GROWTH PERFORMANCE, BLOOD PARAMETERS, CARCASS CHARACTERISTICS AND MEAT QUALITY OF POTCHEFSTROOM KOEKOEK CHICKENS SUPPLEMENTED WITH *LIPPIA JAVANICA* LEAF MEAL

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BSc Animal Science

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DEDICATION

I whole heartedly dedicate this thesis to each and every South African child who lives in absolute poverty, born by two, but raised by a single, illiterate and unemployed parent. This should prove that despite their difficult situation, with enough intent, action, focus, faith, dedication and commitment, they too, can achieve their aspirations.

DECLARATION

I, Tumisang Ben Matshogo, hereby declare that this dissertation is entirely my original work with the exception of references that have been attributed to their authors or sources. This thesis has never been submitted for any degree of examination at any other university.

Candidate's Signature.....

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ABSTRACT

A feeding trial was conducted for a period of 16 weeks, with the objective of determining the effect of *Lippia javanica* leaf meal supplementation on the growth performance, blood parameters, carcass characteristics and meat quality of male Potchefstroom koekoek (PK) chickens. One hundred and sixty male PK chickens were reared at the North-West University Farm. *Lippia javanica* (LJ) leaf meal was chemically analysed and added to a commercial grower diet at a rate of 25 g LJ /kg diet or 50 g LJ /kg diet. An additional two diets, a positive control (commercial grower diet with antibiotics) and a negative control (commercial grower diet without antibiotics) were formulated and thus bringing to four, the total number of dietary treatments. The birds were raised on a commercial starter mash for 4 weeks. At the start of the fifth week, the four experimental diets were offered to the chickens. The experimental unit was a pen holding 8 chickens, which was replicated 5 times per treatment, resulting in a total of 20 floor pens. In week 9, chickens fed diet LJ25 had the highest feed intakes (520.08 ± 18.89 g/kg) compared to other diets. The results on cumulative weight gain (CWG) indicated, a gradual increase in the live weights after week 9 was observed across all treatments. From week 9 to week 15 there was no significant difference in CWG among the birds across dietary treatments. There was a huge decline in feed conversion efficiency (FCE) in all treatments between week 5 and week 6 and a slight decrease in feed conversion efficiency in all treatments thereafter. There were no significant differences in overall FCE between all the treatments ($P > 0.05$). Dietary treatments had no effect ($P > 0.05$) on serum total protein, urea, creatinine, triglycerides, and calcium concentration. However, diet significantly affected ALT, AST, bilirubin, sodium, potassium, cholesterol and magnesium concentration. It was observed that for ALT, chickens offered LJ50 had lowest level (0.10 ± 0.22 IU/L) followed by those on CON- (0.20 ± 0.22 IU/L). The inclusion of *L. javanica* in the diet had no significant influence on haemoglobin concentration and monocytes. However, there were significant differences

with respect to HCT, neutrophils, lymphocytes and normoblasts. It was observed that HCT values were higher in LJ25 and LJ50 (0.39 ± 0.01) chickens compared to CON+ (0.36 ± 0.01) and CON- (0.38 ± 0.01) chickens. Chickens offered LJ25 had the highest lymphocytes value (14.94 ± 1.17 x 10^9/l) compared to chickens fed other dietary treatments. Supplementing chicken with feed containing 50 g/kg of *L. javanica* leaf meal significantly increased carcass mass ($P < 0.05$). However, full gizzard and empty gizzard weights in CON+ and LJ25 chickens were higher ($P < 0.05$) compared to CON- and LJ50 chickens. Meat from CON- (6.07) and LJ50 (6.08) chickens had higher pH than in CON+ (5.9) and LJ25 (5.8) chickens. With regards to meat colour, breast muscle in CON- (53.2) had the highest ($P < 0.05$) lightness (L*) values, while the meat from C0N+, Lj25 and Lj50 the lower values. The addition of *L. javanica* in chicken diets showed no negative effects on the mass of breasts and thighs. The data obtained from this study showed that *L. javanica* can be included in indigenous chicken diets as a nutraceutical without any harmful effect on growth performance, blood parameters and carcass characteristics.

Key words: Growth performance, blood parameters, carcass characteristics, meat quality and *Lippia javanica* leaf meal

LIST OF ABBREVIATIONS

a*	Redness
b*	Yellowness
L*	Lightness
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AOAC	Association of Analytical Chemistry
CP	Crude Protein
CF	Crude Fibre
DM	Dry Matter
GLM	General Linear Model
N	Nitrogen
NDF	Nutrient Detergent Fibre
PK	Potchefstroom Koekoek
SLW	Slaughter Weight
FCE	Feed Conversion Efficiency
NRC	National Research Council
WHC	Water Holding Capacity

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1 CHAPTER ONE - INTRODUCTION

1.1 Background

In rural areas, indigenous chickens are a very important source of income and animal protein in the form of eggs and meat. According to Mtileni *et al.*, (2011), indigenous chickens are also considered to be an important genetic resource for local and commercial farmers. These native chickens' attributes are well-documented and their importance is based on natural scavenging, nesting habits, resistance to disease, tolerance to harsh growing conditions, and a long life span (Hoffmann, 2005; Van Marle-Köster *et al.*, 2009). These important attributes have driven renewed efforts to conserve the indigenous chicken lines and to improve their production and contribution to household food and nutrition security. Indigenous chickens have the potential to provide rural communities with food by converting accessible, cheap feed resources into usable protein in the form of eggs and meat. However, their productivity is considered to be low mainly due to poor management, which result in high mortality rate and slow growth rates. According to Alders *et al.* (2001) and Swatson *et al.* (2001), the main reasons for their poor productivity are poor quality of feed resources, high disease incidences, inadequate foraging ranges and poor management practices by farmers. It is, therefore, important to identify and evaluate alternative, locally available feed resources that may have nutraceutical properties in order to reduce feed costs and improve productivity of indigenous chickens.

According to Choct (2012), the feed intake by an individual animal is a function of the amount of feed offered and the nutrient levels in the diet. Therefore, successful chicken production is dependent upon supplying the birds with a proper feed of the highest quality (Arbor Acres, 2009). In addition, the utilization of non-conventional plants as a source of nutrients as well as health-promoting plant bioactive compounds, could be a solution to problem of poor nutrition and health management. Phytogenic flora and herbal products have traditionally been utilized

in farming against some animal diseases (Viljoen *et al.*, 2005; Ghalamkari *et al.*, 2012). Plants such as *Lippia javanica* that are widely distributed in southern Africa have been used extensively as a source of therapeutic compounds as well as nutritional additives (Viljoen *et al.*, 2005; Oliveira *et al.*, 2007).

In poultry production, feed additives have been used to improve growth rate, health and feed consumption (Engberg *et al.,* 2000; Landy *et al.,* 2011). It is, therefore, important to explore alternative plants which can be utilized as feed additives to improve feed utilization and growth. Some herbal products have received extra attention (Toghyani *et al.,* 2010; Landy *et al.,* 2011; Ghalamkari *et al.,* 2012; Hong *et al.*, 2012) for example *L. Javanica*. *Lippia javanica* has been utilized as an ethno-veterinary drug to treat a wide array of infections that affect the health of livestock mainly in communal areas (Muyima *et al.,* 2004). It is, therefore, important to assess *L. javanica* as a potential alternative feed additive in the production of indigenous chicken. In South Africa, studies to determine the growth and physiological responses of indigenous chickens fed *L. javanica* leaf meal have not been done. This study was, therefore, designed to investigate the effect of *L. javanica* leaf meal, as a dietary additive, on growth performance, haematological profile, serum biochemical indices, carcass characteristics, and meat quality attributes of Potchefstroom koekoek chickens.

1.2 Objectives

The objectives of the study were to:

1 Chemically characterize of *L. javanica* leaf meal and the formulated *L. javanica*-based diets.

2 Determine the effect of *L. javanica* leaf meal supplementation on growth performance and diet utilization in Potchefstroom Koekoek chicken.

3 Examine the haematological parameters and serum biochemical indices of Potchefstroom Koekoek chicken feed diets containing graded level of *L. javanica* leaf meal.

4 Determine the effect of dietary *L. javanica* leaf meal on carcass characteristics and meat quality of the indigenous chicken.

1.3 Research hypothesis

The study was designed to test the null hypothesis that supplementation of Potchefstroom koekoek diets with *L. javanica* leaf meal has no effect on growth performance, haematological indices, serum biochemistry, and meat quality of Potchefstroom koekoek chickens.

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2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

According to Andrews (1991) and Jalaludin (1992), indigenous chickens (*Gallus domesticus*) are the biggest poultry species in rural communities of Africa. They are mainly kept for nutritional, economic and social purposes (Sonaiya *et al.*, 1999). These chickens form a fundamental part of the farming systems in rural communities. However, the number of native chickens reared in households varies significantly, depending on the farmer's managerial skills and availability of resources. Indigenous chickens were first introduced to South Africa in the early 1600's by the early settlers and traders. According to Van Marle-Köster *et al.*, (2009), indigenous chickens are dual purpose birds that have not been exposed to artificial selection in formal breeding programs. They have low production potential when compared to commercial broilers and layers, mainly due to differences in growth rate and feed conversion efficiency. Consequently, the rearing of indigenous chicken is not common among commercial producers. However, indigenous chickens have several advantages, including an inherent ability to scavenge for feed, better resistance to disease, and ability to thrive in harsh growing conditions (Hoffmann, 2005; Van Marle-Köster *et al.*, 2009).

The majority of farmers in South Africa normally rear indigenous chickens using an extensive scavenging system. The chickens select a diet constituting of materials from the surrounding environment, by-products from harvested plant materials and processing of grains as well as cultivated and wild vegetation. The chickens survive under these rather harsh nutritional and environmental conditions (Tadelle & Ogle, 2000). However, it has been shown that their productivity under scavenging conditions is very low (Alders *et al.,* 2001). Possibly the main constraint for indigenous chicken production on a large scale is the wide variations in their productivity when compared to broiler chickens. However, as recommended by Hutanuwat

(1988), there is a need to increase the production potential of indigenous chickens in order to ensure sufficient nutrition for the growing human population. Thus, to ensure sufficient food security, intensive and semi-intensive farming systems seem to be the most viable options to improve the birds' productivity. Hence, there is a need to understand their nutrient requirements under intensive farming systems in order to build up suitable nutritional intervention strategies for optimal growth. With the intensive farming system there is less activities performed by chickens compared to extensive farming as such the growth rate of the chickens can be improved, furthermore, less energy is used under intensive farming. However, because of the chickens' slow growth rate, there is a need to search for less-expensive feed resources with nutraceutical properties that can be used in intensive production systems designed for indigenous chickens. This would guarantee the profitability of the proposed intensification of indigenous chicken production.

2.2 Characteristics of indigenous chickens

Indigenous chickens are genetically slow-growing when compared to broiler chickens, mainly due to balanced nutrition and regular veterinary service. The variation in growth rate and production ability is genetically determined (Nordskog & Briggs, 1967). Indigenous chickens are an integral part of the low-input farming systems (Kitalyi, 1999). However, these production systems have several constraints such as poor management, poor nutrition and disease outbreaks (Permin & Hansen, 1998). Indeed, according to Pousga *et al.* (2005) the production output of indigenous chickens is very low mainly due to the poor genetic potential of the chicken, poor feeding and poor management practices.

Indigenous chickens supply both meat and eggs for human consumption. They are broody and are able to look after their own chicks (Horst, 1989). However, these chickens can only lay a

few eggs per annum, which normally weigh close to 43 grams (Ramlah, 1996). According to (Awuni, 1989) the hen can produce about four clutches per year composed of up to ten eggs per clutch. As concluded by many authors, the low production of indigenous birds is caused by poor nutrition, diseases outbreaks and poor management and genetic factors (Musharaf, 1990). Most farmers normally incubate eggs but hatchability and low survival rates are low. However, Awuni (2002) reported that factors such as predation and high ecto-parasite infestation which discourage brooding were the major reasons for the poor hatchability and survival rate. In most cases, poor management practices such as poor housing, particularly for chicks, affect the growth of chickens (Mwalusanya *et al.,* 2001; Kusina *et al.,* 2001). Some authors have reported that production rate of indigenous chickens are below the standard of commercial layers and broilers along with having smaller bodies (Ebangi & Ibe, 1994; Safalaoh, 2001).

2.3 Role of indigenous chickens

In Africa, poultry production makes an important contribution to household food security. Therefore, the indigenous chickens, as a form of poultry, make a significant role to household food security (Besbes, 2009). The indigenous chickens range freely in the household compound and in most cases they are able to scavenge for their own feed. These chickens can be raised either extensively or semi-intensively exhibiting remarkable adaptation to local environments and diseases (Sonaiya, 2003). The multitude functions of indigenous chickens include the provision of high quality protein meat and eggs, cash from sales, manure and socio-cultural roles. Nhleko *et al.,* (2003) reported that indigenous chickens are among the most adaptable domestic animals that can survive cold and heat, wet and drought, sheltered in cages, unsheltered outside or roosting in trees. Most communal farmers keep these chickens for the production of eggs and meat as well as a source of income.

According to Mapiye (2008) indigenous chickens have various advantages which make them attractive in the context of poverty alleviation and quality protein supply compared to cattle, sheep, goats and pigs. These chickens have high reproduction rate per unit time, they are efficient in transforming feed into usable protein and energy for human food, and they utilize extremely low capital, space and labour (Muchadeyi *et al.*, 2004). Despite their low egg production, the indigenous chickens are a significant component of the rural household livelihood by providing a source of income and nutrition.

2.4 The Potchefstroom koekoek chicken

The Potchefstroom Koekoek chickens were developed and bred at the Potchefstroom Agricultural College in the North West Province during the 1950's, by Marais a researcher. During the development of this breed White Leghorn, Black Australorp and Bared Plymouth Rock were used. This breed is, therefore, known as a locally developed breed. The name "Koekoek" is derived from the barred colour pattern of the chicken. Most off the laying hens that were available during the development period of the Potchefstroom Koekoek laid white shelled eggs, but the consumer demanded brown shelled eggs. Therefore, the Potchefstroom Koekoek was developed for the following specific production traits. The hens should lay a brown shelled egg with an average egg weight of 55, 7 g and the carcass should be attractive with a deep yellow coloured skin. The Potchefstroom Koekoek cocks and redounded hens would be suitable for meat production. Today the meat of this breed is still very popular in local communities and is preferred to that of the broiler breeds. The Koekoek colour pattern is due to a sex-linked gene that is very useful in crossbreeding for egg producing type of hens used for medium input production systems (Fourie & Grobbelaar, 2003). This breed is very popular amongst rural farmers both in South Africa and neighbouring countries for egg and meat production Gondwe and Wollny (2007).

The Potchefstroom chickens can fly onto trees to roost overnight and to be out of the way from ground predators such as the possible attacks by snakes. The Potchefstroom chickens are well adapted to a wide temperature range and they are able to scavenge for food in any area. Most of the time these chickens consume anything from grass seeds, small stones from the ground, household scraps and insects to small rodents. This strain of chicken gets broody and they are able to hatch their own chickens (Fourie & Grobbelaar, 2003).

2.5 Nutrient requirements of chickens

Laohakaset (1997) and Chinrasri (2004) defined nutrient requirements as the amount of nutrients that are necessary for a bird to maintain its performance, maximize its growth and feed utilization efficiency leading, to optimum laying ability and hatchability as well as optimize fat accumulation in the body. The amount of feed consumed by animals on a daily basis contains different nutrients which are vital for body growth and reproduction. A nutrient such as water is vital for body temperature regulation and for the transportation of other nutrients. Nutrients like carbohydrates, lipids and protein that the chicken utilizes as sources of energy or as parts of its metabolic activities are necessary requirements for growth. According to Carlson (1969), growth involves the deposition of bones, muscle and fat, each exhibiting an individual pattern of development of the body. With regard to the percentage increase over the weight at the end of a production cycle, the fast growths or weight gains are take place mainly when the chick is young (Mignon-Grasteaus *et al.*, 2003). As the chick grows older, the weekly increments of weight become materially less although nutrient requirement increase (Mignon-Grasteaus *et al.*, 2003).

Proteins have been described as complex macromolecules composed of 22 different amino acids or their derivatives that are linked by peptide bonds to initially form a primary structure.

As a result of steric constraints this primary structure forms an α-helical structure stabilized by hydrogen bonding as well as by cross-linking of individual amino acid residues. The α-helix that describes the primary structure of the protein has been found to be subsequently folded and arranged into more complex secondary and tertiary structures which, with the specific number and sequences of different amino acids, ultimately determine the three dimensional structure and the corresponding biological characteristics and functionality of the protein (Leeson & Summers, 2001; Horton *et al.*, 2002). Because body proteins are in a dynamic state, with synthesis and degradation occurring continuously, an adequate intake of proteins is essential.

If dietary protein is insufficient there is a reduction or cessation of growth and production and a withdrawal of protein from less essential body tissues to keep the functions of more vital tissues (NRC, 1994). Other factors contributing to difference in protein needs of the chickens include age, body size, sex and breed. Matching the feed protein content with animal protein requirements is important for maximizing animal performance. For example, turkey and broiler chickens have high protein requirements to meet the needs for rapid growth as reported by Sklan and Noy (2003) but on the other hand indigenous chickens such as Potchefstroom Koekoek need less protein because of their slow growth rate and medium body frames (Safaloah, 2001). Male chickens have higher protein needs than females (Thomas *et al.,* 1986; Han & Baker, 1993), because male chickens contain more protein and less fat in their weight gain (Edwards *et al.*, 1973; Han & Baker, 1991). Like the effect of body size and sex, breed differences also affect dietary protein needs in chickens. The NRC (1994) recommended 23, 20 and 18% dietary protein levels for broiler chickens during the starter, grower and finisher phases respectively, for optimal growth and maximum productivity. In contrast, (Tadelle & Ogle 1996) observed that the protein requirement of growing indigenous chickens varies between 16 and 18% during the growing phase for optimal performance. (Chemjor, 1998)

reported that a dietary protein level of 13% is adequate for indigenous chickens aged between 14 and 21 weeks. Kingori *et al.* (2003) observed that indigenous chickens require a protein level of 16 % to optimize feed intake and growth between 14 and 21 weeks of age. Furthermore, Ndegwa *et al.* (2001) reported that indigenous chickens fed diets containing 17 to 23 % CP had similar growth rates and feed intakes, suggesting that a 17% CP diet was sufficient for these chickens. Apparently the information on dietary protein requirements for indigenous chickens is limited and varies with the age. Therefore, there is a need to determine protein levels for optimal productivity in indigenous chickens.

2.6 Improving the growth rate of indigenous chickens under intensive management systems

Poultry feeds are referred to as 'complete' feeds since they contain all the protein, energy, vitamins, minerals and other nutrients essential for proper growth and good health. In order for the birds to have optimal growth, they need feeds which contain the essential nutrients for body function, such as growth and meat production. Therefore, it is very improbable that nutrients in the feed under a scavenging system particularly protein and energy would be present in the same ratio as required by the chickens for good healthy growth. Sometimes protein content may be too low in relation to energy, which places a heavy nutritional burden on the chicken's performance. According to Emmans, (1987) birds have genetically defined requirements for nutrients and are able to consume the desired amount of feed in order to meet their needs for the first-limiting nutrient in the feed. The scavenging rearing system does not meet nutrient requirements adequately. In order to obtain a balanced diet to improve their growth, the intensive rearing system seems to be the most promising alternatives for poultry production. As recommended by Tadelle and Ogle (1996), proper management under intensive rearing system such as regular watering, and feeding regimes, vaccination for common diseases, energy and

protein supplements can stimulate the feed intake and bring significant improvement in the productivity of indigenous chickens. According to Barley and Phororo (1992) supplementing the feeds of indigenous chickens with good energy and protein nutrients can bring a significant improvement in production. As recommended by Hickling *et al.*, (1990), Plavnik *et al.*, (1997) and Rose, (1997) energy and protein are the most important nutrients in a poultry feed as they affect productivity and growth of the birds. According to Larry, (1989) good nutrition is a key factor in determining growth performance and productivity of chickens. Therefore, it is very vital to feed indigenous chickens under intensive production systems with best levels of nutrients to enable them to express their full genetic productive potential.

2.7 Antibiotics as Growth Promoters

2.7.1 Introduction

According to Davey, (2000) antibiotics are chemical substances that are formed from certain fungi, bacteria, and other organisms that are used to inhibit the growth and even destroy, harmful microorganisms that affect the health of the individual. In medicinal chemistry, antibiotics can be produced synthetically. According to previous studies, the effect of antibiotics on microorganisms can be classified as bacteriocidal, (killing bacteria) and bacteriostatic (inhibiting bacterial development) (Hinton, 1988; Norcia *et al.*, 1999). As antimicrobial growth promoters (AGP), antibiotics have been introduced in poultry feed for more than seven decades in the United States and other countries. Early findings indicated that the effects of AGP reported in poultry diets by Moore *et al.* (1946) and in swine diets by Jukes *et al.* (1950). The initial findings were first reported by Starr and Reynolds (1951) after the feeding trial of streptomycin in turkeys. Other preliminary reports were made by Barnes, (1958) then Elliott and Barnes (1959) who described the use of tetracycline as an AGP in poultry diets. From the first findings the use of antibiotics, have been considered in animal

diets as the strategy of improving health of animals. Antibiotics have been added to poultry diets, particularly during the growing stage in order to protect poultry from pathogenic organisms, maintain health, promote growth, facilitate better feed efficiency, and improve meat quality. According to Miles *et al.* (2006) the addition of virginiamycin to a corn-soybean meal diet stimulated improvement in total body weight and the number of absorptive cells per unit length in the intestine of the birds.

The idea of adding antibiotics into feed can lead to improved nutrient absorption and growth rate. However, it has been reported that virginiamycin controlled microbial growth within the lumen of the gastrointestinal tract by disrupting bacterial protein synthesis (Parfait *et al.*, 1978). According to Engberg *et al.* (2000), zinc bacitracin significantly reduced the number of coliform bacteria in the ileum and increased the activities of amylase and lipase in pancreas homogenates. Supplementation with *L. javanica* can result in significant reduction of the growth of *C. perfringens* and *Lactobacillus salivarius* in the gut of broiler chickens (Zulkifli e*t al.*, 2000). High numbers of these lactobacilli may depress the growth performance of broiler chickens related to competition in nutrient uptake or impaired fat absorption due to bile acid deconjugation (Apajalahti *et al.*,2004). Many studies have reported that growth improvement properties of antibiotics are associated with interactions with the microbes in the gut (Blaut and Clave, 2007). AGP can assist in controlling disease outbreaks by modifying and improving the gut microflora, reducing bacterial fermentation and preventing infectious diseases, leading to the improvement of the health status of the animal (Allen *et al.*, 2013). The changes can lead to an increase in nutrient availability for the animal and able to achieve better growth performance (Dibner and Buttin, 2002; Hernández *et al.*, 2006). However, Donoghue (2003) reported that the use of locally available nutraceuticals in poultry diets gives significant economic benefits as

it assists in better production, which allows consumers to purchase high quality poultry products such as meat and eggs at lower prices.

2.7.2 Main mechanisms of action

Antibiotics are needed by both commercial and communal farmers since enteropathogenic microbes interrupt the balance of microflora populations, and compete for accessible nutrients, which are found in the gut, and thus inhibit the growth of livestock. The major reason of including antibiotics in the diets of chickens is to try to inhibit the development of exogenous microflora, thereby promoting the growth rate of chickens. However, the effects of antibiotics are associated with inhibitory effect on pathogenic microbes in the gastrointestinal tract. The decrease of the population of harmful intestinal microflora have favourable effects, such as decline in the occurrence of sub-clinical diseases and financial costs of curing the birds from infections. However, the decline in the quantity of growth depressing metabolites produced by intestinal microbes, results in competition among microbes and hosts for accessible nutrients, which lead to the increase of nutrient uptake by absorptive cells of the gut (Niewold, 2007). Other studies have showed that including antibiotics in the diets controls growth performance and proliferation of exogenous pathogens in various ways (Engberg *et al.*, 2000; Ferket, 2004).

The mechanisms of action of antibiotics differ according to their ability to affect certain disease states of animals. Some mechanisms involve disruption of DNA functions; bacterial permeability or general disruption of other prokaryotic metabolic processes (Parfait *et al.*, 1978). However the mode of action can inhibit bacterial cell wall synthesis (Huber and Nesemann, 1968). Furthermore, the use of antibiotics as treatments of various diseases can dependent on the condition of management. In African countries where poultry are raised in intensive systems with thousands of birds living under confinement on single premises,

antibiotics were needed in very small amount. However, in other countries, where production of birds is less intensive, the occurrences and spectrum of infectious diseases are larger; subsequently the use antibiotics is needed in larger amounts to treat diseases (Castanon, 2007). In addition to controlling proliferation and growth of pathogenic microflora, the addition of antibiotics in the feed can decrease quantity of microbial metabolites that reduce growth and decrease competition of accessible nutrients between microbes and the host (Thomke and Elwinger, 1998).

According to Roura *et al.*, (1992), some toxic substances can be used in small amount to reduce growth efficiency of the host. Consequently, inhibition of the growth of opportunistic pathogens and reduction of toxic bacterial metabolites due to antibiotic supplementation stimulate digestive efficiency and promote the growth rate of the birds. Moreover, the mode of action of antibiotics as growth promoters has also been attributed to the stimulation in absorptive cells growth and improvement in nutrient absorption in the gut (Anderson *et al.*, 1999). In most studies, it has been reported that treating the diets with a small amount of antibiotics stimulates proliferation of absorptive cells in the intestine. Increase in surface area of villus and crypt depth in the duodenum, jejunum, and ileum were observed following antibiotics medication (Iji *et al.*, 2001; Xia *et al.*, 2004). According to Miles *et al.* (2006), improvement in intestinal morphology stimulates better nutrient absorption, resulting in more conservation of energy for tissue maintenance that can be used instead for growth, or improving the absorption of various nutrients.

2.7.3 Alternatives to antibiotics

Various types of feed additives have been evaluated under commercial conditions and in experimental trials with the main objective of achieving the growth improvements and health

of animals (Bozkurt *et al.*, 2009). The alternatives that have been studied include herbs, spices and various plant extracts/essential oils, probiotics or direct-fed microbials (An *et al.*, 2008). Mixtures of two or more of alternative feed additives have been evaluated in many experimental trials with the aim of maximizing the benefits from utilizing them (Hofacre *et al.*, 2003; Choi *et al.*, 2010). Herbs, spices and many plant extracts/essential oils can be utilized as alternatives to replace AGP as they are richin phytochemicals (active compounds) that can be used to stimulate growth and health status of the animals. *In vitro* studies indicated that active compounds in herbs and spices have favourable effects in health status of animals, such as antimicrobial, antifungal, antihelminthic, and anticoccidial properties (Tabak *et al.*, 1999; Araújo and Leon, 2001; Tzakou *et al.*, 2001; Lee *et al.*, 2003; Brr and Mahmoud, 2005; Fernandes *et al.*, 2005; Mekala *et al.*, 2006).

Animal studies showed that the use of active compounds found in feed additives stimulated growth performance (Tabak *et al.*, 1999), resulted better feed efficiency (Lewis *et al.*, 2003), improved nutrient digestibility (Lewis *et al.*, 2004), improved immunity (Alcicek *et al.*, 2004), decreased cholesterol level (Hernández *et al.*,2004), lowered mortality (Cross *et al.*, 2007), improved liveability (Onimisi *et al.*, 2007), increased carcass yield and enhanced meat quality of birds (Rizzo *et al.*, 2008; Windisch *et al.*, 2008; Rahmatnejad *et al.*, 2009). Moreover the use of probiotics has been reported to have properties to replace AGP in feed. Spring *et al.* (2000) reported that some oligosaccharides stimulate the immune system by blocking pathogens binding to oligosaccharides receptors on the mucosal surface. Moreover, Patterson & Burkholder (2003) suggested that mixtures of prebiotics and probiotics known as synbiotics.

2.8 The *Lippia javanica* plant

2.8.1 Description, occurrence, distribution and chemical composition

Lippia javanica is an erect, woody shrub that grows up to two meters in height. The plant is able to grow anywhere in the veld were water is abundant, mostly along rivers and dams. The plant is herbal, with hairy sterms and strong aromatic leaves that have protective function against browsing animals (Mwangi *et al.*, 1991). The plant produces small yellowish white flowers, the flowers are produced in dense rounded heads. This plant is frequently found in several African countries such as Swaziland, Botswana, Zimbabwe and South Africa. Traditionally, Lippia has been frequently utilized as a medicinal plant to treat human ailments, in most African communities (Gerhard and Nemarundwe, 2006).

The available information on the chemical composition of *L. javanica* is associated with high level of essential oils, which have variations in terms of quantitative and qualitative of natural plant populations (Viljoen *et al.*, 2005). There is a lack of data available on the nutritive value of *L. javanica* plants. Essential oils in Lippia are known to contain the largest proportion of the phytochemicals, which are the active ingredients in disease treatment as well as improving growth and the health status of animals. However, the quantity and quality of the essential oils tend to vary due environmental and climatic conditions. Generally, 5 subspecies of *L. javanica* have been identified based on the major chemicals contained. These included the myrcenone rich-type (36–62%), the carvone rich-type (61–73%), the piperitenone rich-type (32–48%), the ipsenone rich-type (42–61%) and the linalool rich-type (>65%) are usually prevalent in South Africa (Viljoen *et al.*, 2005).

2.8.2 Ethno-pharmacology and utilization

Traditionally different parts of *L. javanica* are utilized for different purposes. In South Africa many societies believe that *L. javanica* can be used to treat infections. Venda people, located in Limpopo province, normally use *L. javanica* as a medication against dysentery, malaria and diarrhoea while the Xhosa people use leaves to treats cough and respiratory difficulties (Lekganyane *at al.* 2012). They normally combine milk and warm water with leaves to make a drink. *Lippia javanica* leaves have been utilized as a disinfectant of meat that has been infected with anthrax (Masika *et al.*, 2000). Available evidence indicated that *L. javanica* leaves are effective against asthma and chronic coughs in humans (Viljoen *et al.*, 2005). In addition the *L. javanica* leaves have been utilized by people to treat chest aliments, rashes and stomach problems. Furthermore, *L. javanica* leaves have also been used to treat wounds and fevers (Viljoen *et al.,* 2005). In Botswana, and Zimbabwe, *L. javanica* leaves have been incorporated in caffeine-free tea (Viljoen *et al.,* 2005).

There is a general lack of statistics on the utilization of *L. javanica* to treat animal diseases. However, it appears that most of the farmers, mainly in communal areas treat their livestock by using medicinal plants (Masika *et al.*, 2000; Muyima *et al.*, 2004). In areas where livestock are associated with household wealth, animal diseases have major economic effects in terms of production losses. There is, therefore, need to investigate the potential of *L. javanica* as a nutraceutical component of animal diets.

2.9 Dietary influence on blood parameters, carcass traits, and meat quality in chickens

2.9.1 Blood parameters

The physiology of animals can be affected by several factors, such as environmental features and nutrition (Ajao *et al.*, 2013). However, the nutrient requirements of an animal is dependent on feed intake and the efficiency of metabolic processes (Bamishaiye *et al.*, 2009). According to Schalm *et al.* (1975) blood parameters of livestock can be influenced by many factors, such as nutrients. According to Swenson (1970) and Addass *et al.* (2012) suggested that nutrition affects blood parameters of animals. Ajao *et al.* (2013) reported that haematological values of animals can be affected by nutrition. However, the processing of feed might have an effect on haematological values of animals (Aya *et al.*, 2013). Nutritional content might have an effects on the blood profile of healthy animals (Odunsi *et al.*, 1999; Yeong, 1999; Iheukwumere and Herbert, 2002). According to Aro and Akinmoegun (2012) and Aro *et al.* (2013), the haematological parameters such as haematocrit value, haemoglobin concentration, erythrocyte and, leucocyte count among others are used in routine screening for the health and physiological status of animals. However, Adejumo (2004) reported that haematological traits particularly packed cell volume (PCV) and Haemoglobin (Hb) were correlated with the nutritional status of the animal. According to Isaac *et al.* (2013), the packed cell volume (PCV) is involved in transport of oxygen and absorbed nutrients. Moreover, blood parameters such as blood viscosity are often neglected in routine clinical and physiological investigations. Blood viscosities can also affected by nutrition, particularly, when processed agro-industrial wastes are taken into consideration (Adejumo, 2004). Animal blood, for example, may be subjected to hyperviscosity syndrome consequent on the diets they consume which might eventually affect other blood values like haematocrit and erythrocyte sedimentation rate (Rosencranz & Bogen, 2006; Aro *et al.*, 2013).

2.9.2 Carcass traits and meat quality

Low ultimate pH reduces the importance of myoglobin, resulting in meat which appears less red and yellow (Castellini *et al.*, 2002). The quality of meat is determined using biochemical, physical-chemical and bacteriological parameters. A high ultimate pH is generally indicative of pre-slaughter stress in animals (Dhanda *et al.*, 2003; Muchenje *et al.*, 2009). The rate of pH decline is a good predictor of the colour and drip loss of meat (Aberle *et al.*, 2001; Muchenje *et al.*, 2008). Higher ultimate pH (pHu) in animal can be associated with low glycogen reserve due to inadequate nutrition (Mushi *et al.*, 2009). The age of the animal may be important because myoglobin, the primary muscle pigment, tends to increase with age in chicken (Lyon *et al.*, 2004). However, Smith *et al.* (2002) reported that the colour of broiler breast meat was not affected by age, whereas Lyon *et al.* (2004) reported that meat texture may be affected by age.

2.10 Summary

Indigenous chickens are hardy and they are able to withstand harsh climatic conditions. The indigenous chicken has a long life span compared to the broilers, therefore, there is a need to come up with a strategy which can be used to improve the rearing management and design a less-expensive diet that would guarantee profitability. There is a demand for better tasting organically produced meat in local and foreign markets. Indigenous chicken meat is characterised by lower fat content. Indigenous chickens are vital in rural areas as a source of income and food. The amount of feed that indigenous chicken consume on a daily basis is not adequate particularly in rural areas. Maize remains the main source of feed for indigenous chickens, although chicken are able to utilize other plants as feed resource. Consumers prefer organically-produced meat due to concern regarding artificial growth promoters and antibiotics that are used in broiler chickens. Indigenous chickens can therefore, be used as alternatives if

growth rate and meat quality can be improved. It is, therefore, important to investigate the utilisation of a potential nutraceutical, *L. javanica*, by chickens and assess its impact on growth performance, health and meat quality.

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3 CHAPTER THREE - GROWTH PERFORMANCE AND BLOOD PARAMETERS OF POTCHEFSTROOM KOEKOEK CHICKENS IN RESPONSE TO DIETARY *LIPPIA JAVANICA* LEAF MEAL

Abstract

A feeding trial was carried out over a period of 16 weeks to determine the effects of supplementation of chicken grower diet with *Lippia javanica* leaf meal on growth performance and blood parameters of Potchesfstroom koekoek (PK). One hundred and sixty male PK chickens were reared at the North-West University farm. *Lippia. javanica* leaves were harvested by hand, air-dried and milled into a leaf meal (LJ). The leaf meal was chemically analysed and added to a commercial grower diet at rates of 25 g LJ/kg diet and 50 g LJ /kg diet. Two additional diets; a positive control (commercial grower diet with antibiotics) and a negative control (commercial grower diet without antibiotics), were formulated and thus bringing to four, the total number of dietary treatments. The chickens were raised on a commercial starter mash for 4 weeks. At the start of the fifth week, the four experimental diets were offered to the chickens. The experimental unit was a pen holding 8 birds, replicated 5 times per treatment, resulting in a total of 20 floor pens. No significant dietary treatment (P >0.05) effect was observed with regards to overall FCE. Dietary treatments also had no significant effect ($P > 0.05$) on total protein, urea, creatinine, triglycerides, and calcium. However, diet significantly affected ALT, AST, bilirubin, sodium, potassium, cholesterol and magnesium concentration. Dietary supplementation with *L. javanica* leaf meal in chicken diets had no negative effects on growth performance, blood parameters, carcass characteristics and meat quality.

Key words: *Lippia javanica* leaf meal, chemical composition, Potchefstroom koekoek chicken, feed conversion efficiency, feed intake, haematology and serum biochemistry.

3.1 Introduction

Lippia javanica is widely distributed in South Africa and it is used extensively as a traditional herbal product, particularly in rural areas. According to Viljoen *et al.* (2005) the infusion of the leaves is normally used as a decongestant for colds and coughs. For many decades, people have used plants as the primary therapeutic agent in medicine. The discovery of antibiotics, led to the development of a pharmaceutical industry in the second half of the last century that relied heavily on pure single active natural compounds and synthetic drugs (Eloff, 2000). The widespread use of commercial antimicrobial drugs to treat infectious diseases in chickens has led to the development of multiple drug resistant bacterial strains. Over the decades antibiotics have been used successfully as growth promoters in the poultry industry to increase growth rates through improving digestive health and nutrients utilization (Landy *et al.,* 2011). However, the continuous use of these growth promoters has resulted in accumulation of antibiotic residues in meat and thus increasing health risks of consumers. (Toghyni *et al.,* 2010).

There is current interest in the use of indigenous plants such as *L. javanica* as growth promoters for indigenous chicken. The plant (*L. javanica*) contains large amounts of phytochemicals including terpenoids that have been observed to have analgesic, anti-inflammatory and antipyretic activities (Abena *et al.*, 2003; Manezhe *et al.*, 2004). However, research to improve the productivity of indigenous chickens under intensive management systems is still limited mainly due to high costs of poultry feed. This study thus evaluated *Lippia javanica* as a nutraceutical in indigenous chicken diets. Therefore, the purpose of this study was to investigate the growth performance and blood parameters of Potchefstroom koekoek (PK) chickens in response to dietary inclusion of *L. javanica.* The PK chickens are normally reared under extensive production systems with minimum inputs.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted at North-West University (NWU) experimental farm (Molelwane). The study site is situated in the North-West province of South Africa. The farm is situated between two latitudes 25°86'00''S and 25°64'32''E. Temperatures range from 3°C to 38°C and rainfall ranges between 300 and 500 mm annually.

3.2.2 Geographical description of the harvesting site.

Lippia javanica leaves were harvested from the Mafikeng game reserve. The Mafikeng game reserve is located in a semi-arid region in the North-West province of South Africa, the geographical coordinates are 25°84'00'' S and 25°62'33''E and is located close to the municipal boundary of Mafikeng. The game reserve is 911 to 1762 m above sea level with unpredictable precipitation bordering on average of 500 mm per annum. In general, precipitation occurs mostly during summer season between November and March. Very cold winters may be experienced (average daily minimum in July is 8°C) and in summer the weather may be extremely hot (37°C). The soils in the game reserve are mainly of red sandy-clay type.

3.2.3 Harvesting and processing of leaf material

The *L. javanica* plants were harvested by hand from an area measuring 250 m^2. Harvested plants were immediately taken to the laboratory and spread on open floors to dry at room temperature to constant weight. After drying the leaves were pruned and then finely ground using a 2 mm sieve, producing the *L. javanica* leaf meal. The leaf meal was then analysed for its chemical constituents and used to formulate experimental diets as an additive in commercial grower chicken diets. The *L. javanica* leaf meal was filled in plastic bags and kept on dry

environment, away from direct sunlight in the laboratory. The drying process and packaging of *L. javanica* is depicted in Plate 1

A

B

C

Plate 1 Drying procedure of *Lippia javanica* **(A)**, after pruning of leaves **(B)**, and the leaf meal powder **(C)**.

3.2.4 Chemical composition of the leaf meal and the diets.

The *L. javanica* leaf meal powder and experimental diets were analysed for dry matter (DM), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein and minerals according to the standard analytical methods (AOAC, 2005). For the determination of dry matter, approximately 1 gram of leaf sample was weighed, and placed into a crucible of known weight and dried in an oven for 24 hours at temperature of 105°C. Thereafter, the samples were removed from the oven, desiccated to cool and re-weighed. Moisture content was then

determined as the difference between the initial sample weight and dry weight, while the DM content was calculated as the proportion of dried weight over the initial weight. Dry samples were ash in a muffle furnace set at of 550°C for 12 hours. The ANKOM^{2000} Fibre analyser (ANKOM Technology, New York) was used to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to procedures by Van Soest *et al.* (1991). Total nitrogen was determined by the standard macro-Kjeldahl method (AOAC 1999, method no. 976.06) and were converted to crude protein by multiplying percentage N content by 6.25 and expressed in g/kg DM.

3.2.5 Mineral analysis

The mineral concentration of diets was analysed at the Animal Health Centre laboratory in North West University Mafikeng campus. Mineral concentration was analysed according to the guidelines provided by the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). The same samples that were used to determine the DM were further incinerated in a muffle furnace at 550^{0}C for 12 hours. The ash was weighed and digested with 10 mL of 32% hydrochloric acid and 1 mL of 55% nitric acid using a Microwave Reaction System Model 3000. The samples were digested for 45 minutes, cooled, and transferred into volumetric flasks (100 mL), which were topped-up with distilled water and left standing for 24 hours to permit the sediment to settle at the bottom of the liquid. After 24 hours, the samples were slowly transferred to McCartney bottles without disturbing the sediment. The concentration of calcium (Ca), potassium (K), sodium (Na), and phosphorus (P) was determined using the ICP Mass Spectrometer (Perkin-Elmer, NexION 300Q).

3.2.6 Dietary treatments and experimental design

A total of one hundred and sixty (160) male Potchefstroom chicks were used for the experiment. The birds were raised on commercial starter mash for 4 weeks. At the end of the 4

weeks pre-treatment period, the chicks were randomly sub-divided into four treatment groups to which the four experimental diets were randomly allocated. Each treatment had 5 replicate pens holding 8 birds each with the pen as the experimental unit. Each pen measured 3.5 m x 1.0 m x 1.85 m (L x B x H). The experimental diets for this study were formulated and mixed at North-West University farm. In formulating the dietary treatments, the *Lippia javanica* leaf meal was used to add to a commercial grower diet at rates of 25 g LJ/kg diet (LJ25) and 50 g LJ /kg diet (LJ50). An additional two diets, a positive control (commercial grower diet with antibiotics, CON+ and a negative control (commercial grower diet without antibiotics, CON-) were formulated and thus bringing to four, the total number of dietary treatments. The study was executed in a complete randomised design (CRD). These experimental diets were formulated to be iso-nitrogenous and iso-energetic. The composition of the diets is represented in Table 3.1. The duration of the experimental feeding trial was 16 weeks under natural lighting. Feed and water were offered to the birds *ad libitum* for the duration of the experiment.

Table 3.1. Gross composition (%) of *Lippia javanica* leaf meal (LJ)-based experimental diets

Ingredients	Diet[1]			
	CON+	CON-	LJ25	LJ50
LJ leaf meal (%)	0	0	2.5	5
Yellow maize	69.9	69.9	68.691	67.025
Prime gluten 60	1.8	1.8	1.300	1.033
Full fat soya meal	5.1	5.1	7.167	10.400
Soya bean meal	19.7	19.7	16.800	13.067
Limestone Powder	1.45	1.45	1.418	1.381
MCP/ Mono Cal	0.72	0.72	0.701	0.678
Salt-Fine	0.32	0.32	0.325	0.326
Sodium bicarbonate	0.17	0.17	0.165	0.163
Choline Powder	0.075	0.075	0..075	0.075
Lysine	0.279	0.279	0.279	0.278
L-Threonine	0.041	0.041	0.040	0.071
Methionine	0.187	0.187	0.183	0.179
PX P2 Br Grower with Phytase	0.167	0.167	0.167	0.167
Coxistac	0.05	-	-	-
Olaquindox	0.04	-	-	-

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg.

Table 3.2. Nutrient composition (%) of formulated experimental diets and *Lippia javanica* (LJ) leaf meal

	Diet[1]				
Parameters	CON+	CON-	LJ25	LJ50	LJ
Moisture %	10.94	10.94	11.29	11.18	10.02
Dry matter %	92.35	92.35	91.93	90.91	93.86
ME Broiler	11.79	11.79	12.10	12.10	
Protein	18.94	18.94	17.99	18.01	15.91
Fat	6.24	6.24	4.83	5.36	4.01
Fibre	4.18	4.18	2.59	2.89	
ADF[2]	4.61	4.61	6.01	6.58	29.91
NDF[3]	23.31	23.31	25.72	26.67	40.24
ASH	4.84	4.84	4.83	4.84	
Linoleic	2.97	2.97	2.16	2.43	
Ca	0.85	0.85	0.85	0.85	0.78
P	0.56	0.56	0.51	0.51	0.14
Na	0.18	0.18	0.18	0.18	0.14
Cl	0.3	0.3	0.3	0.3	0.00
K	0.73	0.73	0.76	0.76	2.83

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg

[2]ADF = Acid Detergent Fibre

[3]NDF = Neutral Detergent Fibre

3.2.7 Growth performance measurements

The average daily feed intake per bird was calculated from 5-16 weeks of age by subtracting the weight of the feed refusals from that of the feed offered per day, and dividing the difference by the total number of birds in the particular pen. The initial body weight was obtained by weighing all 8 chickens from each pen at the beginning of the experiment. Subsequently, the average live weight was determined weekly by weighing all the birds in each pen. The live weights were used to calculate growth rates. The average daily feed intake (ADFI) and average daily gain (ADG) were calculated as follows:

$$\text{ADG} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Feeding period (days)}}$$

$$\text{ADFI} = \frac{\text{Feed offered} - \text{feed refusal}}{84 \text{ days}}$$

The feed conversion efficiency (FCE) was also calculated on a weekly basis, the formula is as follows:

$$\text{FCE} = \frac{\text{Weight gained (g)}}{\text{feed consumed(g)}}$$

All chickens in the experimental pens were checked four times daily for sickness and mortality among the birds. Mortalities were recorded daily

3.2.8 Determination of haematology and serum biochemistry parameters

At 15 weeks of age, blood was collected from 2 birds randomly selected from each pen in the morning before feeding. Blood was drawn from the brachial vein into the sample without anticoagulant for serum biochemical analysis and purple tubes with anti-coagulant for haematology. The blood samples were taken to Lancet laboratory for analyses. The

Haematology analyser was used to determine the haematocrit and the full blood count while serum biochemical indices included the total protein, albumin, glucose, cholesterol, chlorine, sodium, potassium, creatinine total bilirubin, and urea, according to the methods outlined by Bush (1975) and WHO (1980).

3.2.9 Statistical analyses

Data on weekly feed intake, cumulative weight gain, and feed conversion efficiency were analysed using the repeated measures procedure of SAS (2010). Overall feed intake, weight gain, growth rate, feed conversion efficiency, haematology and serum biochemical parameters were analysed using the general linear models (GLM) procedure of SAS (2010). Data on cumulative weight gain were analysed using mixed model procedures. The linear model was employed:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where Y_{ij} = observation of the dependent variable (growth parameters, blood parameter), ij, μ = fixed effect of population mean for the variable, D_j = effect of diet, E_{ij} = random error associated with observation ij.

Weekly feed intake, weight gain and feed conversion efficiency data were analysed using the mixed model procedure of SAS (SAS, 2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

Where, Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

3.3 Results

3.3.1 Growth performance

The effect of diet on average daily feed intake (ADFI) is presented in Table 3.3. In week 5, diet had no significant effect ($P>0.05$) on daily feed intake. For all dietary treatments the feed intake differed with time (weeks). In week 9, chickens fed diet LJ25 had the highest feed intakes (520.08 ± 18.89 g/kg) compared to the other diets. Weekly feed intake increased significantly ($P<0.05$) in all treatments from week 6 to 16. However, overall, average daily feed intake over the experimental period was significantly lower in chickens fed diet LJ50 compared to those offered CON+, CON- and LJ25.

The effect of diet on cumulative weight gain (CWG) is presented in Figure1. In the middle of week 6 and week 7, a slight increase in chick weight was observed across dietary experimental treatments. After week 8 towards the end of the feeding trial at week 16, a gradual growth in the chicken weight was observed in all treatments. From week 7 to week 15 there was no significant difference in CWG among the treatments. However, at the end of the experiment chickens from CON- and LJ25 diets had significantly higher ($P < 0.05$) CWG than the CON+ and LJ50 treatments. Feed conversion efficiency of chicken fed different dietary treatments is presented in Table 3.4. There were no differences ($P>0.05$) in the FCE of the chickens across dietary treatments in week 1. Diet LJ50 resulted in higher FCE (0.31 ± 0.01) at 11 weeks of age compared to the other dietary treatments. However, feeding diets CON+ (0.21 ± 0.01) and CON- (0.20 ± 0.01) resulted in higher values ($P<0.05$) in week 15 compared to LJ25 and LJ50 diets. There was a huge decline in FCE across treatments between week 5 and week 6 and a slight decrease in feed conversion efficiency in all treatments thereafter. There were no significant differences in FCE among all the treatments ($P >0.05$) with regards to overall FCE.

Table 3.3. Weekly feed intake (grams) of indigenous chickens fed *Lippia javanica* leaf meal-based diets

	Diet[1]				
Time	CON+	CON-	LJ25	LJ50	S.E
Week 5	196.72	209.28	203.9	211.4	13.63
Week 6	265.02[a]	241.08[a]	274.64[b]	263.66[a]	15.47
Week 7	385.18[a]	370.60[a]	415.14[b]	349.42[a]	21.63
Week 8	420.02[a]	410.08[a]	466.46[b]	407.44[a]	18.01
Week 9	463.60[a]	472.64[a]	520.08[b]	457.24[a]	18.89
Week 10	556.52	552.36	595.38	569.4	28.49
Week 11	561.26[a]	556.20[a]	579.90[b]	529.26[a]	20.62
Week 12	572.24	598.16	601.06	593.64	23.12
Week 13	636.08	679.18	664.46	635.62	29.02
Week 14	694.44[a]	720.12[b]	673.60[a]	641.28[a]	29.3
Week 15	717.94[a]	740.06[b]	736.28[b]	639.64[a]	39.3
Week 16	499.12[ab]	513.44[b]	512.70[b]	446.96[a]	25.88
Overall	5968.06[a]	6063.14[a]	6243.50[b]	5744.94[a]	205.48

[a, b,]Means in the same columns with similar superscripts are not significantly different (P>0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg

Table 3.4. Weekly feed conversion efficiency in Potchefstroom koekoek chickens offered *Lippia javanica* leaf meal-based diets

	Diet[1]				
Time	CON+	CON-	L.J25	L.J50	S.E
Week 5	0.51	0.51	0.64	0.44	0.13
Week 6	0.35[a]	0.39[a]	0.41[b]	0.36[a]	0.02
Week 7	0.32[a]	0.44[b]	0.36[a]	0.35[a]	0.05
Week 8	0.32	0.29	0.31	0.33	0.04
Week 9	0.31[ab]	0.34[b]	0.28[a]	0.32[b]	0.01
Week 10	0.27	0.29	0.27	0.28	0.01
Week 11	0.25[a]	0.27[ab]	0.23[a]	0.31[b]	0.01
Week 12	0.25[a]	0.27[b]	0.25[a]	0.24[a]	0.01
Week 13	0.22[a]	0.25[b]	0.23[a]	0.23[a]	0.01
Week 14	0.19[b]	0.18[a]	0.18[a]	0.16[a]	0.01
Week 15	0.21[b]	0.20[b]	0.16[a]	0.17[a]	0.01
Week 16	0.16[b]	0.14[a]	0.10[a]	0.10[a]	0.02
Overall	0.26	0.27	0.25	0.25	0.009

[a,b]Means in the same row not sharing a common superscript are different (P <0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg

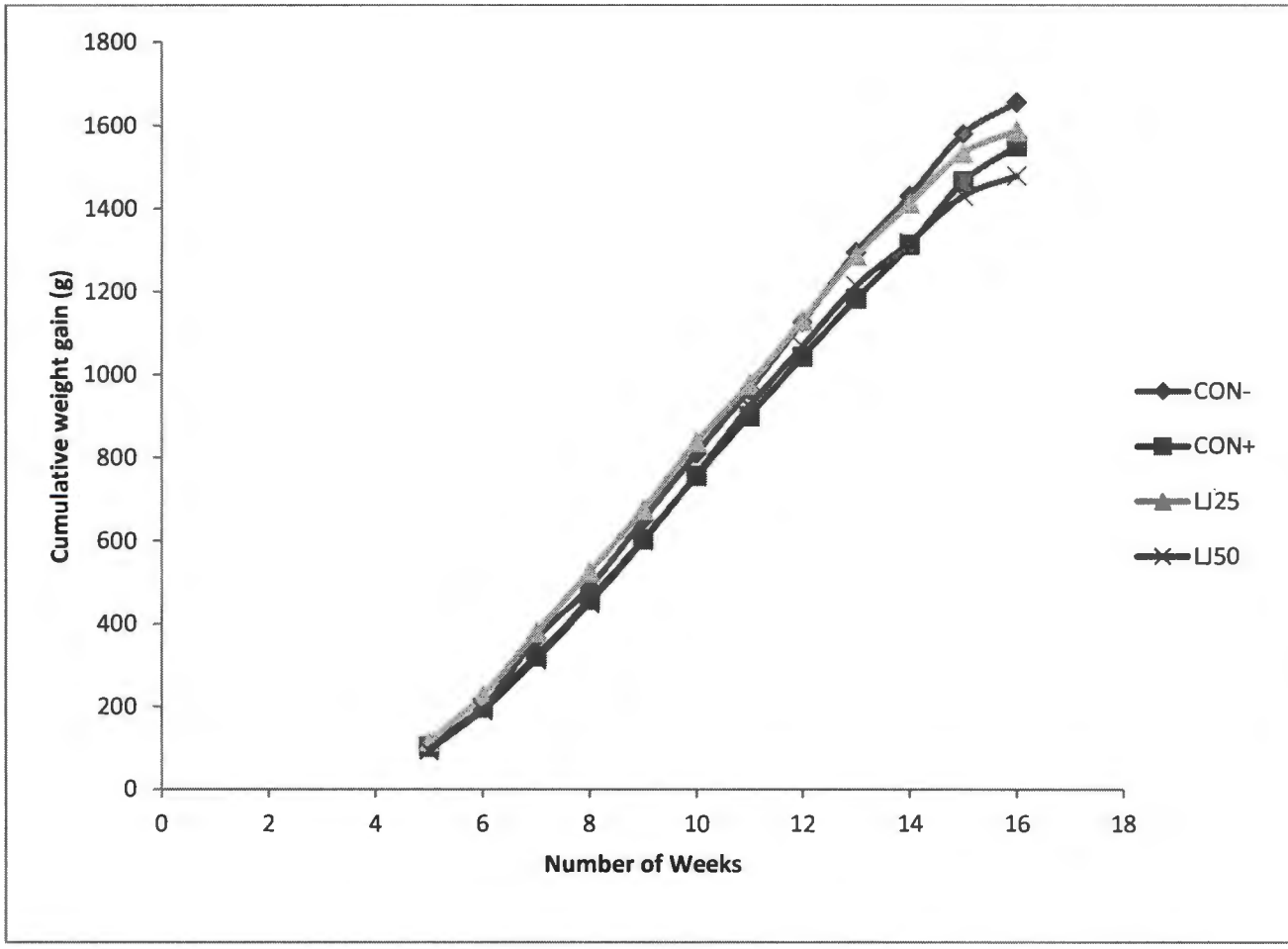

Figure 3.1. The effect of diet on cumulative weight gain of Potchefstroom Koekoek chickens. ([1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg)

3.3.2 Serum biochemistry

The effects of dietary treatments on serum constituents are shown in Table 3.5. Dietary treatments had no significant effect ($P > 0.05$) on total protein, urea, creatinine, triglycerides, and calcium concentration. However, diet significantly affected ALT, AST, sodium, bilirubin, potassium, cholesterol and magnesium concentration. It was observed that for ALT, chickens fed diet LJ50 had lowest level (0.10 ± 0.22 IU/L) followed by those on CON- (0.20 ± 0.22 IU/L). The chickens on CON+ had highest ALT (0.40 ± 0.22 IU/L) levels (P <0.05). Cholesterol levels were highest (2.80 ± 0.12 mg/dl) in chickens fed LJ50 treatment. Chickens on the CON+ diet had the highest magnesium levels (1.02 ± 0.02 mmol/l) whilst those on LJ25 had the lowest levels (0.93 ± 0.02 mmol/l).

Table 3.5. The effect of *Lippia javanica* leaf meal-supplemented diets on serum biochemistry of Potchefstroom koekoek chicken.

	Diet[1]				
Parameters	CON+	CON-	LJ25	LJ50	S.E
Bilirubin	0.28[a]	0.23[a]	0.31[a]	0.42[b]	0.08
ALT[1] (IU/L)	0.70[b]	0.20[a]	0.40[a]	0.10[a]	0.22
AST[2] (IU/L)	186.40[a]	175.10[a]	194.80[b]	192.80[b]	7.94
Total Protein (g/l)	37.20	38.40	37.20	38.00	0.93
Sodium (mmol/l)	150.70[a]	151.90[a]	151.70[a]	153.40[b]	0.61
Potassium (mmol/l)	4.49[b]	3.89[a]	3.68[a]	3.84[a]	0.12
Albumin (g/l)	15.1[a]	15[a]	14.70[a]	16.10[b]	0.42
Urea (mmol/l)	0.19	0.22	0.21	0.25	0.03
Creatinine (mg/dl)	18	18	18	18	0.00
Calcium total (mmol/l)	2.67[a]	2.72[b]	2.65[a]	2.69[a]	0.03
Calcium corrected	3.18	3.22	3.16	3.17	0.03
Cholesterol (mg/dl)	2.35[a]	2.44[a]	2.31[a]	2.80[b]	0.12
Magnesium (mmol/l)	1.02[b]	0.96[a]	0.93[a]	0.98[b]	0.02
Triglyceride (mmol/l)	0.57	0.65	0.53	0.55	0.06
Conjugated-bilirubin	1.2[a]	1.2[a]	1.2[a]	1.21[b]	0.00

[a,b] Means in the same row not sharing a common superscript are different (P <0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg; ALT = Alkaline phosphatase; AST = Aspartate aminotransferase

3.3.3 Blood haematology

As shown in Table 3.6, the inclusion of *L. javanica* in the diet had no significant effect on haemoglobin concentration and monocytes. However, there were significant differences with respect to hematocrit (HCT), neutrophils, lymphocytes, normoblasts and erythrocytes. It was observed that values for HCT were higher in LJ25 and LJ50 (0.39 ± 0.01 l/l) chickens compared to CON+ (0.36 ± 0.01 l/l) and CON- (0.38 ± 0.01 l/l). It was observed that LJ25 chickens had the highest lymphocytes counts (14.94 ± 1.17 x 10^9/l) compared to other dietary treatments. Highest counts for nomoblasts (5670.4 ± 462.1 /100WBC) and (5527.6 ± 462.1 /100WBC) being recorded in chickens offered CON+ and CON- diets compared to LJ25 (4448 ± 462.1 /100WBC) and LJ50 (4808.8 ± 462.1 /100WBC) chickens.

Table 3.6. The effect of *Lippia javanica* leaf meal-supplemented diets on haematology of Potchefstroom chicken.

	Diet[1]				
Parameters	CON+	CON-	LJ25	LJ50	S.E
Erythrocyte Count (x 10^{12}/l)	2.68[a]	2.75[a]	2.88[b]	2.86[b]	0.08
Haemoglobin (g/dl)	10.34	10.64	10.83	10.97	0.39
Hematocrit (l/l)	0.36[a]	0.38[a]	0.39[b]	0.39[b]	0.01
Leucocyte count (x 10^9/l)	12.89[a]	13.66[a]	17.86[b]	17.5[b]	1.37
Neutrophils (x 10^9/l)	0.76[a]	0.95[a]	1.09[a]	1.92[b]	0.26
Lymphocytes (x 10^9/l)	10.35[a]	11.36[a]	14.94[b]	12.88[ab]	1.17
Monocytes (x 10^9/l)	1.47	1.16	1.73	1.74	0.33
Eosinophils (x 10^9/l)	0.31[a]	0.19[a]	0.00	0.81[b]	0.21
Normoblasts (/100WBC)	5670.4[b]	5527.6[b]	4448[a]	4808.4[a]	462.1

[a,b] Means in the same row not sharing a common superscript are different (P <0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg

3.4 Discussion

3.4.1 Feed intake and feed conversion efficiency

This study was conducted to assess the potential of *L. javanica* as an alternative supplement with nutraceutical properties in Potchefstroom koekoek (PK) chickens. The results showed that dietary inclusion of *L. javanica* at levels of up to 50g LJ/kg feed had no effect on growth performance of the chickens as reflected by similar ADFI, ADG and FCE of the chickens across dietary treatment. Similar results were obtained by Mpofu *et al.* (2016) in a study where broiler chickens were also fed diets containing *L. javanica* leaf meal although lower inclusion levels were used. Generally, *L. javanica* contains high fibre content which could influence digestibility of diets, and ultimately, performance of the chickens. Hetland and Svihus (2001) and Hetland *et al.* (2002) reported that high fibre levels above 100 g/kg DM negatively affect feed utilisation in poultry. However, observation has been made that indigenous chickens are able to use a considerable amount of nutrients from high fibre diets compared to genetically improved commercial birds such as broilers (Horsted, 2006). This explains why there were similarities in performance between the *Lippia* fed chickens and those on a commercial diet. The observed huge decline in FCE in all treatments between week 1 and week 2 might perhaps be explained by the fact that the birds were still adapting to the experimental diets.

3.4.2 Haematological parameters

Haematological parameters of livestock have been reported to respond to the diet they are consuming (Madubuike *et al.,* 2006). In the current study all values obtained fell within the normal range for healthy chickens as reported by Animashahun *et al.* (2006). This indicates that the dietary treatments were nutritionally adequate for the PK (Potchefstroom koekoek) chickens. The data obtained from haematological parameters of the Potchefstroom chicken indicated that *L. javanica* had a positive effect on heamoglobin production haematopoiesis and

erythrocytes formation. This is reflected by the increased erythrocyte count in Potchefstroom chicken offered diets containing the *L. javanica* leaf powder, although the increase was not significant. The increase in erythrocytes could be due to the secondary plant metabolites contained in *L. javanica* which are thought to have antioxidative and haematopoietic effects on blood cell formation and functions (Arslan *et al.,* 2004). According to Abdel-Wahhab and Aly (2005), Talebi *et al.* (2005); and Toghyani *et al.* (2010), plants such as *Nigella sativa*, which have similar effects to *L. javanica* have been found to have significant effects on haematological parameters. The findings of the study are also consistent with the observations by Meral *et al.* (2004) and Oluwatosin (2008) who noted that extracts from some plants can have favourable effects on erythrocytes and leucocyte formation in livestock. Among the leucocytes, neutrophils and eosinophils play significant roles by improving the immune system of the chicken. The essential oil found in *L. javanica*, may improve functional effectiveness of the neutrophils and eosinophils in the inhibition of microbial oxygen uptake and oxidative phosphorylation thereby enhancing efficient immune protection (Viljoen *et al.*, 2005).

3.4.3 Serum biochemistry.

Serum biochemical parameters provide useful information for the evaluation of the health status of the chicken and may be reflective of various metabolic alterations in organs and tissues when animals are fed certain diets (Kudair & Al-Hussary 2010). In the current study, dietary inclusion of *L. javanica* inclusion did not affect the serum of the chickens. These findings suggest that *Lippia* had no negative impact on these parameters. Importantly, the serum concentrations were within the range reported for broilers (Ladokun *et al.,* 2008; Mpofu *et al.,* 2016). Total serum protein has been reported to be an important indicator of the adequacy of protein retained in the animal body (Akinola & Abiola, 1991; Esonu *et al.*, 2001). Moreover, total blood protein and creatinine contents have been shown to depend on the

quantity and quality of dietary protein ingested (Iyayi, 1998; Esonu *et al.*, 2001). Serum creatinine concentrations are directly related to muscle volume and activity. The serum creatinine concentration was the same in all chickens across dietary treatments. It was observed that ALT had significant differences among the treatments. However, the lowest values of ALT were observed in chickens fed diet LJ50 (0.10 ± 0.22 IU/L) compared to CON+ chickens (0.70 ± 0.22 IU/L). The elevation in AST may have resulted from handling or muscle injury during the collection of samples, which may have resulted in the leakage of intracellular AST into the blood. However, there was a significant difference in AST levels with LJ25 (194.80 ± 7.94) and LJ50 (192.80 ± 7.94) chickens showing high values compared to CON+ (186.40 ± 7.94) and CON- (175.10 ± 7.94) with lower values. In the current study, the observed variation in AST among the dietary treatments had no effects on the health of the Potchefstroom chickens. The concentration of cholesterol levels in chickens was affected by *L. javanica* leaf meal supplementation. It was observed that LJ50 chickens had high values (2.80 ± 0.12) compared to chickens on CON+, CON- and LJ25 diets. The high level of cholesterol content in LJ50 can be attributed to the effects of essential oil found in the leaves of *L.javanica* which can stimulate or inhibit the action of enzymes that control the synthesis of cholesterol content (kowalska & Bielanki 2009)

3.5 Conclusions

The results of the study showed that most of the parameters measured in PK (Potchefstroom koekoek) chickens fed diets containing *Lippia javanica* leaf meal compared well with the control diet. From the results, the inclusion of *L. javanica* leaf meal in the diet improved the feed intake and growth performance of chickens. Moreover, *L. javanica* inclusion in diets positively influenced haematology and serum biochemistry indices of indigenous chickens, which normally indicates the general health of chickens. The high level of cholesterol could

suggest that the optimum inclusion level of *L. javanica* supplementation can be LJ50, however further research needs to be conducted to confirm this conclusion. The results also showed that *L. javanica* can be included in diets at levels up to 50 g/kg DM without negatively influencing the growth performance of the chickens and, therefore, can be effectively included to reduce the cost of feeding indigenous chickens. The results obtained from this study can be important in improving feed formulations that will improve growth performance and health status in poultry farming. However, it is also important to evaluate the effect of including *L. javanica* leaf meal in the diets of indigenous chickens on carcass characteristics and meat quality since consumer acceptance of poultry products is of paramount importance.

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4 CHAPTER FOUR - CARCASS CHARACTERISTICS AND MEAT QUALITY OF POTCHEFSTROOM KOEKOEK CHICKENS IN RESPONSE TO DIETARY *LIPPIA JAVANICA* LEAF MEAL

Abstract

The current study was undertaken to investigate the effect of inclusion level of *Lippia javanica* leaf meal in Potchefstroom koekoek diets on visceral organs, carcass characteristics, and meat quality. A total of one hundred and sixty (160) Potchefstroom koekoek chickens were randomly allocated to four diets in five replicate pens resulting in total of 20 pens as described in Chapter 3. At the end of the feeding trial described in Chapter 3, all chickens in the pens were starved for 13 hours prior slaughtering to permit the emptying of the GIT. After slaughter, the carcass weight, viscera organ weight and carcass characteristics of the birds were recorded. Breast meat samples were used to measure pH, cooking loss, drip loss, water holding capacity, meat tenderness and meat colour. The data showed that supplementing chicken diets with 50 g/kg of *L. javanica* leaf meal significantly increased carcass weight ($P < 0.05$) compared to supplementing at 25 g/kg. The gizzard weights in CON+ and LJ25 diet fed chickens were higher ($P < 0.05$) compared to CON- and LJ50 chickens. Meat from CON- (6.07) and LJ50 (6.08) diet fed chickens had a higher pH than that chickens fed diet from CON+ (5.9) and LJ25 (5.8) . With regards to meat colour, breast muscle in CON- had the highest ($P < 0.05$) lightness (L*) value (53.2), while the meat from C0N+, LJ25 and LJ50 diet fed chickens had lower L* values. Supplementing with *L. javanica* in chicken grower diets showed favourable effect on the breast and thigh weights. The data obtained from this study showed that *L. javanica* can be included in diets without negatively affecting the viscera organs, carcass characteristics and meat quality of PK chickens.

Keywords: *Lippia javanica* leaf meal, gastrointestinal tract Potchefstroom chickens,

4.1 Introduction

World meat statistics show that poultry products have the highest consumption rate among animal products in many countries (OECD and FAO, 2010). The consumption of poultry meat consumption, when compared to the 2007–2009 base period is projected to grow by 38% by 2019 in developing countries (Rimal, 2005). The rapid renewed demand is expected to be matched by improved producer meat output boosted by the predictable returns and shorter production cycles (OECD and FAO, 2010). The choices of meat by the consumers are driven by nutritional perceptions (Rimal, 2005) that influence willingness to pay. However, the quality of chicken products is the decisive factor that influences the development of the poultry enterprise (Memon *et al.,* 2009).

Meat quality is a description of a range of attributes of meat that makes it acceptable to the consumer (Memon *et al.,* 2009). The meat industry as a whole is primarily driven by consumer preference, where bright red colour is seen as an indicator of freshness and overall wholesomeness (Risvik, 1994; Mancini & Hunt, 2005). Consumer preference research suggests that tenderness is a very important part of eating quality and that variations in tenderness affect the decision to repurchase (Rimal, 2005). The major challenge in chicken production has been the cost of commercial feeds and associated inputs such as growth promoters and prophylactic additives, which may be required in larger quantities, particularly for indigenous chickens whose growth rates are significantly lower compared to broilers. A possible solution is the use of locally available, inexpensive non-conventional feed additives to boost feed utilization and prevent disease outbreaks. Possible candidates include herbal plants such as *L. javanica*, which may serve as sources of nutrients as well as of bioactive plant compounds that enhance growth, health and quality of meat. In addition, the use of these locally available plants with

nutraceutical properties may very well reduce the cost of feeding chickens thus ensuring sustainability as well as profitability.

Lippia javanica is a plant that has been used for management of throat disease in humans in Central Kenya (Njoroge & Bussmann, 2006) and also as a medicinal plant for livestock in a number of African countries (Masika & Afolayan, 2003). Van Wyk (2008) reported that the plant is a very important medicinal plant particularly in Southern Africa. According to Gulmour (1983) *L. javanica* is an important source of vitamin A, ascorbic acid, thiamine and essential amino acids, which can affect meat quality. In the previous chapter, it was observed that inclusion of *L. javanica* leaf meal in the diets of Potchefstroom koekoek chickens improved feed intake, growth performance and health. It was also observed that *L. javanica* inclusion in diets positively influenced haematology and serum biochemical indices of the PK chickens, which normally indicates the general health of chickens. However, carcass characteristics and meat quality are also known to be influenced by changes in diet thus a complete evaluation of the utility of *L. javanica* leaf meal must also include the quality characteristics of the marketed product. Therefore, the aim of this study was to determine the influence of *L. javanica* inclusion in diets on carcass and meat quality parameters in Potchefstroom koekoek chickens that are normally reared in extensive production systems.

4.2 Materials and methods

4.2.1 Study site

The study site was described under Section 3.2.1.

4.2.2 Management of chickens

Four-week old male Potchefstroom koekoek chickens were purchased from a commercial breeder and reared under experimental conditions in the farm for a period of twelve weeks as described in Chapter 3. At the end of the 12-week period the chickens were slaughtered.

4.2.3 Slaughter procedure

At the end of the 16-week experimental period, the birds were fasted for 13 hours to allow for the emptying of the crop (Ari *et al.*, 2013). Subsequently, birds from each pen were taken to the slaughterhouse (Rooigrond, Mafikeng) for the slaughter. The birds were slaughtered humanely using the standard slaughter methods for chickens (including stunning and exsanguination). After slaughtering the birds were put through a de-feathering machine. The chickens were taken back to the North-West University Farm (Molelwane) laboratories for determination of carcass characteristics and internal organ size.

4.2.4 Carcass traits and viscera macro-morphometry

Carcasses from different treatments were placed in labelled plastics bags for identification. The feet, heads and intestines were removed by hand. The carcasses then were weighed to obtain the hot carcass weight (HCW) and after 24 hours of chilling to obtain the cold carcass weight (CCW). The dressing out percentage was calculated according to a standard procedure described by (Uijttenboogaart & Gerrits, 1982). The carcass was then cut to determine the weight of the breast (without skin), upper legs (thigh), lower legs (tibia and foot), and wings. Visceral organs such as proventriculus, gizzard, liver, heart, spleen and caecum were separated by hand before being weighed. The length of small intestines and large intestines were also measured and recorded. After measuring the intestines, the visceral organs were assessed for any injuries, weighed and kept in a cool environment. Breast and thigh samples were collected 24 hours post mortem for proximate analysis and evaluation of the meat quality traits.

4.2.5 Determination of pH in breast muscle

After slaughter, breast meat samples were cut from each bird using a knife and they were stored at 4°C before pH measurements were taken. The post-mortem pH was measured on the breast muscle of each bird 24 hours after slaughter using a portable digital pH meter (CRISON pH24, CRISON Instruments SA, Spain) with a piercing electrode.

4.2.6 Determination of cooking loss

Meat samples weighing between 100 and 110 g were obtained from breast meat. The samples were weighed individually and the weights were recorded as initial weight (W1). The samples were then placed in foil plates and oven broiled (dry heating) at 180°C for 30 min. The broiled samples were then removed from the oven and left to cool for 20 minutes. The samples were then re-weighed and this second weight was recorded as W2 (cooked weight). The cooking loss was calculated as follows:

$$\text{Cooking loss } \% = \frac{(w1-w2)}{w1} \times 100$$

4.2.7 Determination of tenderness

The breast muscle samples that were previously cooked at 180°C for 30 min and used for determination of cooking loss were used for shear force evaluation. The subsamples of 2 cm high × 2 cm width × 15 cm length were sheared perpendicular to the fibre direction using a Meullenet - Owens Razor Shear Blade (A/MORS) mounted on a Texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value in Newtons (N) represented the average of the peak force measurements of each sample.

4.2.8 Measurement of meat colour

The colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was determined on breast meat, 24 hours after slaughter, using a colour-guide 45/0 BYK-Gardener GmbH machine with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. Three readings were taken by rotating the Colour Guide 90° between each measurement, in order to obtain a representative average value of the colour. The guide was calibrated before each day's measurements using the green standard.

4.2.9 Determination of Water holding capacity

The water holding capacity of the meat was measured in duplicate on freshly cut slices of the pectoralis major muscle (PMM) ranging from 8-12 grams. The WHC was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau & Hamm (1957). Water holding capacity was calculated using the equation:

$$WHC\ (\%) = \frac{(initial\ weight - weight\ after\ pressing)}{initial\ weight} \times 100$$

4.2.10 Determination of drip loss

Drip loss measurement was done using the modified procedure of Zhang *et al.* (2009). Briefly breast meat samples were sliced with a knife into blocks weighing between 2-3 grams. The sample weights were recorded as initial weight (W_1). The sample were hooked and suspended on the caps using wool in plastic bottles. The bottles were then stored in a cold room at 4°C for 24 hours. After 24 hours, the samples were taken out gently wiped to remove any liquid on the surface of the meat and the sample were reweighed (W2). The difference in weight of each

sample before and after hanging was expressed as percentage drip loss and calculated as follows:

$$\text{Drip loss (\%)} = \frac{(w1-w2)}{(w1)} \times 100,$$

Where, W1 = initial weight of breast meat samples and W_2 = final weight of suspended samples after 24 hours.

4.3 Statistical analyses

Data on carcass characteristics and meat quality traits were analyzed using general linear models (GLM) procedures of SAS (2010). The linear model was as follows:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where Y_{ij} = observation of the dependent variable (carcass characteristics and meat quality traits) i, μ = population mean for the variable, D_j = fixed effect of diet, E_{ij} = random error associated with observation ij.

4.4 Results

4.4.1 Visceral organs

The weights of viscera of Potchefstroom chicken are presented in Table 4.1. The weights of the proventriculus, liver, gizzard, heart, and spleen were significantly ($P < 0.05$) influenced by experimental diet.Chickens fed diet CON- (9.6 g) and LJ25 (8.7 g) had higher proventriculus weights compared to those from chicken fed diets CON+ (6.8 g) and LJ50 (6.6 g).. The gizzard weights in chickens fed diets CON+ and LJ25 were heavier ($P < 0.05$) compared to those fed diets CON- and LJ50. The heart weight was highest ($P < 0.05$) in CON- (9.5g) and LJ25 (9.8 g), while CON+ (9.2 g) and LJ50 (8.2 g) had the lowest weights. There were no significant

(P<0.05) differences observed in the ceca length and weight as well as in small intestinal length and weight.

Table 4.1.The effects of dietary inclusion of *Lippia javanica* leaf meal on the macro-morphometry of viscera from Potchefstroom koekoek chickens

	Diet[1]				
Parameters	CON+	CON-	LJ25	LJ50	S.E
Proventriculus (g)	6.8[a]	9.6[b]	8.7[b]	6.6[a]	0.6
Liver (g)	25.4[a]	26.1[b]	26.2[b]	23.7[a]	1.1
Full gizzard (g)	51.6[b]	46.3[a]	54.5[b]	40.3[a]	2.7
Empty gizzard (g)	38.6[b]	35.6[a]	41.8[b]	29.8[a]	2.2
Heart (g)	9.2[a]	9.5[b]	9.8[b]	8.2[a]	0.5
Spleen (g)	3.6[b]	3.6[b]	3.3[b]	2.4[a]	0.2
Small intestine length (cm)	133.8	134.0	132.7	130.8	3.3
Small intestine weight (g)	36.8	37.0	36.4	38.8	2.7
Caeca length (cm)	18.8	18.9	18.9	18.9	0.5
Caeca weight (g)	8.3	11.5	9.5	9.0	0.6

[a,b] Means in the same row not sharing a common superscript are different (P <0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg.

4.4.2 Carcass characteristics

The effect of diets on carcass characteristics in male Potchefstroom koekoek chickens is shown in Table 4.2. The weight of the wings, thigh, drum stick, breast as well as in neck and shank length were significantly. It was observed that CON+ (99.5 g) had heaviest wings compared to other treatments. However, LJ25 had the heaviest (P<0.05) thigh weight (124.2 g) compared to other treatments. It was observed that feeding diets CON+ (121.7 g) and CON- (121.1 g) resulted in heavier drum compared to feeding diets LJ25 (113.9 g) and LJ50 (105.4 g). No

significant (P>0.05) dietary effects were observed in terms of shank and head weights and there were significant dietary effects on dressing percentage. Feeding diets CON+ and CON- resulted in higher cold carcass weight (CCW) when compared to feeding diets LJ25 and LJ50.. The same pattern was observed on hot carcass weight (HCW), there were significant differences, CON+ and CON- had higher values (1250.4g) and (1250.7g) when compared to LJ25 and LJ50 which had lower values (1199.9g) and (1121.4g) respectively.

Table 4.2. The effects of dietary inclusion of *Lippia javanica* leaf meal on carcass characteristics of Potchefstroom koekoek chickens

Parameters	Diet[1]				S.E
	CON+	CON-	LJ25	LJ50	
Final body weight (g)	1838.0[a]	1904.2[b]	1756.3[a]	1742.5[a]	54.32
Dressing percent (%)	65.54	64.72	67.53	64.56	2.35
CCW[1] (g)	1208.9±28.1[b]	1235.0±27.1[b]	1184.3±26.27[a]	1121.4±25.8[a]	
HCW[2] (g)	1250.4±27.4[b]	1250.7±26.5[b]	1199.9±25.63[a]	1138.2±24.8[a]	
Wings (g)	99.5[b]	94.8[a]	92.4[a]	89.7[a]	2.9
Thigh (g)	117.8[a]	120.8[ab]	124.2[b]	117.1[a]	5.0
Drum stick (g)	121.7[b]	121.1[b]	113.9[a]	105.4[a]	3.7
Neck (g)	124.1[a]	131.4[b]	128.7[a]	118.8[a]	5.2
Shank weight (g)	45.1	81.0	81.5	83.5	81.1
Shank length (cm)	9.7[a]	10.6[b]	10.6[b]	9.8[a]	0.2
Head (g)	57.4	61.0	62.2	61.2	2.8
Breast (g)	225.2[b]	210.4[a]	196.9[a]	202.8[a]	10.5
Back (g)	192.2[b]	192.4[b]	170.7[a]	172.0[a]	9.3

[a,b] Means in the same row not sharing a common superscript are different (P <0.05).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg; [2]CCW = Cold carcass weight; [3]HCW = Hot carcass weight

4.4.3 Meat quality

The effect of diet on meat quality of Potchefstroom chickens is presented in Table 4.3. The results showed that breast meat initial pH did not differ from chickens across dietary treatments. However, significant dietary induced pH differences ($P < 0.05$) in which the breast meat pH were observed. Breast meat from chickens fed diets CON- (6.07) and LJ50 (6.08) had higher ultimate pH than breast meat from chicken fed diets CON+ (5.9) and LJ25 (5.8).Breast muscle from chicken fed diet CON- (53.2) had the highest ($P < 0.05$) lightness (L*) value, while the meat from CON+, LJ25, and LJ50 had significantly lower values. It was observed that LJ25 (2.3) had the highest values for redness (a*) compared to other treatments. The results also showed that breast meat from chicken fed diet LJ50 had the highest yellowness (b*) value (12.5) compared to meat from chicken fed diets CON+, CON-, and LJ25 chickens whose b* values were 10.1, 11.7, and 11.9, respectively. There was no significant difference observed between experimental diets in terms of drip loss. However, the results of water-holding capacity (WHC), showed that breast muscle from chicken fed diet LJ25 chickens (26.32) had the highest water holding capabilities compared to other treatments.

Table 4.3. The effects of dietary inclusion of *Lippia javanica* leaf meal on meat quality traits of Potchefstroom koekoek chickens

	Diet[1]				
Parameters	CON+	CON-	LJ25	LJ50	S.E
pH_i	7.0	7.1	7.1	7.0	0.03
pH_u	5.9[a]	6.07[b]	5.8[a]	6.08[b]	0.07
Lightness (L*)	51.5[a]	53.2[b]	51.03[a]	51.9[a]	0.8
Redness (a*)	1.6[a]	1.3[a]	2.3[b]	1.3[a]	0.3
Yellowness(b*)	10.1[a]	11.7[a]	11.9[a]	12.5[b]	0.4
WHC (%)[2]	18.65[a]	18.35[a]	26.32[b]	17.29[a]	2.11
Drip loss (%)	8.38	5.09	6.63	7.25	2.08
Cooking loss (%)	33.35[b]	32.69[b]	32.75[b]	28.84[a]	0.96
Meat shear force (N)	15.25[a]	13.54[a]	16.83[b]	12.79[a]	1.45
Bone shear force (N)	26.59[a]	27.15[b]	25.59[a]	23.65[a]	1.41
Bone ASH (%)	36.67	37.79	36.77	37.90	0.46

[a,b] Means in the same row not sharing a common superscript are different ($P < 0.05$).

[1]Diet: CON+ = Positive control diet with antibiotics; CON- = Negative control diet without antibiotics; LJ25 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 25 g/kg; LJ50 = Negative control diet supplemented with *Lippia javanica* leaf meal at the rate of 50 g/kg.

[2]WHC = Water holding capacity; [3]pHu = Ultimate pH; [4]pH_i = Initial pH

4.5 Discussion

4.5.1 Viscera macro-morphometry

The results showing a significant effect of supplementing *L. javanica* on viscera organs contradict findings in previous studies where no differences on viscera macro morphometry were observed (Çabuk *et al.*, 2006; Ahmad *et al.*, 2011). However, in the current study, dietary treatment had an influence on the weight of the proventriculus, liver, gizzard, spleen and heart of Potchefstroom koekoek chickens, while it had no effect on small intestines and caeca weight. Generally, an increase in fibre content as a result of higher inclusion levels of *L. javanica* may be associated with significant changes in the structure and morphology of the gut as an adaptive mechanism to accommodate the fibre and phytochemicals. However, current results are inconsistent with those reported by Hernandez & Madrid (2004) who did not observe any variation between a control diet and those containing plant extracts on organ weight of 42-day-old broiler chickens.

4.5.2 Carcass characteristics

The lack of effect of *L. javanica* supplementation on carcass yield and dressing percentage is steady with results reported (Nikolakakis *et al.,* 2005; Dotas *et al.,* 2014; Mpofu *et al.*, 2016). However, chickens fed the positive control (CON+) had the highest wing, drumstick and breast weights compared to other treatments. This means that the increased amounts of fibre in *L. javanica* could have affected the digestion and nutrient assimilation dynamics, resulting in reduced weight of the carcass cuts.

4.5.3 Meat quality

The pH value of meat has been associated with attributes such as tenderness, WHC, cooking loss and shelf life (Allen *et al.*, 1998). Husak *et al.* (2008) reported that higher pH in meat is more effective for retaining a desirable colour and moisture absorption properties. The ultimatet pH (pH_u) of the meat from all dietary treatments was similar, it ranged from 5.7 to 6.0 suggesting that inclusion of *L. javanica* in PK diets did not negatively influence ultimate meat pH. According to Muchenje *et al.* (2009), the ultimate pH is an indicator of the extent of pH decline 24 hours after slaughter. The pH_u of meat across all dietary treatments was, however, slightly higher than the recommended pH for good quality meat, which is usually from 5.4 to 5.7 (Jaturasitha *et al.*, 2008). The pH of meat from free-range chickens in the previous report was 5.85 (Funaro *et al.*, 2014). High pH values are associated with dark, firm and dry (DFD) meat whilst the low pH values are associated with pale, soft and exudative (PSE) meat (Swatland, 2008). Barnut (2008) reported that rapid post-mortem muscle glycolysis contributes to lower pH in the meat. The current data are in line with the results of Ao *et al.* (2008), which showed that ultimate meat pH ranges from 5.5 to 6.5 in monogastric animals.

The colour of meat is important to consumers when they buy meat products and is an important factor affecting consumer acceptance of meat and meat products (Muchenje *et al.*, 2009, Xazela *et al.,* 2012). In the current study, all meat colour parameters were different across treatments. From the results, it appears that increasing level of *L. javanica* in diets increased the yellowness and redness of the meat. This could perhaps be attributed to the large amount of polyphenols and other pigment compounds in *L. javanica,* which were reported to influence meat colour (Owens *et al.*, 2000; Mpofu *et al.*, 2016). Nevertheless, the meat colour values fell within acceptable ranges (Barbut, 1996; Owens *et al.*, 2000) suggesting that *L. javanica* had no

negative effect on this parameter. The colour of PK meat in this study tended to be more yellow (b*) when compared to the results obtained from broiler meat (b* = 5.84), which were reported by Bianchi *et al.* (2007).

According to Hughes *et al.* (2014), water holding capacity is not vital only for visual and economic reason, it also plays a role in moulding the muscle structure and the ensuing effects on meat quality. The meat from Potchefstroom koekoek chickens fed the control diet had the lowest water retention whilst meat from those chickens supplemented with *L. javanica* had higher water holding capacity. This suggest that diet containing *L. javanica* promoted greater water holding capacity which might be due to essential oil found in *L .javanica* leaves Nzira *et al.,* (2009). With regards to drip loss and cooking, the control diet had higher values and the meat tended to be juicier. The meat shear force of the is an independent measurement of meat tenderness and the lower the shear force values the higher the meat tenderness (Lonergan *et al.*, 2003; Li *et al.*, 2016). The results of shear force indicate that supplementation with *L. javanica* resulted in meat with higher shear force and thus less tender compared to meat from chicken fed CON+ meat. However, the lack of statistically significant differences in terms of tenderness means that all of the dietary treatments promoted similar levels of tenderness in agreement with Schilling *et al.*, (2003).

4.6 Conclusions

The results obtained from the current study showed that supplementing with *L. javanica* can positively affect the size of viscera, external organs, meat quality and slaughter weights of the Potchefstroom koekoek chickens. Chickens fed diet LJ25 had the highest final body weight. Inclusion of *L. javanica* in diets appeared to increase the redness and yellowness of the meat.

Additionally, high values for shear force were observed with the inclusion of *L. javanica*. However, all meat quality parameters fell within the recommended ranges meaning that inclusion of *L. javanica* in diets had no negative effects on the tenderness of the meat.

4.7 References

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5 CHAPTER FIVE - GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

A feeding trial was conducted over a period of 16 weeks to determine the effects of supplementing *Lippia javanica* leaf meal, a plant product with possible nutraceutical properties, on growth performance, blood parameters and meat quality of Potchesfstroom koekoek (PK), an indigenous chicken commonly reared in extensive production systems in South Africa. One hundred and sixty male PK chickens were reared at the North-West University farm. *Lippia javanica* leaves were harvested by hand, air-dried and milled into a leaf meal (LJ). The leaf meal was chemically analysed and added to a commercial grower diet at a rate of 25 g LJ/kg diet and 50 g LJ /kg diet. An additional two diets, a positive control (commercial grower diet with antibiotics) and a negative control (commercial grower diet without antibiotics) were formulated and thus bringing to four, the total number of dietary treatments. The results show that the chickens fed diets supplemented with *L. javanica* leaf meal had similar overall feed intake to the positive and negative control diets. However the results illustrate that the inclusion levels of *L. javanica* in PK diets did not have any negative effect on growth performance. This is vital because inclusion of plant feed resources in chicken diets often tends to reduce diet utilization and hence growth performance due to the presence of fibre and other antinutritional compounds. The utility of any plant-based feed ingredient as a nutraceutical can only be enhanced if inclusion of the plant as a dietary supplement does not negatively affect performance. In this respect, *L. javanica* leaf meal is a good candidate to boost productivity of PK chickens if included in diets at levels up to 50 g/kg. Similar results were obtained by Mpofu *et al.* (2016) in a study where broiler chickens were also fed diets containing *L. javanica* leaf meal. According to Walugembe *et al.*, (2014), high fibre content in chicken diets can lead to the withdrawal from the feed, which could be one of the mechanisms

through which feed intake was affected in the current study. This suggests that high inclusion levels of leaf meals in chicken diets may negatively affect growth parameters; therefore, higher inclusion levels have to be used with caution. However, it appears that the inclusion levels of *L. javanica* leaf meal used in the current study were low enough hence they did not negatively affect digestion and absorption of feed.

Results on the haematology of the Potchefstroom chicken showed that *L. javanica* has a favourable effect on haemoglobin production (haematopoiesis) and erythrocytes formation. This is reflected by the increased erythrocytes count in PK fed offered diets containing the *L. javanica* leaf meal. According to Abdel-Wahhab and Aly (2005); Talebi *et al.*, (2005); and Toghyani *et al.*, (2010), plants such as *Nigella sativa*, which have similar effects to *L. javanica* have been found to have significant effects on haematological parameters. In the current study, the observed effects of *L. javanica* inclusion in the diet on total protein, bilirubin, sodium, potassium, cholesterol and other serum parameters are consistent with the findings in broilers by Mpofu *et al.* (2016) and Ladokun *et al.* (2008). Total serum protein has been reported to be an important indicator of the adequacy of protein retained in the animal body (Akinola & Abiola, 1991; Esonu *et al.*, 2001). The findings of the current study indicates that dietary treatment had an influence on the size of some internal organs of Potchefstroom koekoek chickens, however, the gizzard, proventriculus, and small intestine and caecal sizes were not markedly affected by dietary treatments. The ultimate meat pH (pH_u) ranged from 5.7 to 5.8., which is slightly higher than the recommended pH of a good quality meat, which is usually from 5.4 to 5.7.

5.2 Conclusions

In conclusion, the results of the current study indicated that supplementing chickens with *L. javanica* leaf meal at rates up to 50 g/kg could induce favourable outcomes on chicken growth performance and carcass characteristics. Therefore, *L.javanica* can be used as a neutraceutical ingredient in formulating feed for PK chickens. From observed data the inclusion level of *L. javanica* positively affected the growth performance, carcass characteristic, blood parameters and meat quality. As such, more studies need to be conducted with *L. javanica* in order to investigate other parameters, such as nutrients digestibility and egg quality which were not included in this study. *Lippia javanica* leaf meal concentration could be used to supplement the diets of indigenous chicken.

5.3 Recommendations

The results obtained from the current study showed no side effects from *L. javanica* supplementation with commercial diets. Communal and commercial farmers can use *L. javanica* as a nutraceutical in indigenous chicken diets up to an inclusion level of 50g/kg. However little is known about nutrients from *L. javanica*. In general, it is well-known that feed contributes 70% of production cost, including *L. javanica* in poultry diets can reduce the production costs. Further studies should be investigated for feed production cost with *L. javanica* as a nutraceutical for other animal species.

6 APPENDIX 1

Dependent Variable: GAIN1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1038.05200	346.01733	0.20	0.8944
Error	16	27584.22800	1724.01425		
Corrected Total	19	28622.28000			

R-Square	Coeff Var	Root MSE	GAIN1 Mean
0.036267	39.92428	41.52125	104.0000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	1038.052000	346.017333	0.20	0.8944

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	1038.052000	346.017333	0.20	0.8944

Dependent Variable: GAIN2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1040.202000	346.734000	3.88	0.0294
Error	16	1431.576000	89.473500		
Corrected Total	19	2471.778000			

R-Square	Coeff Var	Root MSE	GAIN2 Mean

R-Square	Coeff Var	Root MSE	GAIN2 Mean
0.420831	9.572962	9.459043	98.81000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	1040.202000	346.734000	3.88	0.0294

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	1040.202000	346.734000	3.88	0.0294

Dependent Variable: GAIN3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6860.51600	2286.83867	0.68	0.5757
Error	16	53622.37600	3351.39850		
Corrected Total	19	60482.89200			

R-Square	Coeff Var	Root MSE	GAIN3 Mean
0.113429	40.61975	57.89126	142.5200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	6860.516000	2286.838667	0.68	0.5757

Source	DF	Type III SS	Mean Square	F Value	Pr > F

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	6860.516000	2286.838667	0.68	0.5757

Dependent Variable: GAIN4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1306.68400	435.56133	0.21	0.8901
Error	16	33671.72800	2104.48300		
Corrected Total	19	34978.41200			

R-Square	Coeff Var	Root MSE	GAIN4 Mean
0.037357	33.98625	45.87464	134.9800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	1306.684000	435.561333	0.21	0.8901

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	1306.684000	435.561333	0.21	0.8901

Dependent Variable: GAIN5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	855.453500	285.151167	1.72	0.2039
Error	16	2658.964000	166.185250		
Corrected Total	19	3514.417500			

R-Square	Coeff Var	Root MSE	GAIN5 Mean
0.243413	8.502085	12.89129	151.6250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	855.4535000	285.1511667	1.72	0.2039

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	855.4535000	285.1511667	1.72	0.2039

Dependent Variable: GAIN6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	264.677500	88.225833	0.24	0.8681
Error	16	5916.028000	369.751750		
Corrected Total	19	6180.705500			

R-Square	Coeff Var	Root MSE	GAIN6 Mean
0.042823	12.13297	19.22893	158.4850

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	264.6775000	88.2258333	0.24	0.8681

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	264.6775000	88.2258333	0.24	0.8681

Dependent Variable: GAIN7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1996.037500	665.345833	2.65	0.0844
Error	16	4021.528000	251.345500		
Corrected Total	19	6017.565500			

R-Square	Coeff Var	Root MSE	GAIN7 Mean
0.331702	10.59645	15.85388	149.6150

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	1996.037500	665.345833	2.65	0.0844

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	1996.037500	665.345833	2.65	0.0844

Dependent Variable: GAIN8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1384.253500	461.417833	1.70	0.2070
Error	16	4340.972000	271.310750		
Corrected Total	19	5725.225500			

R-Square	Coeff Var	Root MSE	GAIN8 Mean
0.241781	10.84116	16.47151	151.9350

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	1384.253500	461.417833	1.70	0.2070

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	1384.253500	461.417833	1.70	0.2070

Dependent Variable: GAIN9

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2443.740000	814.580000	4.48	0.0183
Error	16	2912.092000	182.005750		
Corrected Total	19	5355.832000			

R-Square	Coeff Var	Root MSE	GAIN9 Mean
0.456276	8.801507	13.49095	153.2800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	2443.740000	814.580000	4.48	0.0183

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	2443.740000	814.580000	4.48	0.0183

Dependent Variable: GAIN10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2739.730000	913.243333	2.40	0.1057

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	16	6084.268000	380.266750		
Corrected Total	19	8823.998000			

R-Square	Coeff Var	Root MSE	GAIN10 Mean
0.310486	15.76557	19.50043	123.6900

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	2739.730000	913.243333	2.40	0.1057

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	2739.730000	913.243333	2.40	0.1057

Dependent Variable: GAIN11

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6135.51750	2045.17250	2.58	0.0896
Error	16	12674.68800	792.16800		
Corrected Total	19	18810.20550			

R-Square	Coeff Var	Root MSE	GAIN11 Mean
0.326180	21.08039	28.14548	133.5150

Source	DF	Type I SS	Mean Square	F Value	Pr > F

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	6135.517500	2045.172500	2.58	0.0896

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	6135.517500	2045.172500	2.58	0.0896

Dependent Variable: GAIN12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4588.43200	1529.47733	1.13	0.3657
Error	16	21609.91600	1350.61975		
Corrected Total	19	26198.34800			

R-Square	Coeff Var	Root MSE	GAIN12 Mean
0.175142	56.24545	36.75078	65.34000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	4588.432000	1529.477333	1.13	0.3657

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	4588.432000	1529.477333	1.13	0.3657

Dependent Variable: OveGain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	81446.4520	27148.8173	2.53	0.0939
Error	16	171681.8160	10730.1135		
Corrected Total	19	253128.2680			

R-Square	Coeff Var	Root MSE	OveGain Mean
0.321760	6.607362	103.5863	1567.740

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DIET	3	81446.45200	27148.81733	2.53	0.0939

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DIET	3	81446.45200	27148.81733	2.53	0.0939