viljoen *et al.*, 2005; Ghalamkari *et al.,* 2012).

A natural alternative that has been popularised in many communal households of South Africa in the treatment of livestock disease is fever tea (*Lippia javanica*). The plant is widely distributed in Southern Africa and across the world and it has been used extensively as a medicinal plant for both animals and humans (Viljoen *et al.*, 2005; Oliveira *et al.*, 2007). It contains secondary plant metabolites, particularly terpenoids that have been reported to possess analgesic, anti-inflammatory and antipyretic activities (Abena *et al.,* 2003). The terpenoids were also observed to have inhibitory effects on cultures of *Escherichia coli, Bucillus subtillis* and S*taphylococcus aureus* (Manezhe *et al.,* 2004). The plant has been used as an antihelmintic

(Mabogo, 1990) and has also been found to be active against *Plasmodium falciparum* and *Kblebsiella pneumoniae* (Nkhumeleni *et al.,* 2004; Viljoen *et al.*, 2005).

Information is available on the use of phytogenic plants, with similar medicinal properties as *L. javanica*, as alternatives for antibiotics in broilers and laying hens. For example, *Mentha pulegium L.* in broiler diets has been observed to improve performance and carcass quality (Modiry *et al.,* 2010; Ghalamkari *et al.,* 2012), while neem (*Azadirachta indica*) was observed to favorably influence the immune response of broilers without any adverse effects on growth and carcass quality (Landy *et al.,* 2011). However, despite the potential of *L. javanica* as an alternative to antibiotics in broilers, there has been a dearth of information on its potential use as an antibiotic and growth promotant.

1.2 The objective of the study

This study was designed to assess the effect of different levels of *L. javanica* leaf powder, as an antibiotic alternative, on growth performance, intestinal and carcass characteristics, hematology, fatty acid profiles and sensory characteristics in broiler chickens.

Specific objectives

The specific objectives of study were:

1. To determine the growth performance and survival rates of broilers when they are supplemented with *L. javanica* leaf powder in their diets
2. To evaluate the intestinal characteristics of broilers fed diets supplemented with *L. javanica* leaf powder
3. To examine the haematological indices of broilers fed diets supplemented with *L. javanica* leaf powder

4. To identify of bacteria types in ileum of broilers fed diets supplemented with *L. javanica* leaf powder
5. To evaluate the meat quality, carcass sensory characteristics, and fatty acid profiles of broiler chickens supplemented with *L. javanica* leaf powder.

1.3 Hypotheses

The study investigated the following hypotheses:

1. Inclusion of *L. javanica* leaf powder in diets has no effect on growth, survival rates and meat quality of broiler chickens.
2. Inclusion of *L. javanica* leaf powder in broiler diets has no effect on the intestinal characteristics of broilers.
3. Supplementary feeding with *L. javanica* leaf powder has no effect on sensory characteristics and fatty acid profiles of broiler meat.

2 LITERATURE REVIEW

2.1 Introduction

Poultry is one of the fastest-growing agricultural sectors in South Africa and the world over. It is also the industry that is providing meat that is preferred by almost all cultures, is affordable and of good quality. In addition, to health, chicken provides a meat alternative that is lean and healthy compared to other meat types particularly beef, pork and mutton (SAPA). In South Africa, the poultry farming business has been in existence for a long time and there has been a significant increase in poultry production over the years compared with other livestock due to increasing demand. The broiler industry has grown drastically, reduced the cost and greatly increased the quantity of chicken produced for food (Zuidof, 2010). However, the increase in production has always been associated with the increased use of artificial growth promoters and antibiotics. Consequently, consumers are beginning to question the quality of meat due to associated health concern. This then warrants the need to evaluate the use of natural antibiotics in poultry production. This section deals with broiler production in South Africa, use of antibiotics in broilers production;, nutritional requirements of broilers; meat quality characteristics of broilers; consumer acceptability and sensory evaluation; challenges facing broiler producers; and the occurrence, distribution and chemical composition of fever tea (*L. javanica*) as well as the ethno-pharmacology and utilisation of fever tea.

2.2 Broiler production in South Africa

Broiler production is the largest segment of the South African livestock industry accounting for 17% of the gross income from livestock which is 1.7% of the total gross value of agricultural products (S.A broiler market value chain, 2013). South Africa is also the major broiler producer in Southern Africa accounting for 80% of total broiler production in the region (S.A broiler market value chain, 2013). The industry was formally organized in 1904, when farmers came

together to form the South African Poultry Association (SAPA) in Kimberley (S.A broiler market value chain, 2013). The main objectives of SAPA were to co-ordinate and promote broiler production, to provide an instrument to voice the feelings of the industry, and later to stage egg-laying tests. Over the past five decades up to now, the broiler industry has grown dramatically. Cost of production has significantly been reduced and there has been a marked increase in the quantity and quality of chicken produced for food (Zuidof, 2010). In South Africa, broiler meat is consistently becoming the most preferred source of protein by many consumers and has the highest per capita consumption than all other animal protein sources. The number of chicken slaughtered has significantly increased by about 43% in 2012 compared to the year 2003. About 9.8 million chickens are slaughtered per week and this shows that the broiler industry is developing each and every year (SAPA).

The gross income from broiler meat for 2012 was R29.845 billion. Commercial broiler meat accounts for about 93.6% of the total poultry meat production, while the rest is made up of mature chicken slaughter, small scale and backyard broiler meat production. Currently 7.8 million parents are required to produce commercial progeny for the meat industry from 212 00 grandparents and 4000 pure breed lines. Broiler meat is produced throughout all the provinces of South Africa. Nevertheless, North West, Western & Northern Cape, Mpumalanga and KwaZulu Natal are the largest producers accounting for approximately 85% of the total production. Broiler production is becoming an industry of choice in South Africa due to the faster rate of profit realisation. Moreover, broilers are now very efficient converters of feed to meat and have faster rates of genetics progress. Recent data from the Poultry Research Center, (Irene) shows that through genetic selection, broiler chickens can now grow almost five times as fast as broilers of five decades ago.

2.3 Challenges facing broiler producers

Currently the major challenge facing poultry producers is the loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products. The use of conventional therapeutics, among them antibiotics is a major concern to consumers. The risk of residues in meat has further been exacerbated by the increasing development of antibiotic resistant bacteria. It is therefore imperative to take cognisance of the fact that generally, consumer expectations for high quality products will strongly influence future production methods particularly when it comes to the use of antibiotics (Tierney, 2004). Predictably, there is going to be a shift towards the restriction on the use of antimicrobials as growth promoters. Added to this, the recent understanding of the interaction between nutrients and intestinal health, intestinal microbiota and the immune system – will require nutritionists to change their paradigms (Ferket, 2009). Moreover, complying with the recommendations related to flock health management and farm biosecurity will become increasingly critical. This therefore, means that all the actors in the poultry production chain, including farmers, veterinarians, stockholders and all other partners have to take greater responsibility in producing quality and healthy meat.

2.4 Nutritional requirements of broilers

The broiler nutrition and feeding is an important part of production. An important part of raising broilers is feeding which makes up the major cost of production. Good nutrition is reflected in the broiler's performance and its products. Broiler chickens normally convert feed into food products quickly and efficiently. Poultry chickens require at least 38 nutrients in their diets in appropriate concentrations and balance. There are criteria used to determine the requirement for given nutrients including growth, feed efficiency, prevention of deficiency symptoms and quality of poultry product. Water is one of the most important nutrients that are

required by poultry chickens throughout their entire lives. When dealing with water, there are many factors that influence water intake, they include relative humidity and ambient temperature. Broilers require a large amount of protein in their diet but the levels of protein vary depending on the chickens' function. Energy is usually the most expensive nutritional component of poultry diet. In addition, a higher efficiency in its utilisation will result in lower feed cost. This strategy may allow reducing feed cost and nutrient excretion (Mohen *et al.*, 2005). One of the most important components of broiler diets is the antibiotics which are mainly used to treat diseases and promoting growth performance. However, the continuous use of conventional antibiotics has seen an increased spread of antibiotic resistant bacteria raising concerns from consumers (Hong *et al.*, 2012). Therefore there is need to evaluate natural products as alternatives to the use of such antibiotics.

2.5 Use of antibiotics in broiler production

Antibiotics have been used widely in animal production for decades. They have been used to treat animals diseases and also as growth promoters. Antibiotics were discovered in the 1940s when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved their growth (Castanon, 2007). In poultry, antibiotics have been used for a long time by poultry producers in the prevention of considerable losses in broilers due to mortalities caused by various bacterial and other infectious diseases which may affect broiler chickens (Alavi *et al.*, 2012) Compared to the 1957 broiler modern a broiler requires 9.4g of feed to fabricate 1g of the breast meat and the broilers that were unselected in 1957 required 28g of feed in order for them to obtain the same weight. The use of antibiotic growth promoters (AGP) has been in use for decades and they were used to improve the growth performance of broiler chickens. Supplementing broilers diet with antibiotic growth promoters could increase growth of animals (Roozbeh *et al.,* 2012). In addition, Visek (1978) have also

indicated that antibiotics increase growth rates as a result of improving the gut health which results in better utilization and can decrease feed conversion ratio. The removal of AGP from poultry diets has prompted a search for suitable natural alternatives to combat the increased potential of bacterial disease development in growing flocks especially under conditions of average management quality (Cross *et al.,* 2007). It has also been stated that, the use of various plant materials as dietary supplements, including culinary herbs, may positively affect the health of poultry and their productivity.

The continuous use of antibiotics in animal feeds has, however, seen a rise in antibiotic-resistant bacteria (Hong *et al.*, 2012) raising concerns of the associated effects on consumers. A possibility of the cessation in the use of antibiotics as growth stimulants for farm animals, accompanied by concerns about the possible side effects of their use as therapeutic agents, has produced a climate in which both consumers and manufacturer are looking for alternatives (Kamruzzaman *et al.*, 2005). Whereas the conventional antibiotics are very effective in promoting growth and treating various infections, thereby improving efficiency of broiler production, the current consumer concerns about their health appear to now take precedence over efficiency of production (Donoghue, 2003; Edens, 2003), and many feed manufacturers and broiler producers are now taking cognisance of this. Evaluation of natural alternatives is therefore, imperative.

2.6 Alternatives to antibiotics in broiler production

Many phytogenic compounds and herbal products have been given considerable attention as possible in-feed antibiotic alternatives to the conventional antibiotics (Jamaroz *et al.,* 2005; Windisch *et al.,* 2008; Toghyani *et al.,* 2011; Hong *et al.,* 2012). Among other benefits, phytogenic compounds have been shown to stimulate appetite, enzyme secretion, immune

response and most have antibacterial and antioxidant activities (Toghyani *et al.,* 2011; Hong *et al.,* 2012). However, some of the phytogenic extracts have been reported to contain aromatic properties that have an impact on gut micro-flora, nutrient digestibility, intestinal morphology and meat quality of poultry (Cross *et al.,* (2007). Many sources of useful phytogenic compounds have been identified, particularly the plant sources. These include among others, *Moringa olifera* (Sreelatha and Padma, 2009; Verma *et al.,* 2009), *Achiella talagonica* (Hong *et al.,* 2012), Cinamon and Garlic (Toghyani *et al.,* 2011) and *Lippia javanica* (Muyima *et al.* 2004; Viljoen *et al*., 2005; Oliveira *et al*., 2007). In South Africa and other Southern African countries, *L. javanica* has been observed to be widely abundant and it is currently being used as an ethno-veterinary drug to treat a wide array of infections that affect livestock particularly in communal areas (Muyima *et al.,* 2004). It is therefore, interesting to evaluate *L. javanica* as a possible alternative to the conventional antibiotics in broiler production.

2.7 Occurrence, distribution and chemical composition of fever tea (*L. javanica)*

The *L. javanica* locally known as ''fever tea'', is a small plant that grows in the open veld, in the bush, hillsides, grassland and along roadsides. Mwangi *et al.,* (1991), have described the plant as the onewith very hairy and strongly aromatic leaves which protect the plant from being browsed by animals. This plant is mostly found in South Africa, Botswana, Swaziland, Mozambique, Malawi, Tanzania, Zambia, Kenya, Zimbabwe and east of Ethiopia . Here in South Africa it is normally found in all provinces except Western Cape. The plant has been widely used as a medicinal plant by many African tribes or communities.

Most of the information available on the chemical composition of *L. javanica* relates to the composition of the essential oils which have been found to show quantitative and qualitative variations both within and between natural plant populations (Viljoen *et al*., 2005). Very little,

if any, information is available on the nutritional composition of the plant. The focus on essential oils is pertinent in that the oils hold large portions of the phytochemicals that are effective in disease treatment and also in promoting growth and general health of livestock. The observed variations in quantity and quality of essential oils in *L. javanica* appear to be random and are not associated with geographical distribution of the plant. Generally, 5 chemotypes, a myrcenone rich-type (36–62%), a carvone rich-type (61–73%), a piperitenone rich-type (32–48%), an ipsenone rich-type (42–61%) and a linalool rich-type (>65%) are widely prominent across Southern Africa (Viljoen et al., 2005). Normally, the occurrence of such chemotypes is bound to affect utilisation of the plant and hence any efforts in the use of the plant have to take cognisance of the inherent variability in quality and quantities of the major components.

2.8 Ethno-pharmacology and utilisation of *L. javanica*

The plant has different plant species which are normally used for different treatments. Different cultures use the plant to treat various conditions. The Vhavenda normally use the plant to treat dysentery, malaria and diarrhoea and the Xhosa used the plant leaves to make tea in the treatment of coughs and bronchial problems in general (LeClerc, 2002) In addition, they also add its leaves and stem to milk or water. On the other hand, the plant has also been used for the disinfection of meat of beasts that has been infected with anthrax. It has been proven that when the leaves and stems are burned, and the smoke is inhaled it is effective against asthma and chronic coughs (Van Wyk *et al.*,, 1997). It has also been used against various chest aliments, rashes and stomach problems. In addition, the plant has been used as a remedy for treating malaria, influenza, wounds and fever (Viljoen *et al.*, 2005). In some countries like Botswana the plant leaves are used for making caffeine free tea while in Zimbabwe and Malawi they are also used as nerve tonic.

In the case of animal diseases, there is very little documented information on the use of *L. javanica*. Nevertheless, it appears that the owners of the livestock, particularly in the smallholder sector generally treat their own animals using medicinal plants based on indigenous knowledge that they themselves possess passes on from generation to generation (Masika *et al.,* 2000; Muyima *et al.,* 2004). Diseases of livestock potentially have severe economic impacts in terms of production losses following mortality and morbidity, particularly in the case of cultures where animals are equated to wealth. There is therefore, a need to increase efforts to determine the efficacy of use of *L. javanica* to treat livestock diseases.

2.9 Phytogenic extracts and meat quality characteristics of broilers

Meat quality is one of the most important determinants of consumers' acceptability of meat. With the growing need to produce healthy meat with very little or no residues, serious considerations should be made on the efficacy of the use of phytogenic plants as alternative antibiotics and growth promotants. Besides having therapeutic effects, phytogenic compounds can have influences on quality of meat to the benefit of the consumers. For example, some studies have shown that some phytogenic extracts in meat can reduce the levels of serum cholesterol, low density lipoproteins as well as increasing the levels of high density lipoproteins (Bok *et al.,* 1999; Ting *et al.,* 2011; Hong *et al.,* 2012). These all have the effect of reducing blood pressure and decreasing platelet aggregation (Sterling and Eagling, 2001). Moreover, the phytogenic compounds can increase the antioxidant activity of meat, increasing the body's ability to deal with free radicals that cause cancer (Toghyani *et al.,* 2011, Moyo *et al.,* 2012, Qwefe *et al.,* 2013).

In addition to the increase in antioxidant activity of meat, the compounds can also influence meat quality (chemical composition and colour stability) as well as fatty acid composition of meat (Sreelaths and Padma, 2009; Qwele *et al.,* 2013). Of particular interest, the phytogenic compounds in meat has the benefit of increasing conjugated linoleic acid concentration and yield meat of lighter colour (Mapiye *et al.,* 2011). Further to all this, phytogenic extracts can play a very important role in meat preservation, as natural food preservatives become more popular in the food industry to replace the commonly used synthetics preservatives and have the ability to desirably change flavour (Muyima *et al.,* 2004).

2.10 Summary

The increase in broiler production has always been associated with the increased use of artificial growth promoters and antibiotics. Consequently, consumers are beginning to question the quality and safety of meat due to the associated health concerns hence, the need to evaluate the use of natural antibiotics in poultry production. *Lippia javanica* has been identified as a potential alternative to the use of conventional therapy. However, comprehensive investigations need to be done to evaluate the efficacy of its utilisation particularly in livestock since previous research has mainly focused on its use by humans.

3 MATERIALS AND METHODS

3.1 Geographical description of the study area

The study was carried out at North-West University (NWU) experimental farm. The study site is located in North West Province of South Africa. The North West Province is situated between 25° and 28° South and between 22° and 28° longitude East.

3.2 Harvesting and preparation of the plant material

Lippia javanica foliage (stem and leaves) was harvested from Mafikeng Game Reserve (North West Province, Republic of South Africa) in April 2014. The Mafikeng game reserve is located against the municipal boundary of Mahikeng capital city extending eastwards to cover an area of 4600 ha of open Kalahari grassland and Acacia thorn scrub with bountiful plains for game viewing as its main attraction (Geographical coordinates: 25°85'00'' S, 25°63'33''E). The study area is 920 – 1782 meters above sea level and rainfall in the area is erratic, ranging between 300 and 800 mm annually and the rain is received mostly between November and February. The area is also characterized by seasonal and daily variations in temperature, being very hot in summer (daily average high temperatures of 32°C in January) and mild-to-cold in winter (average daily minimum in July is 0.9°C). The soils are predominantly of the red sandy-clay type but vary across the game reserve.

The plant foliage was collected and dried on open floors at room temperature (20 – 22^{0}C) for 6 days. They were considered adequately dry when they became crisp to the touch. The drying process was done to reduce moisture content, and to prevent the formation of mould during drying. The plants were collected from the same area (250m^2) to avoid harvesting plants from different growth environments. After drying the foliage, dry leaves were pruned from the whole plant. The dried leaves were then ground into a powder to pass through a 1mm sieve to produce *L. javanica* leaf meal. The leaf meal was then packed in white polythene plastic bags and stored

under dry conditions in the laboratory pending chemical analyses and use in feeding experiments.

Figure 3. 1. *Lippia javanica* plant growing in the game reserve (a), immediately after harvesting (b), during the drying process (c), and the ground leaf meal (d).

3.3 Chemical analysis of *L. javanica* leaf powder

Lippia javanica leaf powder was analysed for laboratory dry matter (DM), organic matter (OM), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). All the samples were analysed in triplicate. The determination of DM, approximately 1 g of leaf sample was placed into a pre- weighed crucible and placed in an oven set at 105 ˚C for 24 hours. After 24 hours, samples were removed from the oven, placed in desiccators to cool and weighed. The loss of weight was measured as moisture content and DM was calculated as the difference between initial sample weight and the final weight. Organic matter (OM) content was determined by ashing the dried samples in a muffle furnace set at 550 °C for 24

hours. After ashing, the crucibles were removed, placed in desiccators to cool and weighed. The loss in weight was measured as OM content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by refluxing 0.45 g – 0.05 g samples with neutral detergent solution and acid detergent solution for 1 hour, respectively, using the ANKOM^{2000} Fiber analyser (ANKOM Technology, New York) according to Van Soest *et al.*, (1991). Heater-stable α – amylase was used for NDF analysis. The fibre fractions were expressed as inclusive of residual ash. The content of lignin in ADF was determined by treating ADF residue in ANKOM bags with 72% sulphuric acid to dissolve the cellulose.

3.4 Animals and dietary treatments

One hundred and eighty (180) day old broiler chicks (Cobb 500) were purchased from a local commercial hatchery (Mimosa chicks). They were weighed upon arrival and then randomly divided into four treatment groups; each treatment group was further divided into three replications of 15 chickens per pen. The pen was the experimental unit in this completely randomized experimental design. The day-old chicks were housed in a total of 12 pens measuring 1.5m x 1.5 m (2.25 m^2). The pens met the space requirements for the rearing of broiler chickens. Each pen was equipped with water and feeding troughs while sunflower seed hulls were used as bedding. Infra-red light was provided continuously from day 1 till day 21. On day 22 chickens were reared under a light-dark cycle (from 06:00 am –18:00 pm lights were switched off, then after 18h00 light were switched on) and on day 35 until the end of the experiment, the light was switched off completely.

The experimental design and the procedures were approved by the National Society for the Prevention of Cruelty to Animal of South Africa (NSPCASA). Four dietary treatments were formulated as follows: 1) a negative control made up of the commercial broiler diet without

prophylactic antibiotics (Albac and Aviax) (Negcontrol); 2) a positive control made up of the commercial broiler diet with prophylactic antibiotics (Poscontrol)]; 3) commercial broiler diet without prophylactic antibiotics but supplemented with 5 g of *L. javanica* per kg of feed (Ljav5) and 4) commercial broiler diet without prophylactic antibiotics but supplemented with 12 g of *L. javanica* per kg of feed (Ljav12). Chickens were reared under a 3-phase feeding program consisting of broiler starter mash: 1 to 15 days then grower mash: 16 to 28 days; and finally broiler finisher diet: 29 to 42 days. The diets were formulated according to the nutrient requirements of broilers at different growth phases (Tables 3.1, 3.2, and 3.3). Feed and water were offered *ad libitum* from tube feeders and water troughs, respectively. The chicks were provided with a basal diet formulated to meet or exceed the nutrient requirements for broilers (NRC, 1994). Ethical principles were considered during the whole period of the study to conform to the national and international standards governing research using animals. Ethical approval was sought and obtained from the North-West University Ethics Committee (Ethics Clearance number: NWU-0099-14-S9).

3.5 Experimental design

This study was conducted as a completely randomized design (CRD) with 4 treatments and 3 replicates per treatment. The experimental replicate was a pen holding 15 birds.

Table 0.1: Diet formulation and composition for starter diet (%)

Raw Materials	NegControl	Poscontrol	Ljav5	Ljav12
Fine yellow Maize	60.02	59.44	59.72	59.30
Prime Gluten 60	6.06	6.05	6.03	5.98
Wheat Bran	8.01	8.0	7.97	7.91
Full fat Soya	1.20	1.20	1.20	1.19
Soya bean meal	16.70	16.68	16.63	16.50
Sunflower Oilcake	4.00	4.0	3.98	3.96
Limestone powder	1.20	1.2	1.20	1.19
Potassium Carbonate	0.16	0.16	0.16	0.16
MCP	1.34	1.34	1.33	1.33
Salt-Fine	0.31	0.31	0.31	0.31
Soya oil	0.15	0.15	0.15	0.15
Bicarbonate	0.19	0.19	0.19	0.19
Choline powder	0.078	0.07	0.07	0.07
Lysine	0.30	0.30	0.30	0.30
Methionine	0.04	0.04	0.04	0.04
Broiler Premix	0.25	0.25	0.25	0.25
Albac		0.07		
Aviax		0.05		
Lippia javanica			0.50	1.19

Table 0.2. Diet formulation and composition for grower diet (%)

Raw Materials	Negcontrol	Poscontrol	Ljav5	Ljav12
Fine yellow Maize	62.83	62.75	62.51	62.08
Prime Gluten 60	6.46	6.45	6.43	6.38
Wheat Bran	8.01	8	8.00	8.002
Full fat Soya	1.00	1	1.00	0.99
Soya bean meal	13.10	13.08	13.03	12.94
Sunflower Oilcake	5.01	5	5.00	5.00
Limestone powder	1.20	1.2	1.20	1.19
Potassium Carbonate	0.07	0.07	0.07	0.07
MCP	1.16	1.16	1.16	1.15
Salt-Fine	0.32	0.32	0.32	0.32
Soya oil	0.15	0.15	0.15	0.15
Bicarbonate	0.14	0.14	0.14	0.14
Choline powder	0.06	0.06	0.06	0.06
Lysine	0.25	0.25	0.25	0.25
Methionine	0	0	0	0
Broiler Premix	0.25	0.25	0.25	0.25
Albac		0.07		
Aviax		0.05		
Lippia javanica			0.50	1.19

Table 3. 3. Diet formulation and composition for finisher diet (%)

Raw Materials	Negcontrol	Poscontrol	Ljav5	Ljav12
Fine yellow Maize	73.00	72.86	72.58	72.08
Prime Gluten 60	6.76	6.75	6.72	6.68
Soya bean meal	13.05	13.03	12.99	12.89
Sunflower Oilcake	3.40	3.4	3.39	3.36
Limestone powder	1.10	1.1	1.10	1.09
potassium Carbonate	0.15	0.15	0.15	0.15
MCP	1.10	1.1	1.10	1.09
Salt-Fine	0.30	0.3	0.30	0.30
Soya oil	0.40	0.4	0.40	0.40
Bicarbonate	0.14	0.14	0.14	0.14
Choline powder	0.08	0.08	0.08	0.08
Lysine	0.31	0.31	0.31	0.31
Methionine	0.01	0.01	0.01	0.01
Broiler Premix	0.25	0.25	0.25	0.25
Albac		0.07		
Aviax		0.05		
Lippia javanica			0.50	1.19

3.6 Growth performance and survival rates

Five chickens from each cage (meaning 15 chickens from each treatment) were randomly selected and weighed individually at the beginning of the experiment (initial body weight). Body weight was subsequently determined at 7, 14, 21, 28, 35 and 42 days of age. Feed intake was measured daily and it was calculated as weight of feed offered less the amount of feed refused (this was measured the following morning). Feed conversion ratio was calculated as feed intake divided by weight gain. All the cages were checked three times a day for mortality and chickens that died during the experiment were removed from the cages. Survival rates were calculated by counting dead and culled chickens.

3.7 Determination of haematological parameters

At 28, 35 and 42 days of age, 3 chickens from each cage were selected randomly and blood was collected from the brachial vein using a needle and syringe. The blood was immediately transferred into two different tubes (purple tubes with anti-coagulant for haematology and red tubes without anticoagulant for serum biochemical analysis). Blood samples were collected early in the morning before the chickens had access of feed. The blood samples for haematological parameters were collected and immediately transferred into a sterile vacutainer tube (purple tube) containing anti-coagulants. The Idexx laser cyte (Haematology analyser) was used to analyse the haematological parameters. The haematological indices examined included red blood cells (RBC), white blood cell (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), haemoglobin (HGB), haematocrit (HCT), red cell distribution width (RDW) and platelets (PLT).

3.8 Determination of carcass traits

At the end of the feeding trial, chickens were fasted for 13 hours to allow for the emptying of the crop according to the method by Ari *et al.,* (2013). Thereafter, all chickens were taken to Agrichicks abattoir for slaughter. Upon arrival at the abattoir, chickens were hung upside down on the rail before being stunned. All chickens were slaughtered by cutting the jugular vein with a sharp knife and they were left there for bloodletting. The chickens were then passed through a de-feathering machine. Thereafter all the birds were taken back to the University farm for determination of organ size and length. The following organs were removed by hand before being weighed: proventriculus, gizzard, breast, thigh, liver, pancreas, heart, caecum, and spleen. The length of small intestines was also measured and recorded. All the visceral organs were carefully examined for any damage, weighed and stored. Carcass weight of each chicken was also determined and dressing out percentage was calculated. The weight of organs was expressed as a percentage of the live body weight. Meat samples from the breast were taken for analysis of fatty acids.

3.9 Intestinal tissue histology

Approximately 2 cm of the middle section of the duodenum, jejunum and ileum were removed and placed in neutral formalin (10% formalin solution) for 24 hours, followed by dehydration and embedding in paraffin wax. The duodenum was defined as that portion of the intestine from the gizzard to the pancreatic and bile ducts, jejunum as the portion of the intestine extending from the bile duct entrance to the Meckel's diverticulum, and ileum as the portion from the Meckel's diverticulum to the point 40 mm proximal to the ileocecal junction. On each intestinal slice, 10 points were selected for microscopical investigation of villus height and cryptal depth according to the standard measurements described by Uni *et al.* (1995).

3.10 Identification of bacteria types in ileum

The intestinal contents were collected immediately after slaughter for bacterial analyses on day 42 from 6 birds per treatment. The intestinal contents were diluted in 0.85 % sterile saline solution for identification of *Lactobacilli spp, Clostridium perfringens*, *Escherichia coli, Enterococcus spp,* and *Enterobacteriacae spp.* using the conventional microbiological agar media techniques (nutrient agar and Mac Conkey agar (Baurhoo *et al.*, 2007)). The bacterial analyses were performed in duplicate and the average values were used for statistical analysis. The samples were cultured on two different agar plates and all the plates were incubated anaerobically at 37°C for 24 hours. Pure colonies were examined microscopically by gram stain. Under the microscope the two different shapes of bacteria were examined. The tests were done according to the procedures for confirmation of bacterial and toxin identity (Hannah Gould *et al.*, 2009). The Lactobacilli spp. was confirmed by using API 20 CH kit (Biomeriux_SA, Marcy-l'Etoile/France). *Escherichia coli* were enumerated through the use of plate count Nutrient agar and MacConkey agar. After obtaining the result from the API, they were recorded on apiweb in order to obtain the results.

3.11 Fatty acid profile determination

Total lipid from feed material was extracted with a Soxhlet extraction according to AOAC (2003) procedures for the determination of fats. Total lipid from muscle samples was quantitatively extracted, according to the method of Folch *et al.*, (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene, was added at a concentration of 0.001 % to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a moisture adsorbent. The total extractable fat was determined gravimetrically from the extracted fat and expressed as percent fat (w/w) per 100

g tissue. The extracted fat from feed, subcutaneous fat and muscle was stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and froze then it was storedat–20°C pending fatty acid analyses.

A lipid aliquot (20 mg) from feed, subcutaneous and muscle lipid was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were trans-esterified to form methyl esters using 0.5 M NaOH in methanol and 14 % boron trifluoride in methanol (Park and Goins, 1994). Fatty Acid Methyl Esters (FAMEs) from subcutaneous fat, feed and muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Analysis was performed using an initial isothermic period (40°C for 2 minutes). Thereafter, temperature was increased at a rate of 4°C/minute to 250°C. Finally an isothermic period of 230°C for 10 minutes followed. FAMEs n-hexane (1μl) was injected into the column using a Varian CP 8400 Auto sampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, and Johannesburg, South Africa). Individual fatty acids were expressed as a proportion of total fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio, atherogenicity index.

3.12 Consumer sensory evaluation

Chickens breast meat was prepared using two different methods (boiling and frying) for sensory analysis. Both meat samples were first deboned and cut into small pieces of approximately 2 cm x 2 cm. Meat samples from each carcass were cooked separately. In the first method, pieces of meat were boiled in water using commercial stoves for 40 minutes. In the second method, cooking oil was added to the pan and pieces of meat were fried on a commercial stove until it was ready for consumption. Salt was added to taste in each method of cooking. At the end of the cooking procedure, meat was allowed to cool.

The meat from each method was evaluated separately using a panel of consumers. Each panellist received labelled pieces of meat on a plate from the four dietary treatments. The participants were taught how to record scores for each variable that was tasted and they identified the meat samples that they preferred on the basis of taste, flavour, tenderness and the colour of the meat. The waiting period between meat sample tasting was 3 minutes. All panellists were not given any information about the meat or the experimental treatments and their procedures.

Thirty sensory panellists who included students from Animal Science Department were recruited through personal contact. There were no incentives offered to the sensory panellists. The only requirement was that members of the panel had no objections to consuming chicken meat. The sensory panellists differed regarding gender, tribes and age. All the participants were trained on making inferences and recording the scores for each sample. After tasting the meat, the participants were instructed to rinse their mouth to remove the after taste in order to avoid crossover effects. Meat samples were rated for tenderness, juiciness, meaty flavour, overall flavour and overall acceptability. Sensory panellists awarded scores using a 8 point descriptive

scale to evaluate aroma intensity (1=extremely bland to 8=extremely intense), initial impression of juiciness (1=extremely dry to 8=extremely juicy), first bite (1=extremely tough to 8=extremely tender), sustained impression of juiciness (1=extremely dry to 8=extremely juicy), muscle fibre and overall tenderness (1=extremely tough to 8=extremely tender), amount of connective tissue (1=extremely abundant to 8=none), overall flavour intensity (1=extremely bland to 8=extremely intensive), and atypical flavour intensity (1=none to 8=extremely intense).

3.13 Statistical analysis

Data on growth performance, haematology, carcass traits, fatty acid profiles and sensory evaluation were subjected to analysis of variance procedures using the general linear model procedure (PROC GLM) of SAS (2008). Data on sensory evaluation was log transformed [Log (x +1)] to achieve normality. The sensory results were then reported as back transformed means. Separation of means was done using the probability of difference (PDIFF) option of SAS 2008. The model used was as follows:

$$Y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where Y_{ij} = response variable (growth performance, haematology, intestinal morphology, carcass traits, and fatty acid profiles), μ = overall mean, t_i = effect of diet level i, ε_{ij} = random error.

The model used for the analysis of the effect of diet, cooking method, gender and their interaction on sensory attributes was as follows;

$$Y_{ijkl} = \mu + d_i + cm_j + g_k + (d\ x\ cm)_{ij} + (d\ x\ g)_{jk} + (cm\ x\ g)_{jk} + (d\ x\ cm\ x\ g)_{ijk} + \varepsilon_{ijkl}$$

Where Y_{ij} = response variable (sensory evaluation scores), μ = overall mean, d_i = effect of diet level i, cm_j = effect of cooking method, g_k = effect of gender, $(d \times cm)_{ij}$ = diet by cooking method interaction, $(d \times g)_{ik}$ = diet by gender interaction, $(cm \times g)_{jk}$ = cooking method by gender interaction, $(d \times cm \times g)_{ijk}$ = diet level by cooking method by gender interaction, ε_{ijkl} = random error.

4 RESULTS

4.1 Growth performance and survival rates

The effect of inclusing *L. javanica* in the broilers diets on live weights is presented in Figure 4.1. At the beginning of the experiment, all chicks in the different treatment groups had similar initial BWT (7.2 g/chick). Between week 1 and week 3, a gradual increase in live weights of the chicks was observed across treatments. From week 3 to the end of the experiment at week 6, a sharp and dramatic increase in the live weights was observed. No differences ($P > 0.05$) in weekly weights were observed across the treatments throughout the experiment although the chickens in the negative control tended to have lower weights throughout the experiment. Cumulative body weight-gain followed a similar trend as the weekly live weights. The average daily feed intake (ADFI), daily gain (ADG), feed conversion ratio (FCR) and slaughter weights are presented in Table 4.2. The broilers fed *L. javanica* had significantly ($P < 0.05$) lower ADFI compared to the other two groups. However, the broilers in the positive control and those offered Ljav5 had significantly higher ADG and lower FCR. Slaughter weights were also higher in these 2 treatment groups compared with the negative control and Ljav12. The broilers in the negative control had the lowest average slaughter weights. In terms of survival rates, only a single death was recorded in the Ljav12 treatment group.

4.2 Haematological parameters

The haematological parameters of the broilers are presented in the Table 4.2. Values for red blood cell count (RBC), mean corpusular volume (MCV), mean cell haemoglobin (MCH), white blood cell count (WBC) and % neutrophils (%NEU), monocytes (%MONO) and eosinophils (%EOS) were significantly different ($P<0.05$) across treatments.

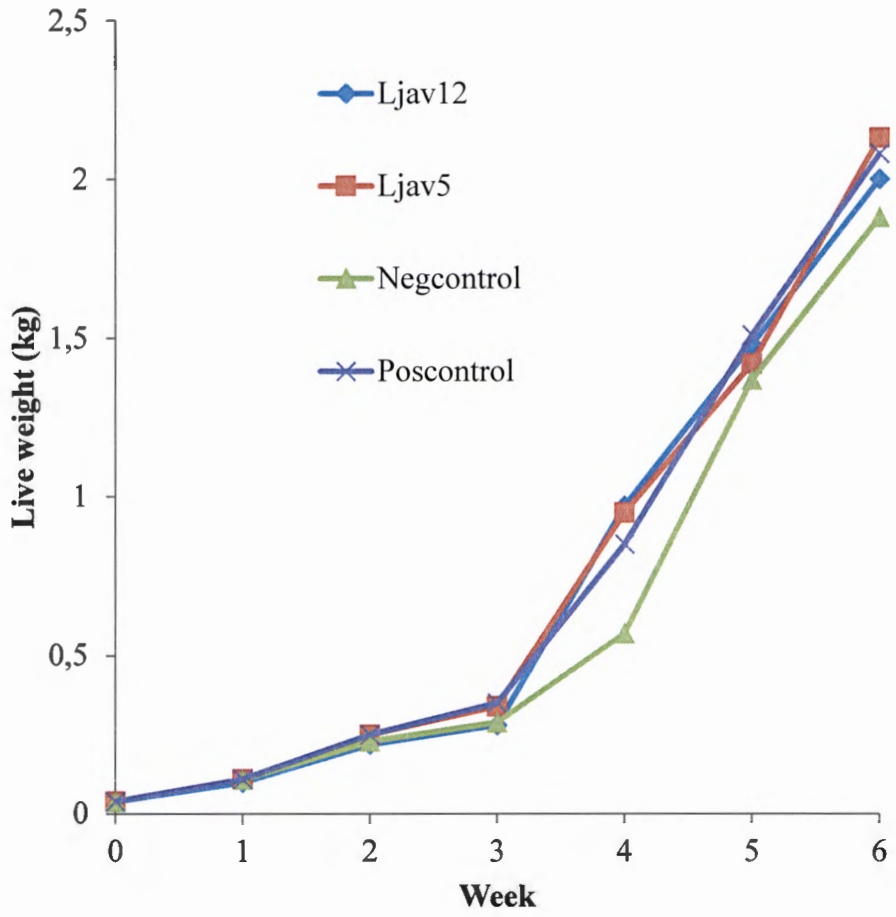


Figure 4. 1. Weekly live body weights for broilers receiving different levels of *L. Javanica* leaf powder. Dietary treatments; Negcontrol = commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic;*Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12)

Table 4. 1. Effects of feeding graded levels of *L. javanica* leaf meal on growth efficiency parameters of broilers chickens

Growth efficiency parameters	Dietary treatment[1]				
	Negcontrol	Poscontrol	Ljav5	Ljav12	SEM
Average daily feed intake (kg/day)	0.100[b]	0.099[b]	0.097[a]	0.097[a]	0.001
Average daily gain (kg/day)	0.045[a]	0.051[b]	0.052[b]	0.048[a]	0.002
Feed conversion ratio	2.192[b]	1.957[a]	1.889[a]	2.013[a]	0.069
Average slaughter weight (kg/day)	1.967[a]	2.163[c]	2.213[b]	2.035[a]	0.072

[abc]Means in the same row with different superscript are significantly different ($P<0.05$)
[1]Dietary treatments; Negcontrol = commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12)

Table 4. 2. Haematology indices of broilers fed different diets containing *Lippi javanica* leaf powder

Haematological parameters	Dietary treatments[2]				
	Negcontrol	Poscontrol	Ljav5	Ljav12	SEM
RBC (× 10^6/µl)	1.84[a]	2.05[ab]	2.10[b]	2.13[b]	4.23
Haematocrit (%)	26.1	27	28.6	29.6	0.58
HGB (mg/100Ml)	15.1	13.2	12.3	13.3	0.40
MCV (fl)	136.1[c]	121.0[a]	126.5[b]	120.5[a]	0.71
MCH (pg)	57.3[b]	51.6[a]	51.4[a]	51.3[a]	0.64
RDW (%)	1.34	0.79	0.96	0.79	0.52
WBC (× 10^3/µl)	24.5[a]	31.6[c]	27.7[b]	27.2[b]	1.09
NEU%	9.44[a]	20.7[b]	19.9[b]	18.9[b]	1.29
LYM%	10.53	4.64	5.31	10.38	50.61
MONO%	3.17[b]	1.65[a]	1.31[a]	4.25[b]	0.77
EOS%	1.06[b]	1.09[a]	1.15[b]	2.78[c]	0.51
BASO%	0.23	0.28	0.20	0.13	0.39
PLT	0.05	0.03	0.04	0.03	1.29

[a,b,c,d]Means in the same row with different superscript are significantly different. Haematological parameters: RBC = red blood cell; HGB = Haemoglobin; MCV = mean corpusular volume; MCH= mean cell haemoglobin; RWD=red cell distribution width;WBC= white blood cell count; NEU= neutrophils; LYM=Lymphocytes; MONO= monocytes; EOS= eosinophils; BASO= basophils; PLT= Platelet. [1]Dietary treatments; Negcontrol = commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic;*Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12)

However, hematocrit (hct), haemoglobin (hgb), red cell distribution width (rdw), lymphocytes % (lym%), basophils (baso%), lymphocyte (lym), basophils (baso) and platelet (plt) did not differ ($P<0.05$) in chickens offered the different dietary treatments. The negcontrol had the lowest RBC ($1.84 \times 10^6/\mu l$) while Ljav12 had the highest RBC ($2.13 \times 10^6/\mu l$). The negcontrol, however, had the highest ($P<0.05$) MCV (136fl) compared to the other treatments. The white blood cell was found to be highest ($P<0.05$) in Ljav5 ($31.6 \times 10^3/\mu l$) while the lowest value ($24.5 \times 10^3/\mu l$) was obtained in negcontrol. Neutrophils percentage and EOS also followed a similar trend as the WBC. Nevertheless, % MONO was found to be higher ($P<0.05$) in negcontrol compared with the other treatments.

4.3 Intestinal morphology and microbiota

Plate 4.1 shows the internal morphology of different sections of the intestines from broilers fed on the 4 different diets. No major differences were observed in terms of structures and arrangement of the microvilli and other cell structures in the epithelia of the duodenum, ileum and jejunum across treatments. No damages were also observed on the microvilli and any other cellular structures within the intestinal lumen. With regards to the microbiota identification, the main microorganism identified was the *E. coli*, which is normally found in healthy birds. No other microorganism of interest was identified. The *E. coli* was also found within bacterial populations as expected of healthy birds (<6 cfg/g) (Spring *et al.,* 2000).

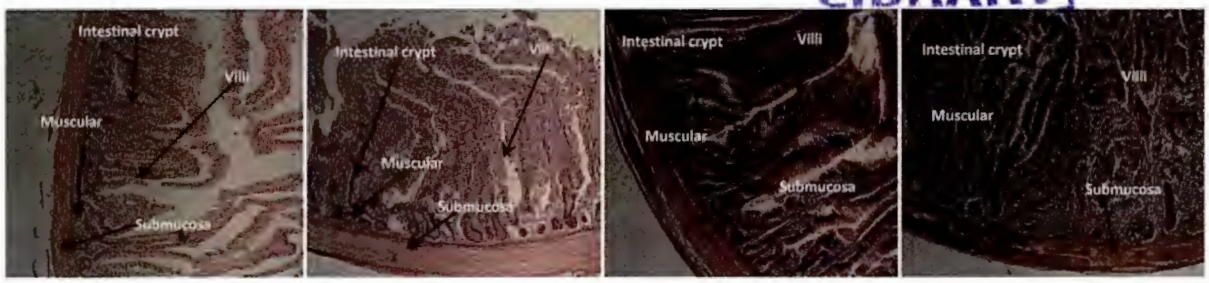
4.4 Internal organs Carcass characteristics

The results for the relative organ sizes are presented in Table 4.3. The weights of proventriculus, gizzard, small intestine, liver, pancreas, caecum and the small intestine length were significantly ($P<0.05$) affected by diet. Broilers that were fed 12 g/kg of *L. javanica* leaf

(a) Duodenum

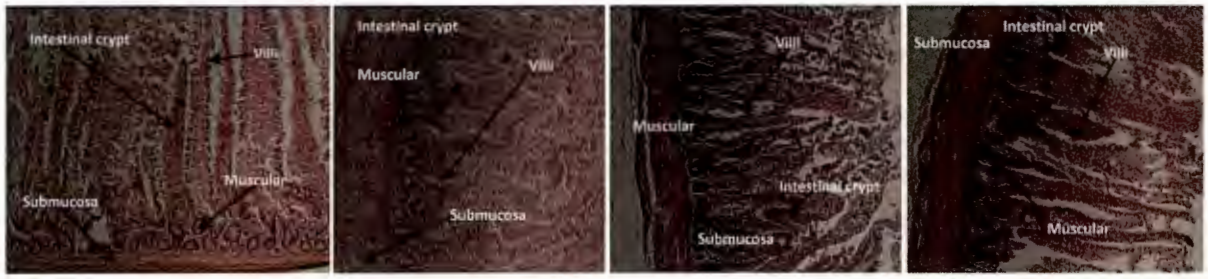
Negcontrol Poscontrol Ljav5 Ljav12

(b) Ileum



Negcontrol Poscontrol Ljav5 Ljav12

(c) Jejunum



Negcontrol Poscontrol Ljav5 Ljav12

Plate 4.1 Morphology of sections of duodenum (a), ileum (b), and jejunum (c) at 42 days in broiler chickens fed different treatment diets

Table 4. 3. The effect of *L. javanica* leaf powder on relative organ weight of broilers at 42 days (all units are recorded in grams)

Parameters	Negcontrol	Poscontrol	Ljav5	Ljav12	SE
Proventriculus	9.20[a]	10.50[a]	9.60[a]	12.70[b]	0.850
Gizzard	35.40[a]	34.10[a]	32.00[a]	46.00[b]	3.400
Small intestine weight	47.70[b]	33.00[a]	40.70[a]	54.00[b]	4.062
Small intestine length	153.80[b]	135.60[a]	121.00[a]	160.20[b]	6.598
Liver	36.70[b]	41.90[b]	36.30[b]	27.00[a]	2.371
Pancreas	3.40[a]	5.20b[a]	4.30[b]	3.00[a]	0.376
Heart	10.90	12.10	11.20	10.20	0.956
Caecum	15.40[b]	12.60[a]	15.50[b]	13.65[a]	0.851
Spleen	2.10	1.90	2.20	1.90	0.206

[a,b,c]Means in the same row with different superscript are significantly different. Means represent 6 chicks per treatment. Standard Error= SE. Dietary treatments; Negcontrol = commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12)

meal had the highest weights for the proventriculus and gizzard as well as the longest small intestines (P<0.05).Nevertheless, the broilers in the Ljav12 treatment group had the lowest weights for the liver and pancreas while the broilers in the poscontrol group had the highest (P<0.05). No differences were observed across the treatments in terms of heart and spleen sizes. *Lippia javanica* inclusion had no effect on the breast weight, thigh weight, carcass weight, and dressing percentage of the broilers (Table 4.4). However, significant differences were observed on the weight of abdominal fat pad, with broilers that received *L. javanica* leaf powder showing significantly higher abdominal fat weight compared with those whose diets did not contain *L. javanica* leaf powder.

Table 4. 4. The effect of *L. javanica* leaf powder on carcass characteristics of broiler chickens(all units are recorded in grams)

Parameters	Negcontrol	Poscontrol	Ljav5	Ljav12	SEM
Breast	355.6	401.3	384	357.8	36.6
Thigh	237.3	232.6	251.2	209.6	14.6
Abdominal fat	36.4^{a}	33.5^{a}	40.5^{b}	39.2^{b}	5.66
Carcass weight	1.49	1.51	1.47	1.48	0.06
Dressing %	77.7	79.9	76.8	78.5	9.21

[a,b,c]Means in the same row with different superscript are significantly different. Dietary treatments; Negcontrol = commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12). SEM = Standard error of the mean

4.5 Fatty acid profiles

The effect of diet on proximate composition of the subcutaneous adipose tissue of broilers is presented in Table 4.5. Diet significantly (P<0.05) affected subcutaneous fat % (SCF %). Birds on the poscontrol had the highest SCF% while those on the Ljav12 diet had the lowest SCF%. Diet, however, had no effect on fat free dry matter (FFDM) and moisture. The results on the composition of individual fatty acids (FAs) in the breast muscle of the broilers are presented in Table 4.6. Across treatments, oleic (30.9–33.9%), followed by palmitic (23.9–25.2%) and linoleic acid (15.8 –16.6 %) were the main FAs found in the breast muscle of the broilers. Diet had no effect on most of the FAs except for heptadecenoic (C17:1c10, eicosatrienoic [C20:3c8, 11, 14 (n-6)], docosadienoic [C22:2c13, 16(n-6)] and docosapentaenoic [C22:5c7, 10,13,16,19 (n-3)] acids. The negcontrol group had the highest value (P<0.05) for heptadecanoic acid while Ljav5 group had the lowest. With regards to eicosatrienoics (n-6), the Ljav12 group had the highest proportion (P < 0.05) whilst the poscontrol had the lowest proportion.

Table 4. 5. Proximate composition (%) of subcutaneous adipose tissue from broilers as affected by dietary treatment

Parameter[1]	Dietary Treatment[2]			
	Negcontrol	Poscontrol	Ljav5	Ljav12
SCF	$1.64^{b} \pm 0.12$	$1.88^{c} \pm 0.13$	$1.59^{b} \pm 0.12$	$1.47^{a} \pm 0.13$
FFDM	22.3 ± 0.29	22.1 ± 0.29	23.5 ± 0.29	22.3 ± 0.29
Moisture	76.1 ± 0.34	76.1 ± 0.34	75.3 ± 0.34	76.3 ± 0.34

[1]Parameter: SCF = Subcutaneous fat; FFDM = Fat free dry matter.
[2]Dietary treatment: negcontrol = Commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12).

Table 4. 6. Total fatty acid composition (%) of subcutaneous adipose tissue from broiler chickens as affected by dietary treatment

Fatty acids	Dietary treatments[1]				
	Negcontrol	Poscontrol	Ljav5	L.jav12	SEM
C14:0	0.3	0.32	0.33	0.3	0.01
C14:1c9	0.05	0.08	0.07	0.05	0.01
C15:0	0.1	0	0.01	0	0.01
C16:0	24.3	23.9	25.2	24.0	0.51
C16:1c9	5.15	5.74	5.28	4.97	0.35
C17:0	0.04	0.04	0.05	0.03	0.01
C17:1c10	0.51^{c}	0.39^{b}	0.27^{a}	0.49^{c}	0.05
C18:0	8.8	7.9	8.59	9.09	0.43
C18:1t9	0.01	0.04	0.02	0.03	0.01
C18:1c9	30.9	33.9	31.9	31.2	1.18
C18:1c7	5.05	5.23	5.16	5.1	0.10
C18:2c9,12 (n-6)	16.6	15.8	15.8	16.2	0.49
C20:0	0.14	0.09	0.1	0.13	0.04
C18:3c6,9,12 (n-3)	0.11	0.1	0.12	0.09	0.01
C20:1c11	0.3	0.29	0.31	0.31	0.02
C18:3c9,12,15 (n-3)	0.46	0.53	0.52	0.44	0.04
C20:2c11,14 (n-6)	0.31	0.2	0.27	0.33	0.03
C22:0	0.01	0	0.01	0.01	0.01
C20:3c8,11,14 (n-6)	1.06^{b}	0.78^{a}	0.91^{a}	1.09^{b}	0.09
C22:1c13	0	0	0	0	0.003
C20:4c5,8,11,14 (n-6)	4.89	3.71	4.11	4.96	0.47
C22:2c13,16 (n-6)	0.02^{b}	0.1^{c}	0.02^{b}	0.01^{a}	0.04
C20:5c5,8,11,14,17 (n-3)	0.39	0.23	0.36	0.39	0.05
C24:1c15	0	0	0	0.02^{b}	0.01
C22:5c7,10,13,16,19 (n-3)	0.33^{b}	0.42^{a}	0.44^{b}	0.47^{b}	0.05
C22:6c4,7,10,13,16,19 (n-3)	0.21	0.18	0.23	0.24	0.04

[a,b,c]Means in the same row with different superscript are significantly different ($P<0.05$).

[1]Dietary treatments: negcontrol = Commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12).

Additionally, the proportions of docosadienoic, another n-6 FA, were highest ($P < 0.05$) in the poscontrol group and lowest in the Ljav5 and negcontrol groups. Most of the n-3 FAs were not affected by diets except for the docosapentaenoic, which was found to be higher ($P < 0.05$) in the Ljav5 treatment group and lowest in the negcontrol. The effects of diet on total saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) fatty acids, omega-3 (n-3) fatty acids, PUFA: SFA and n-6: n-3 ratios of broiler breasts are presented in Table 4.7. The broilers in the Negcontrol group and Poscontrol had a significantly ($P<0.05$) higher total SFA compared to the Lippia fed broilers. On the contrary, the broilers in the lippia-containing treatment groups had higher ($P<0.05$) total PUFA, total n-3 FAs and PUFA: SFA ratio and also had significantly lower n-6:n-3 ratios compared to the other two treatment groups. No differences were however, observed with regards to total MUFA and total n – 6 FAs.

Table 4. 7. Total (%) and fatty acid ratios of subcutaneous adipose tissue from broiler chickens as affected by dietary treatment

Fatty acids[1]	Dietary treatments[2]				
	Negcontrol	Poscontrol	Ljav5	Ljav12	SEM
Total SFA	33.5[ab]	34.3[a]	32.3[b]	33.5[ab]	0.62
Total MUFA	42.1	45.7	42.9	42.3	1.34
Total PUFA	22.3[a]	22.1[a]	24.7[b]	24.2[b]	1.02
Total n-6	22.8	20.6	21.1	22.6	0.99
Total n-3	1.59[a]	1.37[a]	1.65[b]	1.64[b]	0.08
PUFA:SFA	0.66[a]	0.68[a]	0.73[b]	0.72[b]	0.03
n-6: n-3	14.3[b]	15.36[b]	12.9[a]	13.9[a]	0.82

[ab]Means in the same row with different superscript are significantly different ($P<0.05$).

[1] Fatty acids: SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6 = omega-6 fatty acids; n-3 = omega-3 fatty acids.
[2]Dietary treatments: negcontrol = Commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12).

4.6 Sensory evaluation

The effects of diet, cooking method, gender and their interactions on various sensory attributes of broiler meat are shown on Tables 4.8 and 4.9. The results from the current study showed that diet and its interaction with cooking method significantly affected sensory scores of both the boiled and fried meat ($P<0.05$). Notably, in all treatments, the boiled meat was rated more superior than the fried meat (Table 4.8). Of particular interest were the high scores for muscle fibre and overall tenderness and amount of connective tissue given to the Ljav5 and Ljav12 treatment groups, which indicated that the meat was tenderer with less connective tissue in it (Table 4.8). Significant interaction effects of gender and cooking method on sensory scores were also obtained (Table 4.9). Notably, male consumers appeared to give superior scores to most of the sensory attributes across treatments irregardless of the cooking method.

Table 4. 8. Effect of *Lippia javanica* inclusion and cooking method on consumer sensory scores of meat from broiler chickens.

sory ibutes[1]	Dietary treatments[2]							
	Negcontrol		Poscontrol		Ljav5		Ljav12	
	Boiled	Fried	Boiled	Fried	Boiled	Fried	Boiled	Fried
OI	4.53^{b}±0.32	3.59^{a}±0.32	4.14^{b}±0.32	4.34^{b}±0.32	4.37^{b}±0.32	4.04^{b}±0.32	4.43^{b}±0.32	3.83^{b}±0.32
	5.02^{b}±0.27	3.22^{a}±0.27	4.66^{b}±0.27	4.53^{b}±0.27	4.99^{b}±0.27	3.76^{a}±0.27	5.51^{b}±0.27	3.04^{a}±0.27
	3.54^{a}±0.27	3.77^{a}±0.27	3.80^{a}±0.27	4.76^{b}±0.27	4.36^{b}±0.27	4.13^{ab}±0.27	5.44^{bc}±0.27	3.48^{a}±0.27
	4.52^{ab}±0.30	3.25^{a}±0.30	4.44^{a}±0.30	4.64^{bc}±0.30	4.76^{bc}±0.30	3.66^{a}±0.30	3.64^{ab}±0.30	3.77^{a}±0.30
OT	5.28^{c}±0.26	3.52^{a}±0.26	4.97^{b}±0.26	4.67^{b}±0.26	5.02^{c}±0.26	4.02^{b}±0.26	5.81^{a}±0.26	3.87^{a}±0.26
T	4.82^{a}±0.28	4.46^{a}±0.28	4.84^{a}±0.28	5.40^{b}±0.28	4.65^{a}±0.28	5.58^{b}±0.28	5.47^{bc}±0.28	5.17^{b}±0.28
	5.34^{c}±0.28	4.40^{b}±0.28	5.36^{c}±0.28	5.16^{c}±0.28	4.88^{b}±0.28	4.51^{b}±0.28	5.77^{c}±0.28	3.97^{a}±0.28
YPIFI	3.19^{b}±0.40	2.93^{a}±0.40	3.21^{b}±0.40	3.43^{b}±0.40	3.66^{b}±0.40	3.15^{b}±0.40	3.86^{b}±0.40	2.44^{a}±0.40

[Ab]Means in the same row with different superscript are significantly different ($P<0.05$)

[1]Sensory attributes: AROI = Aroma intensity, FB = First bite, IJ = Initial impression juiciness, SJ = Sustained impression of juices, MFOT = Muscle fiber & overall tenderness, ACT = Amount if connective tissue, OFI = Overall flavour intensity, TYPIFI = Typical flavour intensity.

[2]Dietary treatments: negcontrol = Commercial broiler diet without prophylactic antibiotic; poscontrol = commercial broiler diet with prophylactic antibiotic; *Lippia javanica* leaf powder 5 g/kg (Ljav5); *Lippia javanica* leaf powder 12 g/kg (Ljav12).

Table 4. 9. Effect of consumer gender and cooking method on sensory scores of broilers.

Sensory attributes	Female		Male	
	Boiled	Fried	Boiled	Fried
Aroma intensity	4.33±0.21	4.07±0.21	4.43±0.24	3.81±0.24
First bite	5.11^{d}±0.18	3.53^{a}±0.18	4.96^{c}±0.20	3.76^{b}±0.20
Initial impression juiciness	3.91^{a}±0.18	3.92^{a}±0.18	4.69^{b}±0.20	4.11^{a}±0.20
Sustained impression of juiciness	4.49^{c}±0.20	3.79^{b}±0.20	5.23^{d}±0.23	3.83^{a}±0.23
Muscle fiber & overall tenderness	5.28^{d}±0.17	4.05^{b}±0.17	5.26^{c}±0.19	4.01^{a}±0.19
Amount if connective tissue	4.59^{a}±0.20	5.09^{b}±0.20	5.34^{b}±0.23	5.17^{a}±0.23
Overall flavour intensity	5.56^{c}±0.19	4.41^{a}±0.19	5.08^{b}±0.21	4.67^{a}±0.21
Typical flavour intensity	3.78^{a}±0.27	3.43^{b}±0. 27	3.18^{a}±0.30	2.54^{a}±0.30

^{Ab}Means in the same row with different superscript are significantly different ($P<0.05$); ^{abcd}Means with the same superscript are not significant different

5 DISCUSSION

5.1 Growth performance and survival rates

The current study was conducted to evaluate the potential of *L. javanica* as an alternative growth promotant and antibiotic in broilers. The results revealed that the broilers fed *L. javanica* had lower ADFI compared to the positive control. Despite this, the broilers fed 5 g/kg *L. javanica* had similar ADG and slaughter weights as those in the positive control. This indicates that *L. javanica* improved the feed efficiency given the reduced feed intake and improved the ADG and ultimately slaughter weights. This is consistent with the findings of Hong *et al.* (2012) that made similar observations in broilers fed essential oils from some natural plants (oregano, anis and citrus peel). Toghyani *et al.*, (2011) and Khodanazary *et al.*, (2013) also demonstrated that natural plant supplements such as cinnamon in broilers provided satisfactory results on performance indices and may have the potential to be applied as an alternative for in-feed antibiotics. Notably, broiler chickens that were fed 12 g/kg of *L. javanica* leaf powder showed the lowest feed intake and highest FCR over the course of the experimental period. This could be attributed to the increased amount of fibre in the diet, which could have affected feed intake. With high fibre diets, it has been observed that broilers tend to increase feed intake as a way to compensate for the reduced nutrient concentration in feed (Edwards, 1995; Walugembe *et al.,* 2014). However, the increased feed intake coupled with reduced degradation of the fibrous diets in chickens inevitably results in increased bulk of digesta in the intestinal tract. This ultimately leads to the withdrawal from the feed by the broilers and hence feed intake is affected (Walugembe *et al.,* 2014). This might imply that at such inclusion levels and above, growth parameters may be affected and hence higher inclusion levels have to be used with caution. Nevertheless, the inclusion levels of *L. javanica* were too low to raise any alarm with regards to digestion and absorption of feed. Generally, fibre levels above 100g/kg have been observed to affect feed utilisation (Hetland and Svihus, 2001; Hetland *et*

al., 2002). The fact that a single mortality was recorded over the experimental period is commendable as this reflects the generally good hygienic and biosecurity practices that were being observed throughout the study

5.2 Haematological parameters

Results on haematology of the broilers blood showed that *L. javanica* has a favourable effect on red blood cell (haematopoiesis) and white blood cell (erythropoiesis) formation. This is reflected by the increased red blood cell count and high haematocrit percentage in broilers offered diets containing the *L. javanica* leaf powder. This might have a significant positive effect on the general health of the broilers. Plants such as *Nigella sativa* with similar effects as *L. javanica* have been found to significantly decrease haematological disorders (Abdel-Wahhab and Aly, 2005; Talebi *et al.*, 2005; Toghyani *et al.,* 2010). In addition to increased red blood cell count, diets with *L. javanica* also improved the white blood cell count, neutrophils and eosinophils, which play a very important role in the immune system of the broilers. This is consistent with the findings of Meral *et al,.* (2004) and Justine and Oluwatosin (2008) who observed that certain plant extracts can have positive influences on RBC, WBC and PCV in animals. The desirable effect of *L. javanica* could be due to the presence of highly active components with strong antioxidant characteristics that positively influences the general health of the animals (Arslan *et al.,* 2004). Active components in *L. javanica* oils such as terpenoids (particularly carvacrol and thymol), have been shown to be effective inhibitors of microbial oxygen uptake and oxidative phosphorylation (Viljoen *et al.* (2005). In addition, other phenolic compounds contained in *L. javanica* such as flavonoids, including isogancaonin C, bolusanthin III, bolusanthin IV, derrone, medicarpan, genitein and gancaonin have shown antibacterial potency and have been observed to strongly inhibit *E. coli, B. subtilis, S. aureus,* and *Candida mycoderma* (Erasto *et al.*, 2004; Viljoen *et al.*, 2005).

5.3 Intestinal traits and microbiota

Results on structure and arrangement of the microvilli and other cell structures in the epithelia of the duodenum, ileum and jejunum revealed no observable damage due the activities of microorganism within the gut or the phytochemical components of *L. javanica*. This could have been due to the absence of infectious levels of bacteria within the gut. From the results, the main microorganism identified was *E coli,* whose levels were within the normal range for healthy birds. Generally, extracts and oils from *L. javanica* and other associated plants are known to have antimicrobial effects in the gastrointestinal systems of chickens (Burt, 2004; Si *et al.,* 2006; Hong *et al.,* 2012). The lack of alteration in the intestinal epithelia and absence of microbiota in the intestines could also be a reflection of optimum housing conditions and high level of biosecurity observed during the experiment.

5.4 Internal organs and carcass characteristics

The results of the relative organ weights showing the significant effect of *L. javanica* inclusion on some internal organs are contrary to previous studies, in which there were no differences in relative organ weights caused by essential oils or other herbal extracts (Hernandez *et al.,* 2004; Çabuk *et al.,* 2006; Demir *et al.,* 2008; Amad *et al.,* 2011 and Kirkpinar *et al.,* 2011). Nevertheless, the observed high weights of the proventriculus and gizzard as well as the longer small intestines observed in the broilers fed *L. javanica* at the rate of 12 g/kg could be a result of an adaptive mechanism to deal with the increased amounts of feed that would ultimately optimise digestion and absorption. However, this needs further verification since the high weights of some of the internal organs are not supported by a concomitant increase in size of the liver, which plays an important role in the detoxification of increased amounts of phytochemical in the feed (Adamu *et al.*, (2012). Alternatively, it may be suggested that the amounts of phytochemicals were insignificant to cause an increase the surface area in the liver

(Dotas *et al.,* 2014). The lack of effect of *L. javanica* inclusion levels on carcass yield, dressing percentage, thigh and breast weight is consistent with findings from similar studies elsewhere (Moschini *et al.,* 2005; Nikolakakis *et al.,* 2005; Dotas *et al.,* 2014). Nevertheless, the observed high abdominal fat values for the broilers fed *L. javanica* could be a cause for concerns with regards to the high amounts of undesirable fat.

5.5 Fatty acids analysis

Results for the profiles of long chain fatty acids of the broilers revealed that oleic acid (C18:1t9) was the most abundant followed by palmitic (C16:0) and linoleic acid (C18:2C9, 12). Similar observations were made in broilers fed *Camelina sativa* oil by Jaskiewicz *et al.,* (2014). Conjugated linoleic acid has been found to affect body composition, specifically a reduction in body fat mass and an increase in lean body mass. Specifically it has been found to have anticarcinogenic, antiatherogenic, antidiabetogenic, and immune modulating properties (Rainer and Heiss, 2004). While palmitic acid has been found to increase blood cholesterol level, oleic has been found to have an opposite effect on the blood cholesterol levels (Pena *et al.,* 2009). Finding from the study also showed that the broilers receiving *L. javanica* free diets have significantly higher proportions of some n-6 fatty acids. Interestingly and in contrast, the broilers receiving *L. javanica* diets had high proportions of some n-3 fatty acids, which are critical with regards to human health.

Generally, plasma cholesterol concentration is influenced by the FA composition of dietary fat with high levels of long chain SFA increasing plasma cholesterol level compared to high levels of MUFA and PUFA (Grundy and Denke 1990; Muchenje *et al.,* 2009; Banskalieva *et al.* 2000;; Marume *et al.,* 2012). The present study reveals that, generally, the broilers receiving *L. javanica* in their diets had lower total SFA, higher total PUFA and total n-3 fatty acids which

may have potential benefits to human nutrition. Moreover, the PUFA: SFA ratios were high in broilers receiving *L. javanica* whilst the n-6: n-3 ratios were significantly lower. The PUFA/SFA and *n*-6/*n*-3 ratios are commonly used to evaluate the nutritional and health value of meat for human consumption (Aldai *et al.* 2007; Alfaia *et al.* 2007). The recommended PUFA/SFA ratio in human diets should be above 0.4 (Higgs 2002). In the present study, the PUFA/SFA ratios obtained from all treatments were above the recommended value although the higher amounts in the *Lippia*-fed broilers could significantly decrease the chances of development of cardiovascular and some chronic (Grundy and Denke 1990; Banskalieva *et al.* 2000; Mapiye *et al.* 2011). Although the *Lippia* fed broilers had significantly lower n-6/n-3 ratios than the other two, it appears that all the ratios were well above the recommended n-6: n-3 ratio of 5:1 in human diets. However, this could be characteristics of broiler meat as similar observations were made by Jaskiewicz *et al.,* (2014).

5.6 Sensory evaluation

The fact that meat from broilers fed *L. javanica* was rated superior is significant particularly with regards to its influence on intramuscular fat composition and hence sensory characteristics of the meat. Tenderness and juiciness are complex attributes that are often affected by diet and intramuscular fat composition of meat (Calkins and Hodgen, 2007). It appears, therefore, *L. javanica* was able to increase and modify the intramuscular fat thereby increasing the marbling of the meat. This can also be confirmed by high abdominal fat pad observed in the *Lippia* fed broilers in the current study. Of particular interest were the high ratings for muscle fibre intensity and overall tenderness and connective tissue given to the *Lippia* fed broilers which confirms the favourable effect of *L. javanica* on the sensory quality of the meat. Ideally, meat quality levels should combine the capacity to retain high nutritional value and to excel in functional roles such as tenderness and juiciness of the cooked product among other roles (Calkins and Hodgen 2007; Muchenje *et al.* 2008; Marume *et al.,* 2012).

The results on the effect of cooking method on sensory attributes showing that boiled meat received higher ratings in all treatment groups are in conflict with the finding by Xazela *et al.,* (2011) who observed that fried meat had higher scores than boiled meat. This could be attributed to differences in backgrounds. The consumer panel used by Xazela *et al.,* (2011) were mainly of Xhosa background whereas the one used in the current study were mainly of the South African Tswana community. Observations have previously been made that background and attitudes of consumers influence how consumers perceive different preparations of meat (Nicklaus *et al.,* 2004). Moreover, relationships have been observed between age, gender, education and place of living with regards to perceptions of different cooking methods (Radderand le Roux, 2005). This could also explain why, in the current study, males were giving superior scores to most of the sensory attributes across treatments.

6 CONCLUSION

The findings from the current study revealed that supplementing feed with *L. javanica* can positively affect ADG and slaughter weights of broilers. The broilers fed Ljav5 obtained the highest final body weight amongst the other treatment groups. Results on the haematology of broilers blood appear to give an impression that *L. javanica* has a favourable effect on red blood cell (haematopoiesis) and white blood cell (erythropoiesis) formation. Results on the structure and arrangement of the microvilli showed no major difference observed among the treatments and no damage was observed on the microvilli. The observed high weights for the proventriculus and the gizzard as well as longer small intestines observed in the broilers fed Ljav12 could be a result of an adaptive mechanism to deal with the increased amounts of fibre and phytochemicals that would ultimately optimise digestion and absorption. Finding from the study also showed that the broilers receiving *L. javanica* free diets have significantly higher

proportions of some n-6 fatty acids. Interestingly and in contrast, the broilers receiving *L. javanica* diets had high proportions of some n-3 fatty acids which are critical with regards to human health. Meat from broilers fed *L. javanica* was rated as superior. The high ratings for muscle fibre intensity and overall tenderness and connective tissue given to the *Lippia* fed broilers confirmed the favourable effect of *L. javanica* on the sensory quality of the meat. Overall, the findings from the study tentatively confirm the efficacy of the use of *L. javanica* as both an antibiotic and growth promotant, although further investigations needs to be done to confirm this. In addition, further investigations are needed to test *L. javanica* leaf powder in the indigenous chickens to observe their performance. Also a comparison can also be done on both broilers and indigenous chickens

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8 APPENDICES

Appendix 1. Meat Sensory Evaluation form

Evaluation sheet: growth performance, carcass characteristics, and meat quality in broilers supplemented with fever tea *(Lippia javanica)* leaf powder

Each panellist was receiving two evaluation forms as there was two different method of cooking meat.

Meat Sensory Evaluation Form

Animal Science Program

School of Agricultural Sciences

Faculty of Agriculture, Science and Technology

North-West University

Mafikeng Campus

Meat Sensory Evaluation Form

Choose your answer by marking with an "X" **Date; .....**

Age;

≤ 20	
21-25	
26-30	
≥ 30	

Ethnic group;

Xhosa;.....,Tswana;.....,Sotho;.....,Zulu;.....Venda;.....,Shona;.....Pedi;.....Swati;.....,-

Other; .....

Gender;

Male....., Female.....

Please evaluate the following samples of meat for the designated characteristics.

Choose your answer between 1 & 8 and write it on the block written "answers";

	Characteristics	**Rating scale**	**ANSWERS**			
			TRT 1	**TRT 2**	**TRT 3**	**TRT 4**
1	**Aroma intensity;** Take a few short sniffs as soon as you remove the foil. Typical chicken aroma	**1=Extremely bland** **2= Very bland** **3= Fairly bland** **4= Slightly bland** **5=Slightly intense** **6= Fairly intense**				

		7= Very intense **8=Extremely intense**				
2	**Initial impression of juiciness;** The amount of fluid exuded on the cut surface when pressed between the thumb and forefinger	**1= Extremely dry** **2= Very dry** **3= Fairly dry** **4= Slightly dry** **5=Slightly juicy** **6= Fairly juicy** **7= Very juicy** **8=Extremely juicy**				
3	**First bite;** The impression that you form on the first bite	**1=Extremely tough** **2= Very tough** **3= Fairly tough** **4= Slightly tough** **5=Slightly tender** **6= Fairly tender** **7= Very tender** **8=Extremely tender**				
4	**Sustained impression of juiciness;** The impression of juiciness that you form as you start chewing	**1= Extremely dry** **2= Very dry** **3= Fairly dry** **4= Slightly dry**				

		5=Slightly juicy **6= Fairly juicy** **7= Very juicy** **8=Extremely juicy**				
5	**Muscle fibre & overall tenderness;** Chew sample with a light chewing action	**1=Extremely tough** **2= Very tough** **3= Fairly tough** **4= Slightly tough** **5=Slightly tender** **6= Fairly tender** **7= Very tender** **8=Extremely tender**				
6	**Amount if connective tissue (Residue);** The chewiness of the meat	**1=Extremely abundant** **2= Very abundant** **3=Excessive amount** **4= Moderate** **5= Slight** **6= Traces** **7= Practically none** **8= None**				

7	**Overall flavour intensity;** This is the combination of taste while chewing and swallowing- referring to the typical chicken flavour	**1=Extremely bland** **2= Very bland** **3= Fairly bland** **4= Slightly bland** **5=Slightly intense** **6= Fairly intense** **7= Very intense** **8=Extremely intense**				
8	**A- Typical flavour intensity**	**1= None** **2= Practically none** **3= Traces** **4= Moderate** **5= Slightly intense** **6= Fairly intense** **7= Very intense** **8=Extremely intense**				

ANY COMMENTS;

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