

**EFFECT OF CITRIC ACID SUPPLEMENTATION ON GROWTH PERFORMANCE,
CARCASS CHARACTERISTICS AND PHYSICO-CHEMICAL ATTRIBUTES OF
MALE VENDA CHICKENS**

BY

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DECLARATION

I declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Animal Production has not been submitted by me for a degree at this or any other university, this is my own work in design and execution, and that all materials contained herein has been duly acknowledged.

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ABSTRACT

Two experiments were conducted to investigate the effect of citric acid supplementation on growth performance, carcass and physicochemical features of male Venda chickens. Day-old chicks were vaccinated against diseases at hatchery. Sick chickens were isolated and treated accordingly by the veterinarian. On Experiment 1, birds were assigned to four dietary supplemented with varying inclusion levels of citric acid. Treatment description was as follows: CA1: 0g/kg DM of feed, CA2: 12.5g/kg DM of feed, CA3: 25g/kg DM of feed, and CA4: 50g/kg DM of feed, where 50 chicks were randomly assigned to each treatment.

In the present study, citric acid did not affect ($P>0.05$) DM feed intake and feed conversion ratio of male Venda chickens aged one to 30 days. However, citric acid supplementation affected ($P<0.05$) growth rate and live weights of male Venda chickens aged one to 30 days. The growth rates and live weights of male Venda chickens aged one to 30 days were optimised at citric acid supplementation levels of 2.392 and 2.536g per kg DM of the diet. Citric acid supplementation levels of 12 and 25g per kg DM improved ($P<0.05$) DM feed intake, growth rate, feed conversion ratio, and live weight of male Venda chickens aged 31 to 90 days. Optimal growth rate, feed conversion ratio, and live weights of male Venda chickens aged 31 to 60 days were optimised at citric acid supplementation levels of 2.250, 2.373, and 2.308g per kg DM of the diet. Citric acid supplementation levels of 1.560, 2.167, 2.332, and 2.272g per kg DM of the diet resulted in optimised DM feed intake, growth rate, feed conversion ratio, and live weights of male Venda chickens aged 61 to 90 days. Citric acid supplementation improved ($P<0.05$) live weight, carcass weight and dressing pieces of male Venda chickens aged 90 days.

On experiment 2, the effect of citric acid supplementation on meat pH, thawing loss, cooking loss and shear force of male Venda chickens were determined. Supplementing Venda chickens with citric acid had affected ($P<0.05$) cooking loss, shear force and meat pH of male Venda chickens aged 90 days. Male chickens supplemented with 50g of citric acid per kg DM outperformed 0, 12.5 and 25g of citric acid per kg DM in terms of physicochemical features, implying that 50g of citric acid

per kg DM can help improve meat pH, thawing loss, cooking loss and shear force values of male Venda chickens.

It can be concluded that citric acid supplementation of 12.5, 25g per kg DM can be utilized in the diet of Venda chickens aged one to 90 days. However, 50g of citric acid resulted in lower feed intake and weight loss this might be because high levels of citric acid supplementation may be too sour and made the feed to appear unappealing to the chickens. However, more research is needed to confirm these findings.

Keywords: citric acid, weights, meat pH, cooking loss, chickens.

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LIST OF ABBREVIATIONS

AGP	Antibiotic growth promoter
ABF	Antibiotic free birds
GIT	Gastrointestinal tract
PUFA	Polyunsaturated fatty acids
CA	Citric acid
L*	Lightness
A*	Redness
B*	Yellowness
WHC	Water holding capacity
PSE	Pale, soft, and exudative
G	Grams
Kg	Kilograms
WBC	Weight before cooking
WAC	Weight after cooking
pHu	Ultimate pH
n-6:n-3	Omega 6 to omega 3 ratio
MJ	Megajoule
ME	Metabolisable energy
DM	Dry matter
ANOVA	Analysis of variance
AOAC	Association of Analytic Chemists
ARC	Agricultural Research Council

$^{\circ}\text{C}$	Degree cetrigrade
r^2	Coefficient of determination
FRC	Feed conversion ratio
D	Dry matter
SPSS	Statistical Package of Social Sciences
WBSF	Warner-Bratler Shear Force
HSD	Honest Significant Difference

CHAPTER 1
INTRODUCTION

1.1 Background

According to Robinson *et al.* (2015), 70% of the world's population will live in cities by 2050, resulting in a 70% increase in demand for animal-derived food, needing high levels of efficient production to meet these demands. Total population living in cities is estimated at 2.36 billion, representing 52% of world urban population in 2022 (Demographia World Urban Areas, 2022). Global meat production has expanded dramatically, with poultry and pig production accounting for the majority, particularly in developing nations (Thornton, 2010). Over the past 50 years, it has become standard practice to use antibiotic growth promoters (AGP) to increase animal performance (Gollnisch *et al.*, 2001). Antibiotics have been found to minimize subclinical and clinical infections by decreasing the number of bacteria in the gastrointestinal tract (GIT). The decreased number of bacteria in the GIT decreases food competition, stimulates the immune system, thins the intestinal wall, and promotes nutritional digestibility (Economou and Gousia, 2015). In contrast, antibiotic resistance has become a continuous source of concern (Hamid, 1992). The introduction and extensive usage of antibiotics has had a substantial impact on animal health and well-being (Gorforth and Goforth, 2000). Long-term antibiotic use in poultry has been called into question since it can result in adverse effects such as meat residues and the development of microbial resistance (Muaz *et al.*, 2018).

With the removal of antibiotics from various poultry sectors and the introduction of antibiotic-free birds (ABF), the industry is faced with an immediate demand to replace antibiotics with comparable abilities (Cheng *et al.*, 2014). Organic acids including citric acid, acetic acid, propionic acid, and formic acid have the ability to accelerate poultry growth and improve other productivity indices (Islam, 2012). In addition, organic acids improve animal welfare and meat quality qualities (Menconi *et al.*, 2013). Most common bacteria that affect the intestinal health of poultry are Salmonella, Campylobacter and Escherichia coli which can be controlled by supplementation of citric acid in diet, therefore enhancing growth performance and carcass characteristics (Dittoe *et al.*, 2018).

Current meat consumption trends highlight the significance of meat quality control in the poultry industry (Mari *et al.*, 2012). The most common chicken carcass components are the breast and thigh muscles (Yu *et al.*, 2005). It was observed that

the use of an acidifier can improve the development rate and carcass quality of broiler chicken (Mellor, 2000). However, information on the use of citric acid to improve growth and quality of Venda chickens' carcass and meat is limited and not conclusive. Therefore, there is a need to assess the effect of citric acid supplementation on the carcass features and physicochemical qualities of male Venda chicken.

1.2 Problem statement

Indigenous chickens are extremely significant nutritionally, commercially, and culturally in South Africa (Alabi, 2013). The meat from such chickens is highly sought after all over the world (Choo *et al.*, 2014; Walley *et al.*, 2015). However, indigenous chickens, such as Venda chickens, have poor growth rates (Alabi *et al.*, 2013). As a result, it is critical to boost indigenous chicken growth rates and find ingredients to improve their carcass characteristics and physico-chemical attributes. The use of an acidifier may improve broiler chicken development and carcass quality (Mellor, 2010) since they have been tested in laying hens, broiler chickens and in pils and the results have shown that organic acids can improve poultry performance (Jahanian and Golshadi, 2015; Dehghani-Tafti and Jahanian, 2016; Long *et al.*, 2018)

1.3 Rationale

Organic acids such as citric acid, acetic acid, propionic acid, and formic acid are excellent naturally occurring growth boosters which could be incorporated in poultry diets (Islam, 2012). According to Menconi *et al.* (2013), organic acids such as citric acid may improve animal welfare and economic problems in the chicken business by lowering body weight loss and enhancing meat quality qualities. Several studies found that adding organic acids like citric acid to broiler feeds increased weight gain (Nourmohammadi and Afzali, 2013; Fazayeli- Rad *et al.*, 2014). Citric acid supplementation in feeds reduces microscopic organisms and organisms within the gastrointestinal tract, increasing feed intake and digestion and thereby improving broiler chicken growth rate and carcass qualities (Papatsiros *et al.*, 2013; Dittoe *et al.*, 2018).

Kim *et al.* (2015) showed that increasing the citric acid concentration had an antibacterial effect on the growth of microorganisms in the physicochemical properties of sous vide chicken breast at 2% and 5% concentrations. Citric acid is used in

marinades to increase the water retention and softness of broiler birds. It acts as a chelator to regulate the action of pro-oxidant metals (Ke *et al.*,2017). Aktas *et al.* (2016) discovered that marinating meat with citric acid reduces shear force value in chickens. However, research on the effect of citric acid supplementation on carcass traits and physicochemical properties of Venda chickens is sparse, to the best of our knowledge.

1.3.1 Aim

The aim of the study was to identify the supplementation level of citric acid that might be used to improve growth performance, carcass characteristics and physicochemical attributes of male Venda chickens.

1.3.2 Objectives

The objectives of the study were to determine:

- I. the effect of citric acid supplementation on feed intake, growth intake, feed conversion ratio and live weight of male Venda chickens
- II. the effect of citric acid supplementation on live weight, carcass weight and dressing pieces of male Venda chickens.
- III. the effect of citric acid supplementation on meat pH, shear force and cooking loss of male Venda chickens.

1.3.3 Hypotheses

The hypotheses of the study were as follows:

- I. Supplementation of citric acid has no effect on feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens.
- II. Supplementation of citric acid has no effect on live weight, carcass weight and dressing pieces of male Venda chickens.
- III. Supplementation of citric acid has no effect on meat colour, meat pH, shear force and cooking loss of male Venda chickens.

CHAPTER 2
LIRETATURE REVIEW

2.1 Introduction

Indigenous chickens are the most extensively domesticated type of livestock in resource-limited rural parts of Southern Africa. In South Africa, Venda chickens are abundant in the Limpopo province (Mtileni *et al.*, 2011). Traditionally, resource-poor farmers with limited resources reared these types of chickens. This is due to their remarkable illness resistance, flexibility, scavenging abilities, and ability to live without scheduled feeding (Ajayi, 2010). With local communities producing around 80% of chicken products, the indigenous poultry industry is crucial to the national economy (Sharma, 2010). Chicken flesh is regarded to be the most popular poultry product (Sharma, 2010). Chicken consumption is expected to rise year after year because of high demand, low pricing, few or no religious restrictions, excellent digestion, good taste, and low calorie content (Raphulu *et al.*, 2015).

Indigenous chickens play an important role in Southern African rural communities with limited resources. They transform readily available feed ingredients into highly nutritious and valued products and functions. Chicken meat and eggs account for a considerable part of animal protein consumed in rural areas of Southern Africa (Swatson, 2003). Poultry meat and eggs provide protein that is important especially to children, elderly and pregnant women, therefore making a significant contribution in areas malnutrition particularly in rural areas (Martin *et al.*, 2012). According to Muchadeyi *et al.* (2007a) most villages rely only on indigenous chickens for income. They sell meat and eggs to the neighbours or at village market. Chicken meat and product sales offer the households a pathway out of poverty, income to purchase food and items and pay school fees. Indigenous chickens are sold at higher price than broilers (Mtileni *et al.*, 2009). The reason could be because of the multiple uses of the indigenous chickens including for cultural and ritual purposes as well as food (FAO, 2010).

Venda chickens are typically reared using scavenging production system adopted by subsistence farmers, and to a lesser extent through semi-intensive production systems (Muchadeyi *et al.*, 2004; Mtileni *et al.*, 2009). Venda chickens have poor reproductive success, low growth rates, diseases and high mortality (Salum *et al.*, 2002; Conroy *et al.*, 2005). Despite the fact that they are a slow-growing species with

a low carcass weight, consumers prefer them compared to the improved breed (Missohou *et al.*, 2002).

The growth of nutritionally based health issues has raised consumer awareness of broiler meat quality (Ncube *et al.*, 2018). As a result, because of their greater meat quality, local chicken breeds are chosen (Sheng *et al.*, 2013). Furthermore, there is high demand of meat and meat products because of increased human population leading to shortage of meat (FAO, 2015). Health issues have contributed to an increase in demand for healthier chicken meat. Slow-growing bird meat is healthier nutritionally (less fat and higher n-three polyunsaturated fatty acid (PUFA) content) and hence may better match customer expectations for organic products (Sirri *et al.*, 2011).

Meat quality control in the broiler sector is important (Gaya *et al.*, 2011). Chicken meat is also widely available as retail chunks or processed meat (Le BihanDuval *et al.*, 2008). The most common chicken carcass components are the breast and thigh muscles (Yu *et al.*, 2005). According to Lang hout. (2000), using an acidifier may improve the development rate and carcass quality of broiler chickens.

2.2 Description of Venda chickens

Lebowa-Venda chicken breed was first observed in the Venda district of Limpopo Province (Figure 2.01). (Mogesse, 2007a). Lebowa-Venda is a multicolored chicken with basic colors similar to the white, black, and brown of the region's indigenous cattle and goats (Van Marle-Koster and Nel, 2000). It has a single comb but can also have a rose comb and five toes. Lebowa-Venda produces few eggs but is broody and has outstanding mothering ability (Mogesse, 2007a; Mngonyama, 2012). The LebowaVenda chicken is fairly large in contrast to other indigenous chicken species and lays huge, colorful eggs. These chickens reach sexual maturity at 143 days, weighing an average of 2.1 kg in cocks and 1.4 kg in hens at 20 weeks of age (Mogesse, 2007a). The average weight of the cockerels and hens can reach up to 2.9-3.6 kg and 2.4-3.0 kg (Manyelo *et al.*, 2020). These Venda chickens are resilient and have a restricted reproductive potential (Van Marle-Koeste *et al.*, 2008). (Norris *et al.*, 2007).



Figure 2.01 The structure of Venda hen and rooster. Source: Anonymous (2010a)

2.3 Rearing of indigenous chickens under communal systems and their meat quality attributes

The production system of a poultry farm has a considerable impact on the quality of poultry carcasses and meat (Fanatico *et al.*, 2005a; Husak *et al.*, 2008; Dal Bosco *et al.*, 2012; Bogosavljevi-Bokovi *et al.*, 2012). In villages, there are comprehensive systems for maintaining chickens, which exposes them to weather extremes, predators, thefts, and diseases, as well as unregulated breeding (Goraga *et al.*, 2016; Olwande *et al.*, 2010). In communal areas, there are no organized feeding systems in place. Chickens roam around in-search of feed resources to meet daily nutritional needs. Indigenous chickens roam free and scavenge for food, but supplementary feeds based on cereal grains such as maize and sorghum are often springled on the ground for the chickens to eat (Gondwe and Wollny, 2007; Melesse, 2014).

Various research on indigenous chicken production (Dana *et al.*, 2010a; Mengesha, 2006) have identified the most common production system as one with small flock sizes, minimal input requirements, generally good output, and infrequent disease outbreaks. In this technique, indigenous chicken genotypes are employed, allowing them to graze freely or forage on neighbouring lands to meet their nutritional needs. The feed that the indigenous chickens eat is determined by local food supplies

scavenged. When rearing indigenous chickens, a house may not always be available, and if it is, it is created from locally produced materials (Mtileni *et al.*, 2009).

Indigenous chickens reared in full free range had a higher breast percentage when compared to intensively reared chickens (Sanka and Mbage, 2014). This is because of low stocking density and increased physical activities which reduce abdominal fat and increase the breast muscle (Sanka and Mbage, 2014; Cheng *et al.*, 2008). Consumers believe that poultry meat from extensive production system is flavorful because the diet of the chickens contains additional nutrients that chickens consume while scavenging which has a positive effect on flavour, aroma as well as color of the meat (Sossidou *et al.*, 2015). As a result of the low production costs, homesteaders choose the large production system (Magothe *et al.*, 2012). However, this method cannot ensure or verify chicken quality, especially in terms of live body weight, carcass proportion, meat quality, and meat safety (Wattanachant, 2008).

Allowing indigenous birds to wander has a significant impact on body weight gain and breast yield, as well as the lightness of the breast meat and the redness of the leg meat (Tong *et al.*, 2015). Puchala *et al.* (2015) revealed that free-range chickens ingest plants containing xanthophyll, which accumulates in subcutaneous fat, boosting carcass color intensity and boiled broth of such carcasses, making them yellower and thus more appealing to consumers. Furthermore, Fanatico *et al.* (2005a) discovered that free range management of indigenous chickens increases skin color intensity. Furthermore, Puchala *et al.* (2015) discovered a decrease in carcass fatness in Greenleg Partridge and Rhode Island Red chickens raised under intensive management, but an increase in the quantity of both omega 6 to omega 3 ratio (n-6:n3) polyunsaturated fatty acids (PUFA) in the breast and leg muscles without affecting saturated fatty acid content.

2.4 Citric Acid (CA)

2.4.1 Citric acid description

Citric acid ($C_6H_8O_7$) (Figure 2.02) is a weak organic acid that occurs naturally in all citrus fruits and has a pH of 0.2 (Makut and Ekeleme, 2018). It gets its name from the Latin word "citrus" and is produced in living cells through a biochemical reaction known as the Krebs cycle (Swain *et al.*, 2011). It is an ever present metabolic intermediate

product that can be found in almost most living organisms (Papagianni, 2007). Citric acid is available in the form of colourless crystals or a white or almost white crystalline powder that is nearly odourless (Commission Directive, 2008). Citric acid is highly soluble, colourless, and solid at room temperature in its pure form (Angumeenal and Venkappayya, 2013). Due to the prevalence of triple carboxylic acid functional groups in its structure, this has a molar mass of 210.14 g/mol with three different pKa levels around pH 3.1, 4.7, and 6.4. (Papagianni, 2007). It was first extracted from lemon juice around 1784 and is really a crucial metabolic outcome of the tricarboxylic acid (or Krebs) cycle that can be found in tiny amounts in almost all living organisms. (Max *et al.*, 2010).

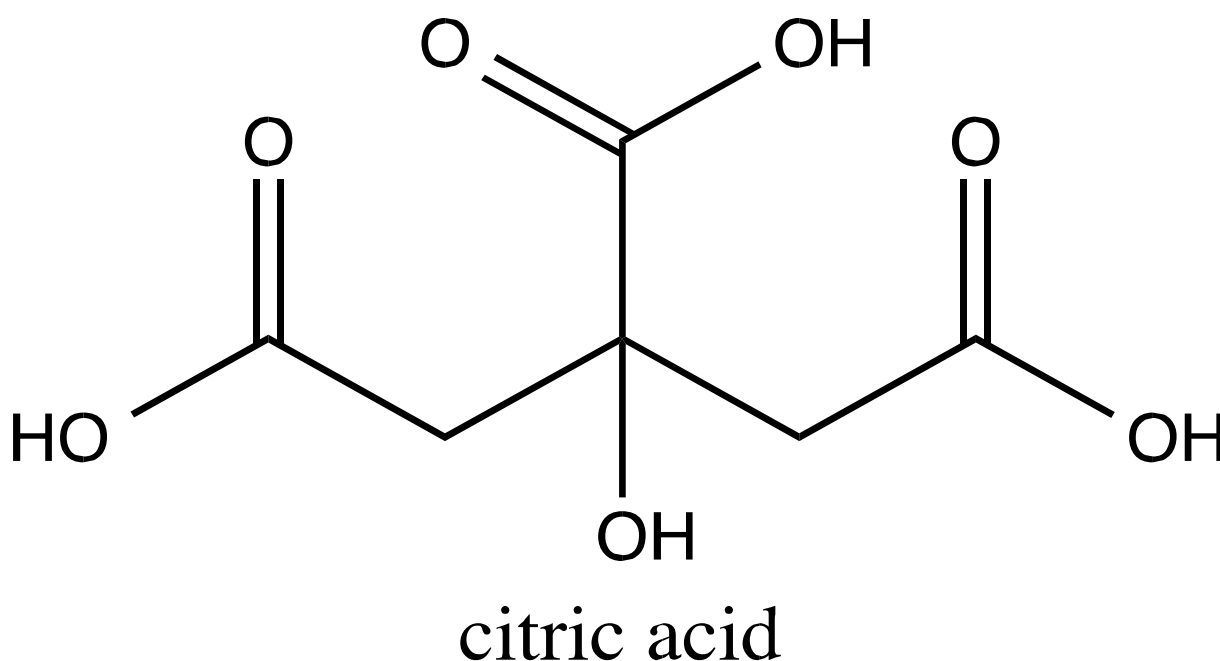


Figure 2.02 Citric acid molecular structure. ChemBioDraw (2014).

2.4.2 Production of citric acid

CA can be produced through either solid state or submerged fermentation (Adham, 2002). Citric acid is commercially produced via a microbiological approach that typically involves submerged fermentation with *Aspergillus niger* (Prasad *et al.*, 2013; Yadegary *et al.*, 2013). Citric acid is a bioengineered and biochemical substance that is usually produced in tones and has a yearly output of 1.6 million tonnes (Nadeem *et al.*, 2010; Nwoba *et al.*, 2012). CA is produced by a wide range of microbial taxa, including bacteria, fungi, and yeast. Table 2.01 show some bacteria that produce citric acid. Nonetheless, the majority of them are unable to offer commercially viable yields. Today, most citric acid is produced by the fungus *A. niger* (Ali *et al.*, 2002). The

reasons for choosing *A. niger* over all other potential future citric acid manufacturing microorganisms include its increased citric acid productive capacity at low pH well without release of harmful compounds (Nwoba *et al.*, 2012; Haider,2014), ease of handling (Nadeem *et al.*,2010), and ability to metabolize a variety of cheaper products such as brewers spent seed (Femi-Ola and Atere, 2013), orange peels ((Majumder *et al.*, 2010; Pawar and Pawar., 2014).

Table 2.01 Microorganisms capable of producing citric acid. Source: Swain *et al.* (2011).

Fungi	Yeast	Bacteria
<i>Aspergillus niger</i>	<i>Candida tropicalis</i>	<i>Arthrobacter paraffinens</i>
<i>A. aculeatus</i>	<i>C.oleophila</i>	<i>Bacillus licheniformis</i>
<i>A. carbonarius</i>	<i>C.guilliermondii</i>	<i>Corynebacterium spp</i>
<i>C. citroformans</i>	<i>C. citroformans</i>	
<i>A. foetidus</i>	<i>C. intermedia</i>	
<i>A. luchensis</i>	<i>C. parapsilosis</i>	
<i>Penicillium spp</i>	<i>C. fimbriae</i>	
Mutant strains	<i>C. lipolytica</i>	
<i>A. niger</i> YW-112	<i>Yarrowia lipolytica</i>	
<i>A. niger</i> GCB-75	<i>Hansenula anamola</i>	

2.5 Citric acid as growth promoter

Costa *et al.* (2013) demonstrated the ability of organic acids to improve growth in a variety of food animals, including pigs, poultry, and fish, with implications for animal health and productivity. Although the effects of acids are not limited to pigs, many studies have been undertaken on their use. In one study, piglets' growth, average daily feed intake (ADFI), and feed conversion ratio (FCR) were all improved, and postweaning oedema illness was reduced when compared to a negative control. According to the findings, where antibiotics are not permitted, the acids tested (lactic and citric acids) should be utilized as feed supplements instead of antibiotics (Tsiloyiannis *et al.*, 2001a). During a post-weaning diarrhoea epidemic in pigs, six different acids (propionic, lactic,formic,malic,citric, and fumaric acids) significantly increased feed intake compared to a negative control diet.

2.6 Carcasses characteristics of indigenous chicken

In places where local chicken is becoming more popular, most are sold whole, and in the poultry trade, consumers prefer whole chicken over processed chicken (Zhao *et al.*, 2012). Carcasses can be sold entire after slaughter, or separately as bone fragments or sections. To fulfil the increased demand for higher quality and more processed poultry, the poultry industry had to adapt its feeding method. Most chicken products are currently marketed in order to generate high-value goods such as breast meat and boneless fillets (Young *et al.*, 2001). Broiler lineage, gender, and slaughter age are all thought to increase meat quality. As a result, poultry growers must be able to forecast yield patterns (Young *et al.*, 2001).

Chickens must have high slaughter efficiency and an ideal carcass structure to fulfil the needs of consumers and the slaughter industry (Bogosavljevic Boskovic *et al.*, 2010). Common parameters for broiler carcass output include live weight, slaughter weight, dressing weight, refrigerator weight, and partial yield (Agbede and Aletor, 2003; Gadzirai *et al.*, 2012). Poultry carcasses are the empty bodies of chickens that have been slaughtered and are utilized for food or further processing. Several configurations can be created when processing chicken carcasses, and the components generated usually depend on the value of the parts, which depends on the consumer's taste (Owens *et al.*, 2000). The edible yield of breasts, drumsticks, thighs, and wings can be calculated as a percentage of the overall carcass weight. It is often reported as a percentage of the carcass weight. In short, the dressing %, the yield rate of the parts, and breakdown qualities of the pieces effectively represent the carcass structure.

The poultry industry is significantly reliant on the ability of the producer to enhance the proportion of the most relevant components of the carcass. These include enhanced pectoral muscle production and a decrease in carcass fat (Guerrero-Legarreta, 2010). Customers today are willing to accept and pay a premium for a product's convenience and partial preparation (Owens *et al.*, 2010). According to Young *et al.* (2001), wing and drumstick growth was inconsistent with age as compared to the breast, thighs, flanks, and forelimbs, which grew with age at slaughter. The line system and feed have the greatest influence on the composition of poultry carcasses. These two elements have also been demonstrated to have an impact on meat quality (Jaturasitha

et al., 2008b). Traditional chickens are raised in a litter production method and have various behaviours.

Native chickens gain less weight than improved chickens, one of the reasons for that is that improved strains are raised under intensive production system which favour productivity (Lawrie and Ledward, 2006). However, many production systems (local chicken production system) are subjected to high temperatures and increased forage area activities (Fanatico *et al.*, 2005). As a result, if birds from the intense production system were sacrificed at the same age as those from the extensive production system, it is expected that they would fully develop at a younger age and provide larger slaughter weight and fatter carcasses. According to Castellini *et al.* (2002) extensive birds exhibited lower development rates and carcass weights than intensive birds.

In South Africa, there is limited information available on the carcass features and portion yields of domestic chicken lines. Van Marle-Köster and Webb (2006) compared the carcass characteristics of domestic South African birds to those of a commercial broiler line (the Potchefstroom Koekoek, New Hampshire, Naked-Neck, LebowaVenda, and Ovambo chicken lines). The chickens were fed a commercial broiler diet for 11 weeks (77 days) before 10 birds from each line were chosen at random for study. The Naked-neck had the highest breast muscle yield, while the Ovambo had the highest dressed carcass weight (939.8g) (18.03 percent). Similar outcomes were obtained by (Jaturasitha *et al.*, 2008a; Jaturasitha *et al.*, 2008b; Hagan and Adjei, 2012).

2.7 Effect of inclusion of citric acid on meat quality

Consumers describe meat quality as the attributes they value, such as visual, sensory, and health characteristics, as well as more intangible qualities such as environmental effects and welfare status (Becker, 2000). The quality of the product is determined by a carcass with greater fat or muscle proportions (Madruga *et al.*, 2009). The first significant stage in judging the quality of a product happens when consumers purchase meat. As a result, they are included in the definition of meat quality (Joo *et al.*, 2013). Consumers are looking for meat that will add to their own satisfaction.

Meat colour, water holding capacity, and fat content, according to Muchenje *et al.* (2009), are the most important visual and palatability markers that influence a consumer's attention in the first place. Aesthetic, sensory, and nutritional aspects, as

well as carcass conformation, should be considered when evaluating meat quality (Bogosavljevic-Boskovic *et al.*, 2010). Only a few of the fundamental and non-basic factors that might affect the various qualitative attributes of chicken meat are genotype, breed, age, rearing system, feeds, chemical composition, structure, muscle quality, and processing conditions (Fletcher, 2002). Birds generate meat, and because freerange chickens display fewer stress-related factors, customers feel they create greater meat quality because they exercise more, behave more naturally, and may be healthier than intensively raised chickens.

2.7.1 Meat pH

Because high muscle temperatures paired with rapid pH fall have a negative impact on meat pH, it is an essential indicator of meat quality (Kim *et al.*, 2014). Aside from muscle structure, breed, maturation level, and sex, pH can be influenced by fasting, eating, cooling, and electrical stimulation. Handle birds antemortem and postmortem to ensure that the pH of the muscles is appropriate (about pH 5.7) (Evaris *et al.*, 2017)

At the time of slaughter, oxygen and nutrition are cut off to the circulatory system. Lactic acid is formed from glycogen in an anaerobic environment. When lactic acid builds up in muscle, the pH is lowered, which promotes muscle conversion to meat. The pH of postmortem muscle tissue is usually around 5.5, but the pH of postmortem chicken meat is usually higher, reaching pH 6.0 at 2 to 4 hours after slaughter (Nissen and Young, 2006; Warriss, 2010). The pace at which pH drops will affect the color, softness, water holding capacity (WHC), loss of moisture during cooking, the juiciness, and the microbiologic stability of a food product (Honikel, 2004).

A high protein water binding capacity influences the physical structure and reflectance qualities of meat (Hughes *et al.*, 2014). A rapid drop in post-mortem pH raises the probability of PSE (pale, soft, and exudative) meat, which is associated with a lower water holding capacity and light meat (Castellini *et al.*, 2002a). A higher pH_u increases the likelihood of dark, hard, and dry meat that is darker and more vulnerable to bacterial invasion, resulting in a shorter life span (Lawrie and Ledward, 2006; Husak *et al.*, 2008). In general, meat with a higher pH retains color better, absorbs moisture better, and has a better flavor (Lawrie and Ledward, 2006; Husak *et al.*, 2008; Warriss, 2010). A higher pH_u value indicates less post-mortem proteolysis and tougher beef products (Fletcher, 2002; Lawrie and Ledward, 2006; Warriss, 2010). Because

indigenous chickens are more susceptible to stress, pH drop occurs in breeds such as the Koekoek more than in improved birds such as broiler chicks, resulting in lower pH (Castellini *et al.*, 2002a; Debut *et al.*, 2003; Berri *et al.*, 2005; Debut *et al.* 2005).

Owing to the existence of more glycogen at the point of slaughter, the flesh of chickens bred in large production systems is frequently reported to have a lower pH (Castellini *et al.*, 2002a; Wang *et al.*, 2009). The pH of local chicken meat was the same as that of intensive meat, according to Ponte *et al.* (2008) and Poltowicz and Doktor. (2011), while Husak *et al.* (2008) observed no significant variation in pH between intensively grown and unrestrained broilers.

The animals' greater activity throughout extensive upbringing may result in more (red) muscle fibres with a higher glycogen content. As a result, specific muscles, particularly the thigh, would have an increased anaerobic glycolytic potential during post-mortem glycolysis, resulting in a lower pH post mortem (Lawrie and Ledward, 2006). A rise in citric acid content lowers the pH of chicken meat, reducing microbial burden (Meltem *et al.*, 2017).

2.7.2 Shear force

Shear force determination is a reliable method for evaluating meat tenderness, and the extent of myofibrillar protein proteolysis is dependent on it (Marcinkow-skalesiak, *et al.*, 2016). Shear force is a softness measurement, with greater values suggesting tougher or less tender meat (Yang *et al.*, 2010). Broilers that grew slowly produced softer breast meat than broilers that expanded swiftly, according to Fanatico *et al.* (2009). This was linked to bodyweight differences in each genotype, which resulted in variable rates of post-mortem stiffness.

The age, gender, muscle positioning, live weight, breed, and antemortem stress of the bird all influence shear force variation (Muchenje *et al.*, 2009). Meat softness and toughness are linked to two components of muscle: muscle fibers and connective tissue (Kerth, 2013a). Sarcomere shortening and the amount of myofibrillar protein metabolism influence the softness of myofibrillar proteins in muscle fibers (Kerth, 2013a). Longer sarcomeres require less shear force than shorter sarcomeres, leading in a favorable connection between increased tenderness and muscle sarcomere

length. Because shorter sarcomas have more actin and myosin overlap and hence more actomyosin cross bridges, a higher shear force is needed (Weaver *et al.*, 2009).

To achieve acceptable levels of tenderness in chicken breast meat, a period of aging is required before the skeletal muscle is removed from the carcass. Since deboning time has the greatest impact on meat quality characteristics, aging for 4-6 hours after slaughter is recommended (Sams and Owens, 2010). Broilers raised to older ages had higher shear rates, and differences in growth toughness may increase toughness (Brewer-Gunsaulis and Owens, 2013). Mehaffey *et al.* (2006) reported that deboning at 2 hours after slaughter resulted in higher shear force, pH values, and L* values regardless of age, compared to decontamination 4 hours after death.

Depending on the cooking method, the tenderness of the meat may vary. Cooking softens the collagen in the muscle tissue, making the connective tissue more fragile. Softening occurs between fractures of the pelvis between 20 and 500 °C and loss of resistance of the surrounding collagen fibers (McCormick, 2008). Longer cooking durations at lower temperatures reduce meat stiffness and are advised for connective tissue muscles (Lawrie and Ledward, 2006). Powell *et al.* (2000) discovered that the heating rate and endpoint temperature had an effect on muscular connective tissue during cooking. Combes *et al.* (2003) discovered that cooking temperature had a substantial effect on mechanical softness characteristics, with higher cooking temperatures increasing beef toughness. Myofibrillar proteins harden when collagen gelatinizes at high temperatures, therefore a balance of cooking time and temperature is critical for excellent meat suppleness. Instrumental methods for evaluating chicken tenderness data include the Warner-Brasler shear force method, Allo-Kramer shear method, Meullenet-Owens Razor Shear (MORS) test, and instrumental Texture Profile Analysis (TPA) (Lyon *et al.*, 2010). According to Honikel (2004), elevated levels of citric acid lowered the pH of meat, resulting in less red and more yellow color, with less shear strength or tenderness.

2.7.3 Chicken cooking loss

Cooking losses are the overall losses caused during meat cooking, including dripping and evaporation losses (Obuz and Dikeman, 2003). The amount of moisture released while cooking is determined by the pH of the meat. Meat products are deemed dry because meat with a high pH loses less water while cooking than meat with a low pH

(Warriss, 2010). (Honikel, 2004; Lawrie & Ledward, 2006). Chicken muscles that have been subjected to significant degrees of stress before sacrifice have a lower pH and more meat loss after cooking (Castellini *et al.*, 2002a; Debut *et al.*, 2005; Lawrie & Ledward, 2006). Meat moisture content drops as cooking losses increase, although this is undesirable for buyers (Abu *et al.*, 2015).

According to Chartrin *et al.* (2006), fattier breast muscles had larger cooking losses. Castellini *et al.* (2002a,b) discovered that scavenging birds had higher cooking losses than intensively bred birds due to low muscle pH. Husak *et al.* (2008) discovered that washing chicken breasts from intensively farmed chicken breasts resulted in greater water retention and less cooking loss. Domestic chicken had higher cooking losses than upgraded species (Fanatico *et al.*, 2005a; Lonergan *et al.*, 2003). This has been related to the increased muscle fat composition of slow-growing birds. Cooking losses through water, water, and oil vary depending on the cooking procedure (Kumar and Albersberg, 2006). It is impossible to determine the cooking loss at the time of purchase. Acute stress causes more meat to be lost during the slaughter process (Berri *et al.*, 2005).

2.8 Limitations of using Citric Acid as growth promoter

Citric acid may reduce feed palatability, resulting in reduced feed intake and hence poorer development rates in animals (Salgado-Tránsito *et al.*, 2012). Organic acids damage metals poultry equipment, necessitating constant replacement. Bacteria are known to develop acid resistance when exposed to acidic environments over a lengthy period of time (Sorvari *et al.*, 2010). The presence of other antibacterial agents can reduce its efficacy. Organic acids can, to some extent, boost the buffering capacity of dietary components.

2.9 Conclusion

Indigenous chicken production is one of the most important enterprises in low-income areas. Chickens produce protein, generate revenue, and have social and cultural functions. Despite their significance, they are produced at a slow pace in terms of quality and quantity, which is hampered by inadequate feeding standards. Improving nutrition management is the only approach to achieve peak productivity. It is essential to do research and development on antibiotic alternatives as feed additives. Citric acid

is a potential alternative growth promoter that can be used as a feed additive in place of antibiotics when antibiotics are not authorized in poultry diets. Citric acid is a weak organic acid that lowers the pH of the gastrointestinal system, resulting in increased nutrient absorption and higher growth rates and carcass quality in chickens. The effects of CA supplementation in indigenous chickens are unknown. As a result, determining optimal supplementation levels and evaluating the effects of CA supplementation levels as a potential growth enhancer in indigenous chickens is crucial.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study site

The study was conducted at the University of Limpopo, Aquaculture (1312m altitude, 23°53'33.06"S latitude and 29°45'46.29"E longitude), Limpopo Province, South Africa. Summer minimum temperatures are relatively high, exceeding 13 °C. Winter minimum temperatures can be cold (0.6 °C) with a mean summer temperature of 27 °C and a mean winter temperature of 18 °C. The mean annual rainfall of the reserve varies between 400 mm and 600 mm (with a mean of 500 mm). Mean annual potential evaporation is between 2092 and 2122 mm (SA Weather Service, 2015).

3.2 Preparation of the house

The experimental house was thoroughly cleaned using water and a disinfectant (Virokill, Angel feed, Polokwane). Post cleaning, the house was left empty for seven days to break the life cycle of any disease-causing organisms not eliminated. The house was separated into 20 floor pens after adequate drying. The floor was covered with 7cm of fresh saw dust. The residence was heated using 250-watt infrared lights.

3.3 Acquisition of materials and management of chickens.

A total of 200 male day-old Venda chicks were purchased from the Agricultural Research Council (ARC) in Pretoria, South Africa, and delivered to the University of Limpopo, Aquaculture, in the morning utilizing a well-ventilated van. Before the experiment began, Angel Feed in Polokwane, South Africa, provided household disinfectants, 250-watt infrared lights, feeds, and drinkers. Prestige Laboratory Supplies supplied the citric acid utilized for feed augmentation. The day-old chicks were vaccinated against diseases at hatchery. Sick chickens were isolated and treated accordingly by the veterinarian. Dead chickens were taken immediately from the experimental house to the laboratory for post-mortem by Veterinarian.

3.4 Experimental diets, designs, and procedures

Experiment 1 determined the effect of citric acid supplementation on male Venda live weight, carcass weight, and dressing pieces. Males were used in the study because they grow quicker than females. Venda chicks were sexed at hatching (Kaminski and Wong, 2017). Four dietary treatments with various citric acid levels were randomly assigned to 200 male day-old Venda chicks: CA1 (0g/kg DM of feed), CA2 (12.5g/kg DM of feed), CA3 (25g/kg DM of feed), and CA4 (50g/kg DM of feed) (Table 3.01). In a completely randomised design, each treatment was replicated 5 times with 10 chicks

each replicate, making a total of 50. The NRC-recommended iso-energetic and isonitrogenous diet consisted of maize-soybean meal (1994). The experimental diet (Table 3.02) was formulated to meet nutritional requirements of Venda chickens, and isoenergetic and iso-nitrogenous (12.14 MJ ME/kg DM diet and 180g CP/kg DM diet, respectively). Partial analysis of variance (ANOVA) for the experiment is represented in Table 3.03.

Experiment 2 determined the effect of citric acid supplementation on meat pH, shear force and cooking loss of male Venda chickens. The treatment, design, diet and experimental layout were the same as to those experiment 1 described in section 3.4.

Table 3.01. Dietary treatments for the experiment (1-90 days old chickens)

Diet code	Diet description
MCA ₀	Male Venda chickens fed a maize-soybean meal-based diet without citric acid supplementation
MCA _{12.5}	Male Venda chickens fed a maize-soybean meal-based diet supplemented with 12.5g of citric acid/kg DM of feed
MCA ₂₅	Male Venda chickens fed a maize-soybean meal-based diet supplemented with 25g of citric acid /kg DM of feed.
MCA ₅₀	Male Venda chickens fed a maize-soybean meal-based diet supplemented with 50g of citric acid/kg DM of feed.

	Starter				Grower				Finisher			
	Control	12.5	25	50	Control	12.5	25	50	Control	12.5	25	50
Soya oil cake 47%	37.20	37.20	38.00	38.65	35.00	35.00	35.00	34.00	31.00	31.00	32.00	33.00
Sunflower 38%	3.00	3.00	3.00	1.00	2.00	2.00	2.00	1.50	1.50	1.50	1.50	1.50
Yellow maize	50.23	48.48	46.43	45.00	53.00	51.23	50.00	49.03	57.21	55.43	53.18	49.16
Soya oil	5.50	6.00	6.00	7.00	6.50	7.00	7.00	7.00	7.00	7.50	7.50	8.00
Salt	0.50	0.50	0.50	0.35	0.40	0.40	0.40	0.40	0.35	0.35	0.35	0.35
MCP	0.90	0.90	0.90	0.90	0.70	0.72	0.75	0.82	0.79	0.82	0.82	0.84
Limestone	1.70	1.70	1.70	0.95	1.30	1.30	1.25	1.10	1.10	1.10	1.10	1.10
Valine	0.10	0.10	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine HCL	0.25	0.25	0.25	0.30	0.25	0.25	0.25	0.30	0.25	0.25	0.25	0.25
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.25	0.25	0.25	0.25
Threonine	0.02	0.02	0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Citric acid	0.00	1.25	2.50	5.00	0.00	1.25	2.50	5.00	0.00	1.25	2.50	5.00
Analysis												
Moisture (%)	9.97	9.77	9.50	9.34	9.99	9.78	9.65	9.26	10.04	9.83	9.35	9.30
Protein (%)	23.03	23.00	23.00	23.00	21.95	21.75	21.75	21.00	20.21	20.07	20.00	20.49
Fat (%)	7.22	7.67	7.90	8.55	8.25	8.93	8.93	9.56	8.80	9.24	9.00	9.58
Fibre (%)	3.15	3.12	3.00	2.66	2.86	2.75	2.75	2.62	2.62	2.60	2.60	2.89
Ash (%)	1.68	1.68	1.53	1.30	1.29	1.30	1.30	1.31	1.28	1.30	1.31	1.32
AMEN (kcal/kg)	3017.45	3009.76	3008.50	3010.87	3137.79	3125.00	3125.00	3100.10	3219.39	3210.87	3210.00	3110.00
Lysine (%)	1.40	1.40	1.43	1.44	1.33	1.33	1.33	1.32	1.22	1.22	1.24	1.26
Methionine (%)	0.65	0.65	0.64	0.63	0.63	0.62	0.62	0.61	0.56	0.56	0.56	0.56
CA	0.81	0.90	0.79	0.63	0.71	0.71	0.71	0.66	0.64	0.65	0.65	0.66
P	0.67	0.66	0.65	0.64	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
NA	0.19	0.19	0.17	0.13	0.15	0.15	0.15	0.15	0.13	0.13	0.13	0.13
CL	0.28	0.28	0.25	0.19	0.22	0.22	0.22	0.22	0.19	0.19	0.19	0.19

Table 3.02 Ingredients and nutrient composition of the diet for the experiment

The active ingredients contained in the vitamin premix were as follows (per kg of diet): vitamin A 12000IU, vitamin D3 3500 IU, vitamin K3 2.0 mg, vitamin B12 0.02 mg

Table 3.03 Partial analysis of variance (ANOVA) for the experiment

Source of variation	Sum of Squares (SS)	Degrees of Freedom (d.f)	Mean of Squares (MS)	Variation Ratio (F)
Citric acid		(t-1)= (4-1)=3		
Error		(n-t)=(200-4)=196		

3.5 Growth performance

Mean live weights were calculated from the weekly measurements by dividing the total weight with the number of chickens in that pen. Average daily gains were calculated by subtracting the initial weight of the chicken from the final weight and the answer was divided by the number of days. The voluntary feed intake was measured by subtracting the difference in weight of leftovers from that offered per day and the total was divided by the total number of chickens per pen. The feed offered per day and leftovers were measured using the electronic weighing balance used. Daily average feed intake and weight gain were used to calculate feed conversion ratio. Average feed intake was divided by average weight gain to find the FCR value (McDonald *et al.*, 2010). Feed conversion ratio (g DM feed/g live weight gain) = Average feed intake/average weight gain.

3.6 Slaughter and study design

The initial live weights of the chicks were measured at the beginning of the experiment; thereafter weekly live weights were taken until day 90. A total of 140 chickens from a population size of 200 were slaughtered at animal unit laboratory following sampling equation described by Yamane (1967). The statistical model used for sample size was as follows:

$$n = 1 \frac{N}{e^2} + N(e)^2$$

Where n is the sample size, N is the population size, and e is the level of precision.

Cervical dislocation method was used to slaughter the chickens as it one of the most prevalent methods for slaughtering individual birds and it is perceived to be humane by users, easy to learn and perform, and does not require equipment (Mason *et al.*, 2009; Martin, 2015; Martin *et al.*, 2016) the help of Veterinarian.

3.7 Measurement of carcass characteristics

The weights of the carcass, breast meat, drumstick, thigh, and wings were measured. Each chicken was weighed using an electronic weighing balance (AE ADAM) to

measure live weights prior slaughter. Slaughtered chickens were then placed in a bucket with hot water at 60-66°C for 45-90 seconds (Shung *et al.*, 2022) before being removed for defeathering by hand. The carcasses were sliced open at the abdominal region, and the digestive tracts of the birds were removed from their abdominal cavities. The carcasses were sliced on the joints into drumsticks, wings, and thighs, as well as across the shoulder area to remove the backbone from the breast; the cuts were then weighed using an automated weighing balance. Carcass weight was recorded, and the live weight were taken, and dressing percentage was obtained by dividing the carcass weight by live weight of the chicken and expressing the result as a percentage.

3.8. Physico-chemical attributes measurements

Immediately day after slaughter, the breasts were cut-off to measure physico-chemical attributes (meat pH, shear force and cooking loss). Breast meat was put in trays as per dietary treatment and stored in a refrigerator at 4°C. Two trays from each treatment were removed from the refrigerator after every 24 hours of storage to evaluate changes in breast meat pH, meat tenderness and cooking loss. The pH was determined using the digital pH meter (Crison, Basic 20 pH Meter). The shear force was measured using Warner-Bratzler Shear Force (Novaković and Tomašević, 2017). Chicken breast samples were cooked on an electronic stove at 80°C for 30 minutes to calculate the cooking loss. Cooking losses was measured right after cooking by recording weight before cooking (WBC) and weight after cooking (WAC). Cooking loss % was calculated as: $((WBC-WAC)/WBC) \times 100$ (Ngambu *et al.*, 2013).

3.9 Data analyses

Effects of citric acid supplementation on feed intake, growth rate, feed conversion ratio, live weight, carcass characteristics, meat pH and shear force of Venda chickens were computed using analysis of variance (ANOVA) of the Statistical Package for the Social Sciences version 26 (SPSS, 2019). Means were considered different when ($P < 0.05$), the treatment means was separated using Tukey's (HSD) test at $P < 0.05$. The fit was performed by using nonlinear regression by means of NLIN of Statistical Analysis Software version 9.2 (SAS, 2008) The model $Y_{ij} = \mu + T_i + e_{ij}$ will be applied where Y_{ij} = response variables live weight, growth rate, FCR, carcass yield, meat pH

and shear force; μ = overall mean; T_i = fixed effect of citric acid inclusion level; e_{ij} = the residual effect (error).

The optimal responses in Venda chicken body weight, growth rate, FCR, carcass weight, and meat pH, shear force and cooking loss to the level of citric acid supplementation was modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2 + e$$

Where Y = response variable (carcass characteristics, meat characteristics); a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x = level of citric acid supplementation; e = random error and $-b_1/2b_2 = x$ value for optimal response. The linear quadratic model will be used because it is the most commonly used tool for quantitative predictions of dose dependencies (McMahon, 2018).

CHAPTER 4
RESULTS

4.1 Nutrient composition of the diets

Results of the nutrient composition of the experimental starter diets are shown in Table 4.01. The protein content of the diet for treatment 1 was 23.05 and for treatment 2,3 and it was 23.00%. Citric acid supplementation levels were 0, 12.5, 25 and 50g per kg DM.

Results of the nutrient composition of the experimental grower diets are presented in Table 4.02. The protein content of the diet was 21.95% for treatment 1, 21.81% for treatment 2, 21.71% for treatment 3 and 21.00% for treatment 4. Citric acid supplementation levels were 0, 12.5, 25 and 50g per kg DM.

Results of the nutrient composition of the experimental finisher diets are presented in Table 4.03. The protein content of the diet was 20.21% for treatment 1, 20.07% for treatment 2, 20.00% for treatment 3 and 20.49% for treatment 4. Citric acid supplementation levels were 0, 12.5, 25 and 50g per kg DM.

Table 4.01 Nutrient composition of the starter diet

Nutrient	Citric acid supplementation level (g/kg DM of feed)			
	0	12.5	25	50
Moisture (%)	9.97	9.77	9.50	9.35
Protein (%)	23.03	23.00	23.00	23.00
Fat (%)	7.22	7.67	7.90	8.55
Fibre (%)	3.15	3.12	3.00	2.66
Ash (%)	1.68	1.68	1.53	1.30
Amen (kcal/kg)	3017.45	3009.76	3008.50	3010.87
Lysine (%)	1.40	1.40	1.43	1.44
Methionine (%)	0.65	0.65	0.64	0.63
Calcium (%)	0.81	0.90	0.79	0.63
Phosphorus (%)	0.67	0.66	0.65	0.64
NA (%)	0.19	0.19	0.17	0.13
CL (%)	0.28	0.28	0.25	0.19

Table 4.02 Nutrient composition of the grower diet

Nutrient	Citric acid supplementation level (g/kg DM of feed)			
	0	12.5	25	50
Moisture (%)	9.99	9.78	9.65	9.26
Protein (%)	21.95	21.81	21.75	21.00
Fat (%)	8.25	8.69	8.93	9.56
Fibre (%)	2.86	2.83	2.75	2.62
Ash (%)	1.29	1.30	1.30	1.31
Amen (kcal/kg)	3137.79	3129.59	3125.00	3100.10
Lysine (%)	1.33	1.33	1.33	1.32
Methionine (%)	0.63	0.63	0.62	0.61
Calcium (%)	0.71	0.71	0.71	0.66
Phosphorus (%)	0.60	0.60	0.60	0.60
NA (%)	0.15	0.15	0.15	0.15
CL (%)	0.22	0.22	0.22	0.22

Table 4.03 Nutrient composition of the finisher diet

Nutrient	Citric acid supplementation level (g/kg DM of feed)			
	0	12.5	25	50
Moisture (%)	10.04	9.83	9.35	9.30
Protein (%)	20.21	20.07	20.00	20.49
Fat (%)	8.80	9.24	9.00	9.58
Fibre (%)	2.62	2.60	2.60	2.59
Ash (%)	1.28	1.30	1.31	1.32
Amen (kcal/kg)	3219.39	3210.87	3210.00	3110.00
Lysine (%)	1.22	1.22	1.24	1.26
Methionine (%)	0.56	0.56	0.56	0.56
Calcium (%)	0.64	0.65	0.65	0.66

Phosphorus (%)	0.60	0.60	0.60	0.60
NA (%)	0.13	0.13	0.13	0.13
CL (%)	0.19	0.19	0.19	0.19

4.2 Effect of citric acid supplementation on production performance of male Venda chickens aged one to 30 days

The results of citric acid supplementation level on feed intake, growth rate, feed conversion ratio (FCR), and live weight of male Venda chickens aged one to 30 days are presented in Table 4.04. Chickens fed incremental inclusion levels of citric acid in diets had the same DM feed intake and feed conversion ration ($P < 0.05$). Citric acid supplementation did not affect ($P > 0.05$) DM feed intake and feed conversion ratio of male Venda chickens aged one to 30 days. However, citric acid supplementation affected ($P < 0.05$) growth rate and live weight of male Venda chickens aged one to 30 days. Chickens supplemented with 25g citric acid per kg DM had a higher ($P < 0.05$) growth rate than those supplemented with 0, 12 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had a higher ($P < 0.05$) growth rate than those supplemented with 50g of citric acid per kg DM. However, male Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had the same ($P > 0.05$) growth rate. Similarly, Venda chickens supplemented with 0, 12.5 or 50g of citric acid per kg DM had the same ($P > 0.05$) growth rate. A 2.393g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal growth rate of Venda aged one to 30 days (Figure 4.01 and Table 4.05).

Venda chickens supplemented with 25g citric acid per kg DM had heavier ($P < 0.05$) live weight than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had a heavier ($P < 0.05$) live than those supplemented with 50g of citric acid per kg DM. However, Venda chickens fed diets supplemented with 0, 12.5 or 25g of citric acid per kg DM had similar ($P > 0.05$) live weight. Similarly, Venda chickens supplemented with 0, 12.5 or 50g of citric acid per kg DM had the same ($P > 0.05$) live weight. A 2.536 of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal live weight of Venda aged one to 30 days (Figure 4.02 and Table 4.05).

Table 4.04 Effect of citric acid supplementation on DM feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens aged one to 30 days

Variable*	Diet#			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
Feed intake (kg/bird/day)	0.543±0.0440	0.498±0.0576	0.512±0.0201	0.466±0.0296
Growth rate (kg/bird/day)	0.027 ^{ab} ±0.0007	0.026 ^{ab} ±0.0043	0.031 ^a ±0.0014	0.025 ^b ±0.0008
FCR (kg DM feed/kg live weight gain)	1.397±0.1067	1.446±0.5972	1.056±0.1111	1.381±0.3002
Live weight (kg/bird aged 1 to 30 days)	0.563 ^{ab} ±0.0150	0.555 ^{ab} ±0.0911	0.648 ^a ±0.0299	0.525 ^b ±0.0173

* : Values presented as mean ± standard deviation a, b, c, :

Means in the same row not sharing a same superscript are significantly different (p > 0.05)

: Diet codes are described in Chapter 3, Table 3.01

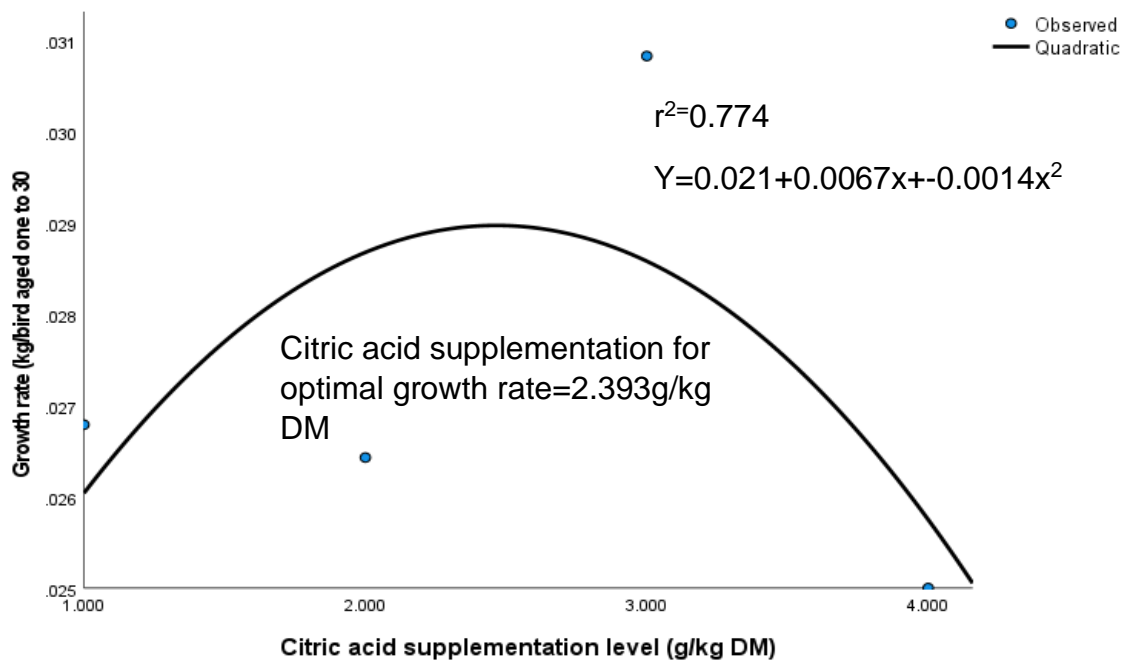


Figure 4.01 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged one to 30 days

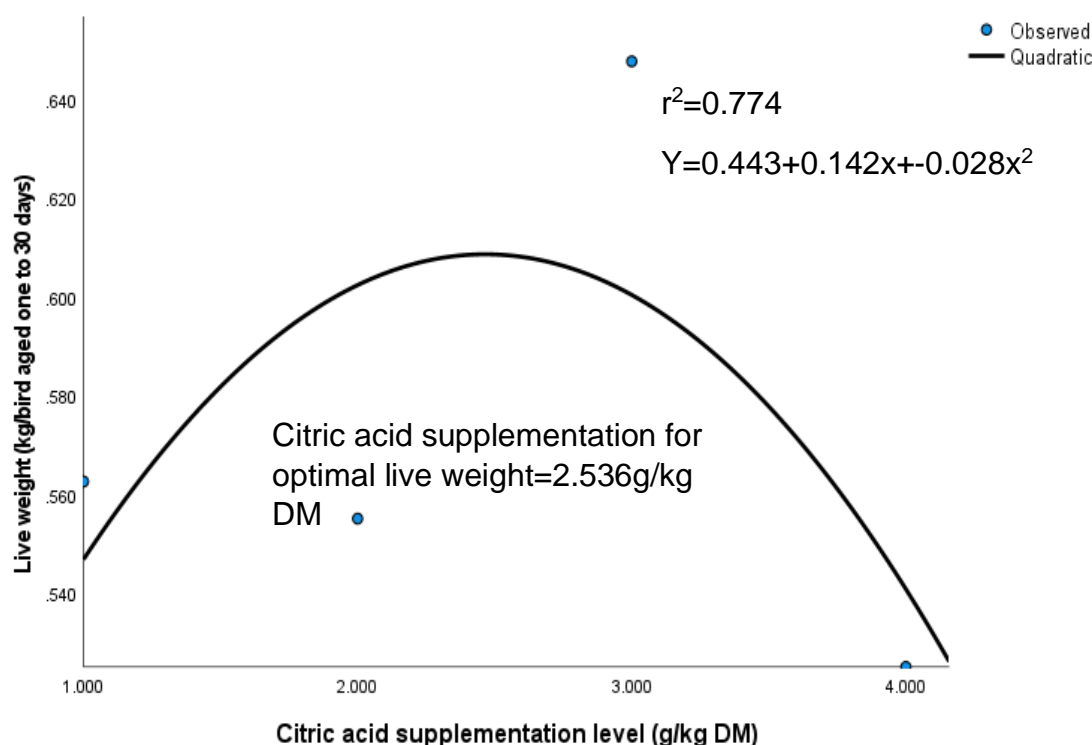


Figure 4.02 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged one to 30 days

Table 4.05 Citric acid supplementation level for optimal growth rate, and live weight of male Venda chickens aged one to 30 days

Variable	Formula	X	Y	r ₂	Probability
Growth rate (kg/bird/day)	$Y=0.021+0.0067x+-0.0014$	2.393	0.0290	0.401	0.774
Live weight (kg/ bird aged 30 days)	$Y=0.433+0.142x+-0.028$	2.536	0.653	0.401	0.774

r² : Coefficient of determination

4.3 Effect of citric acid supplementation on production performance of male Venda chickens aged 31 to 60 days

Results of the effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio and live weight at 60 days of Venda chickens are presented in Table 4.06. Citric acid supplementation affected (P<0.05) DM feed intake, growth rate, feed conversion ratio and live weight of Venda chickens aged 31 to 60 days. Venda

chickens supplemented with 25g of citric acid per DM had higher ($P<0.05$) DM feed intake than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 50 g of citric acid per DM had higher ($P<0.05$) DM feed intakes than those supplemented with 12.5g of citric acid per kg DM. However, Venda chickens supplemented with 0, 25 or 50g of citric acid per kg DM had similar ($P>0.05$) DM feed intake. Similarly, Venda chickens supplemented with 0, 12.5 or 50g of citric acid per kg DM had similar ($P>0.05$) DM feed intake.

Results of the present study indicate that Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had higher ($P<0.05$) growth rate than those supplemented with 0 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) growth rate than those supplemented with 50g of citric acid per kg DM. A 2.250g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal growth rate of Venda aged 31 to 60 days (Figure 4.04 and Table 4.07).

Venda chickens supplemented with 50g of citric acid per DM had higher ($P<0.05$) feed conversion ratio value than those supplemented with 0, 12.5 or 25g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 25g of citric acid per DM had higher ($P<0.05$) feed conversion ratio value than those supplemented with 12.5g of citric acid per kg DM. However, Venda chickens supplemented with 0, 25 or 50g of citric acid per kg DM had similar ($P>0.05$) feed conversion ratio value. Similarly, Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had similar ($P>0.05$) feed conversion ratio value. A 2.373g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal feed conversion ratio value of Venda aged 31 to 60 days (Figure 4.09 and Table 4.07).

Results of the present study indicate that Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had heavier ($P<0.05$) live weight than those supplemented with 0 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had heavier ($P<0.05$) live weight than those supplemented with 50g of citric acid per kg DM. A 2.308g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal growth rate of Venda aged 31 to 60 days (Figure 4.10 and Table 4.07).

Table 4.06 Effect of citric acid supplementation on DM feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens aged 31 to 60 days

Variable*	Diet#			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
Feed intake (kg/bird/day)	1.092 ^{ab} ±0.0283	1.033 ^b ±0.0301	1.120 ^a ±0.0280	1.047 ^{ab} ±0.0575
Growth rate (kg/bird/day)	0.025 ^b ±0.0030	0.031 ^a ±0.0010	0.030 ^a ±0.0015	0.020 ^c ±0.0017
FCR (kg DM feed/kg live weight gain)	2.485 ^{ab} ±1.0244	1.322 ^b ±0.1695	1.549 ^{ab} ±0.1864	2.949 ^a ±0.9248
Live weight (kg/bird aged 31 to 60 day)	1.033 ^b ±0.1255	1.288 ^a ±0.0403	1.243 ^a ±0.0634	0.843 ^c ±0.0699

* : Values presented as a mean ± standard deviation (SD) a, b,

c, : Means in the same row sharing a common superscript are significantly similar ($p > 0.05$)

:Diet codes are described in Chapter 3, Table 3.01

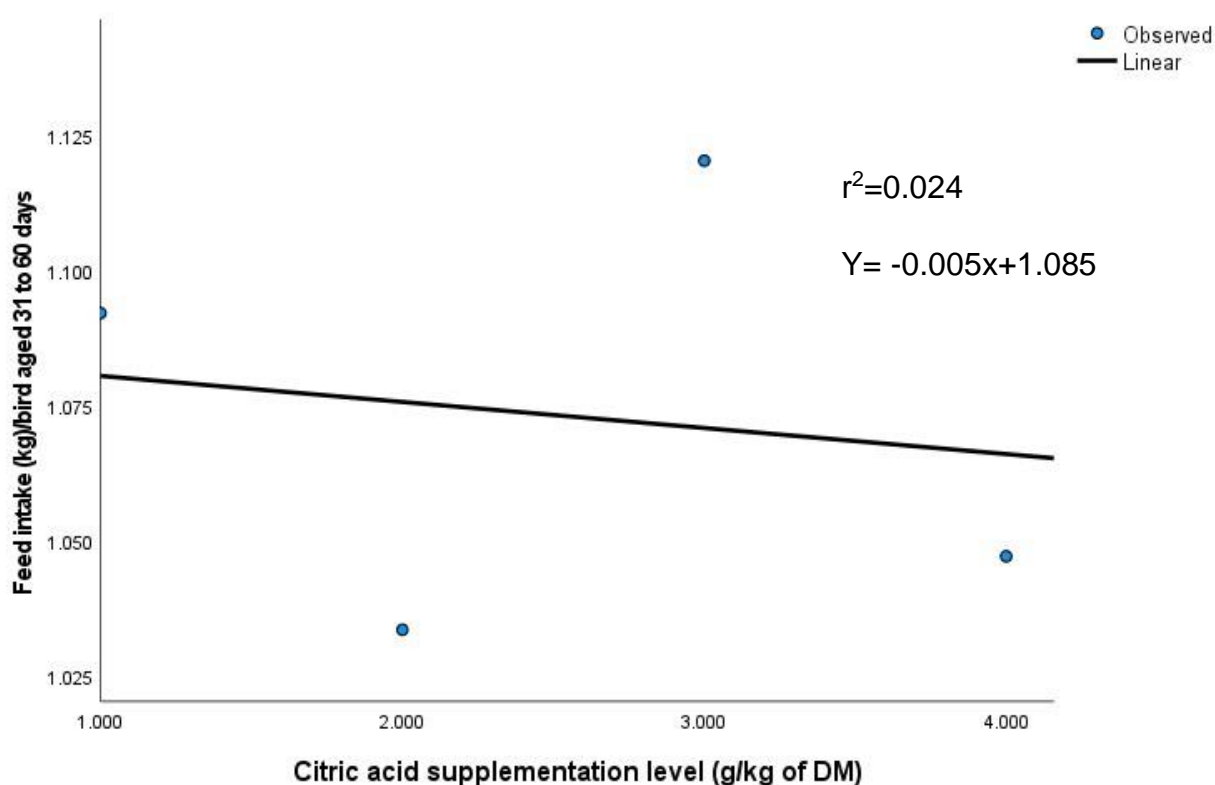


Figure 4.03 Relationship between citric acid supplementation in a diet and feed intake of male Venda chickens aged 31 to 60 days

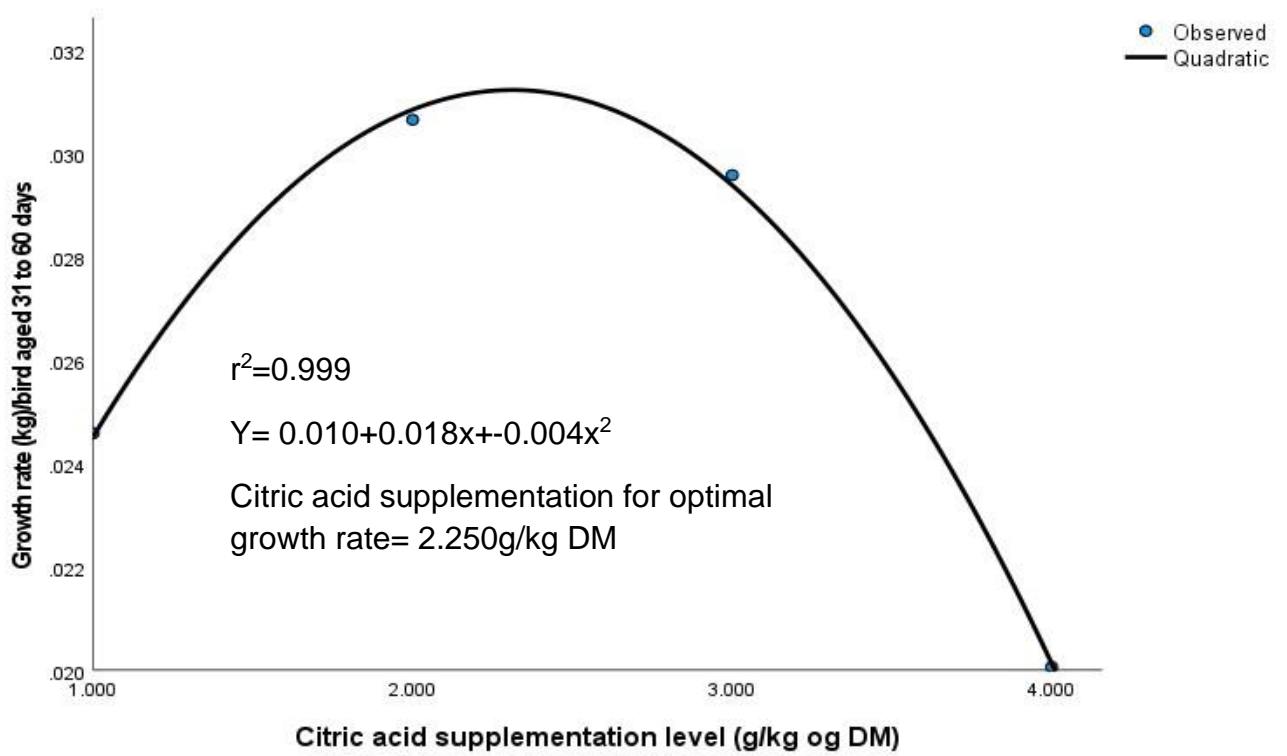


Figure 4.04 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged 31 to 60 days

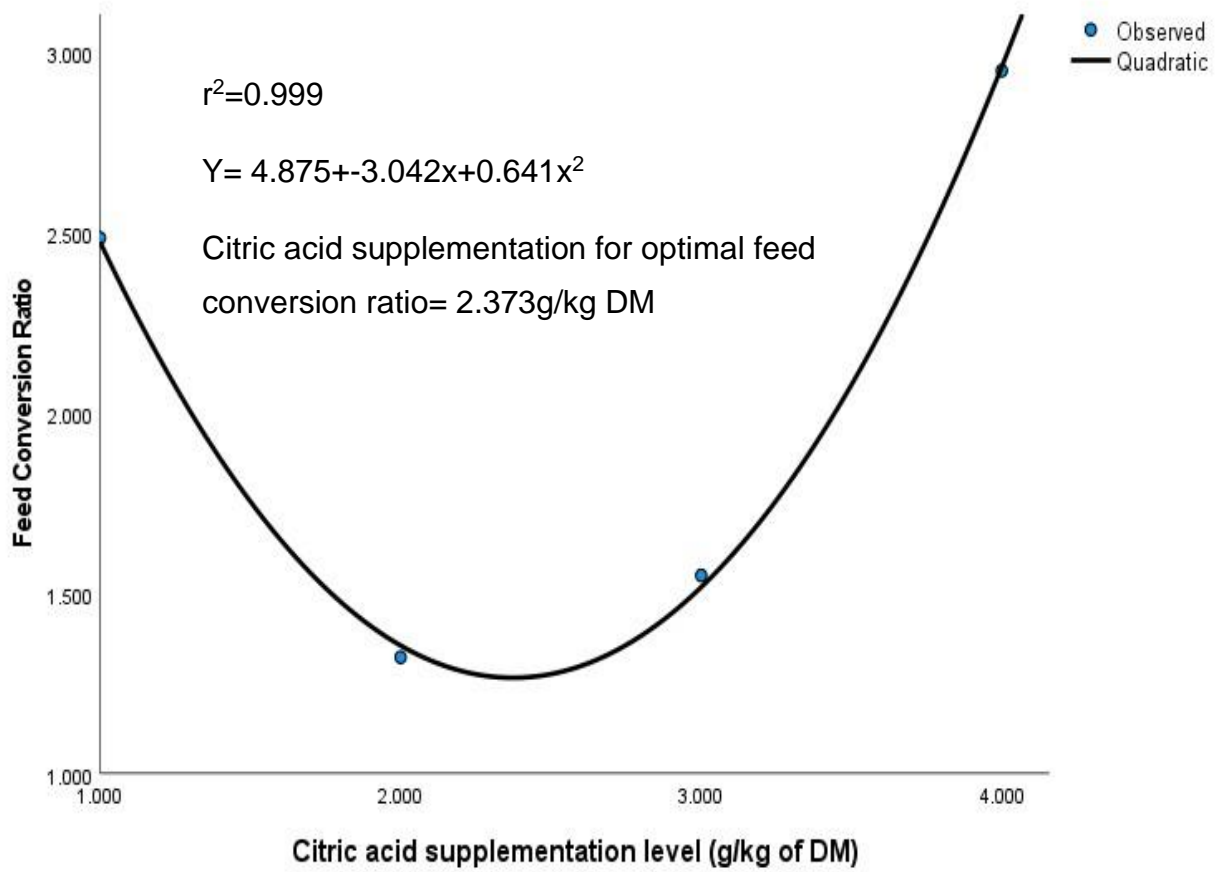


Figure 4.05 Effect of citric acid supplementation in a diet on feed conversion ratio of male Venda chickens aged 31-60 days

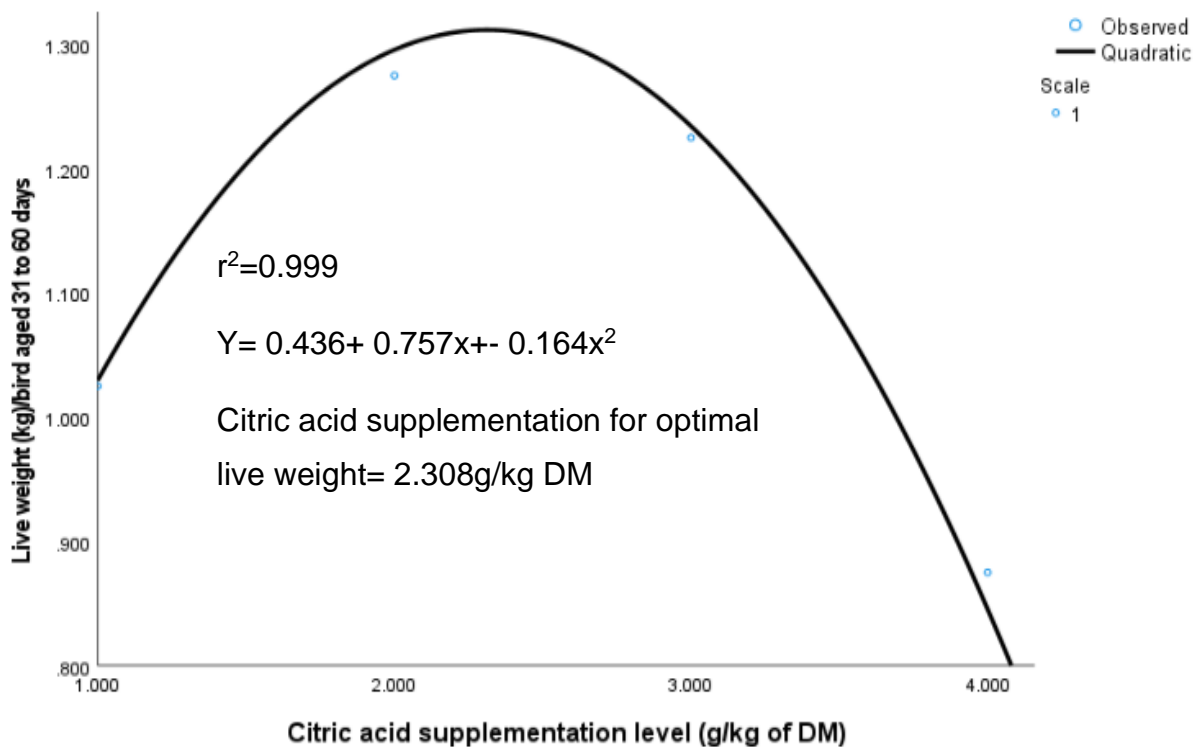


Figure 4.06 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged 31 to 60 days

Table 4.07 Citric acid supplementation level for optimal feed intake, growth rate, feed conversion ratio, and live weight of male Venda chickens aged 31 to 60 days

Variable	Formula	X	Y	r ²	Probability
Growth rate (kg/bird/day)	$Y=0.010+0.018x-0.004x^2$	2.250	0.031	0.999	0.350
FCR (kg DM feed/kg live weight gain)	$Y=4.875+-3.042x+0.641x^2$	2.373	1.266	0.999	0.036
Live weight (kg/bird aged 31 to 60 days)	$Y=0.436+0.757x-0.164x^2$	2.308	1.310	0.999	0.035

r² : Coefficient of determination

4.4 Effect of citric acid supplementation on production performance of male Venda chickens aged 61 to 90 days

The results of citric acid supplementation level on DM feed intake, growth rate, feed conversion ratio, and live weight of Venda chickens aged 61 to 90 days are presented in Table 4.08. Citric acid supplementation affected ($P < 0.05$) DM feed intake, growth rate, feed conversion ratio and live weight of Venda chickens aged 61 to 90 days.

Venda chickens supplemented with 12.5g of citric acid per kg DM had higher ($P<0.05$) DM feed intake than those supplemented with 0, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) DM feed intake than those supplemented with 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 25g of citric acid per kg DM had higher ($P<0.05$) DM feed intake than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had the same ($P>0.05$) DM feed intake. Similarly, Venda chickens supplemented with 0 or 25g of citric acid per kg DM had the same ($P>0.05$) DM feed intake. A 1.566g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal feed intake of Venda aged 31 to 60 days (Figure 4.07 and Table 4.09).

Results of the current study indicate that Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had higher ($P<0.05$) growth rate than those supplemented with 0 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) growth rate than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$) growth rate. A 2.167g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal growth rate of Venda aged 61 to 90 days (Figure 4.08 and Table 4.09).

Venda chickens supplemented with 50g of citric acid per kg DM had higher ($P<0.05$) feed conversion ratio value than those supplemented with 0, 12.5 or 25g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) feed conversion ratio value than those supplemented with 12.5 or 25g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 50g of citric acid per kg DM had the same ($P>0.05$) feed conversion ratio value. Similarly, Venda chickens supplemented with 0, 12 or 25g of citric acid per kg DM had the same ($P>0.05$) feed conversion ratio value. A 2.332g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal feed conversion ratio of Venda aged 61 to 90 days (Figure 4.09 and Table 4.09).

Results of the current study indicate that Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had heavier ($P < 0.05$) live weights than those supplemented with 0 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had heavier ($P < 0.05$) live weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had the same ($P > 0.05$) live weight. A 2.272g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal live weight of Venda aged 61 to 90 days (Figure 4.10 and Table 4.09).

Table 4.08 Effect of citric acid supplementation on DM feed intake, growth rate, feed conversion ratio, and live weight of male Venda chickens aged 61 to 90 days

Variable	Diet ^{*#}			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
Feed intake (kg/bird/day)	1.488 ^{ab} ±0.0658	1.524 ^a ±0.0271	1.418 ^b ±0.0181	1.307 ^c ±0.0445
Growth rate (kg/bird/day)	0.026 ^b ±0.0016	0.031 ^a ±0.0006	0.029 ^a ±0.0009	0.022 ^c ±0.0014
FCR (kg DM feed/kg live weight gain)	1.476 ^{ab} ±0.1428	1.192 ^b ±0.0859	1.275 ^b ±0.0688	1.622 ^a ±0.2249
Live weight (kg/bird aged 31 to 60 day)	1.578 ^b ±0.0946	1.840 ^a ±0.0356	1.763 ^a ±0.0532	1.340 ^c ±0.0841

* : Values presented as a mean ± standard deviation (SD) a, b,

c, : Means in the same row sharing a common superscript are significantly similar ($p > 0.05$)

:Diet codes are described in Chapter 3, Table 3.01

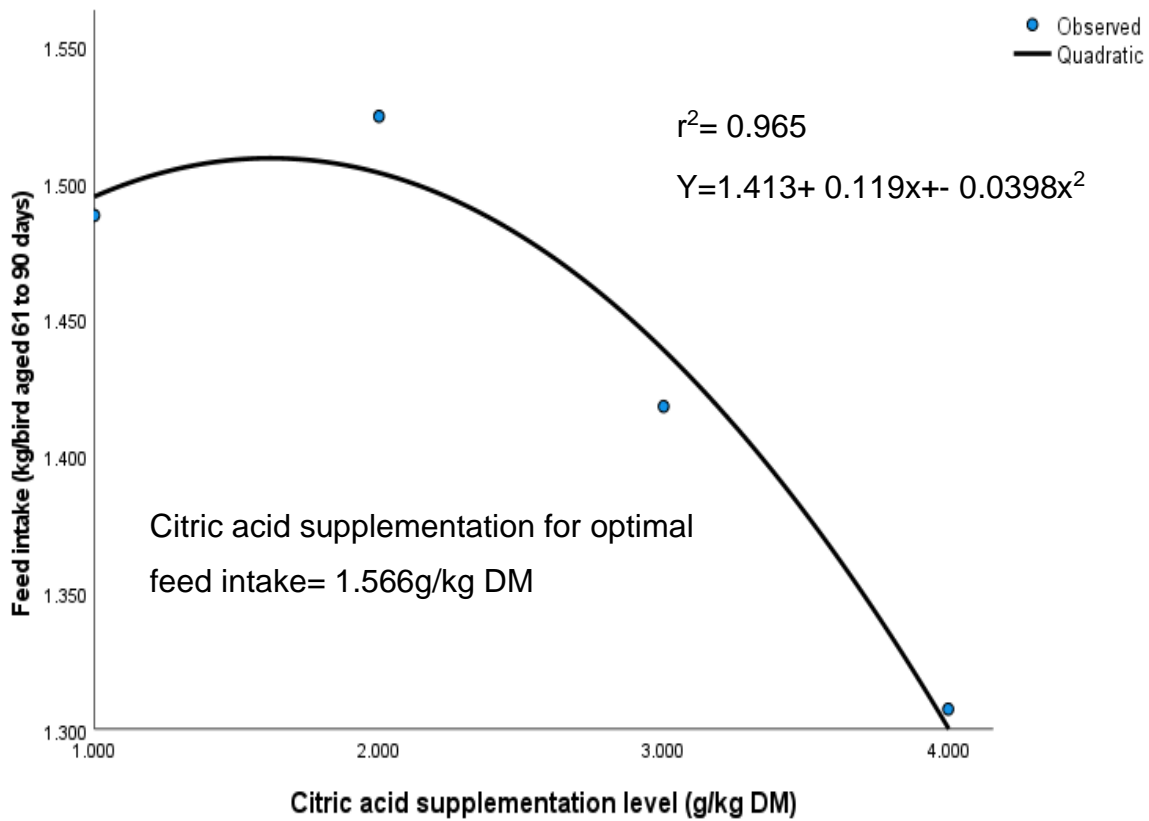


Figure 4.07 Effect of citric acid supplementation in a diet on feed intake of male Venda chickens aged 61 to 90 days

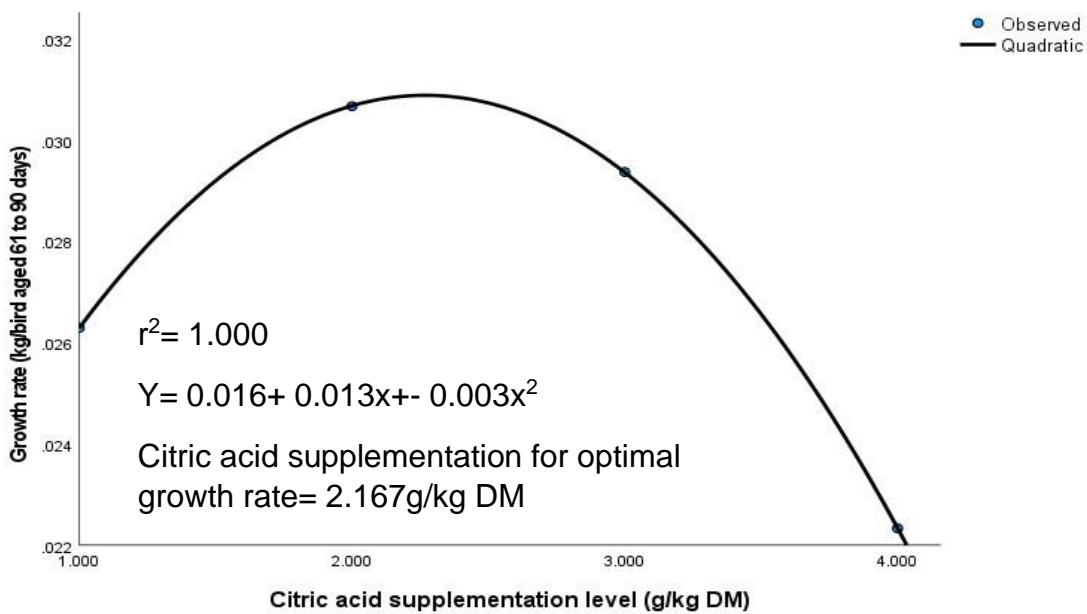


Figure 4.08 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged 61 to 90 days

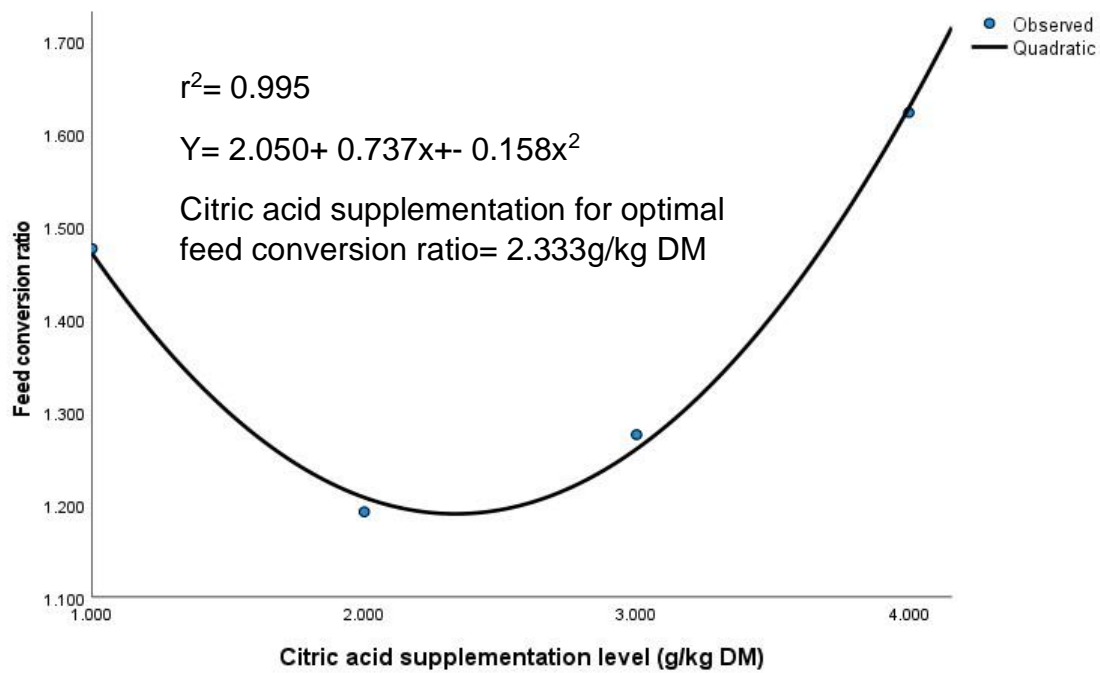


Figure 4.09 Effect of citric acid supplementation level in a diet on feed conversion ratio of male Venda chickens aged 61 to 90 days

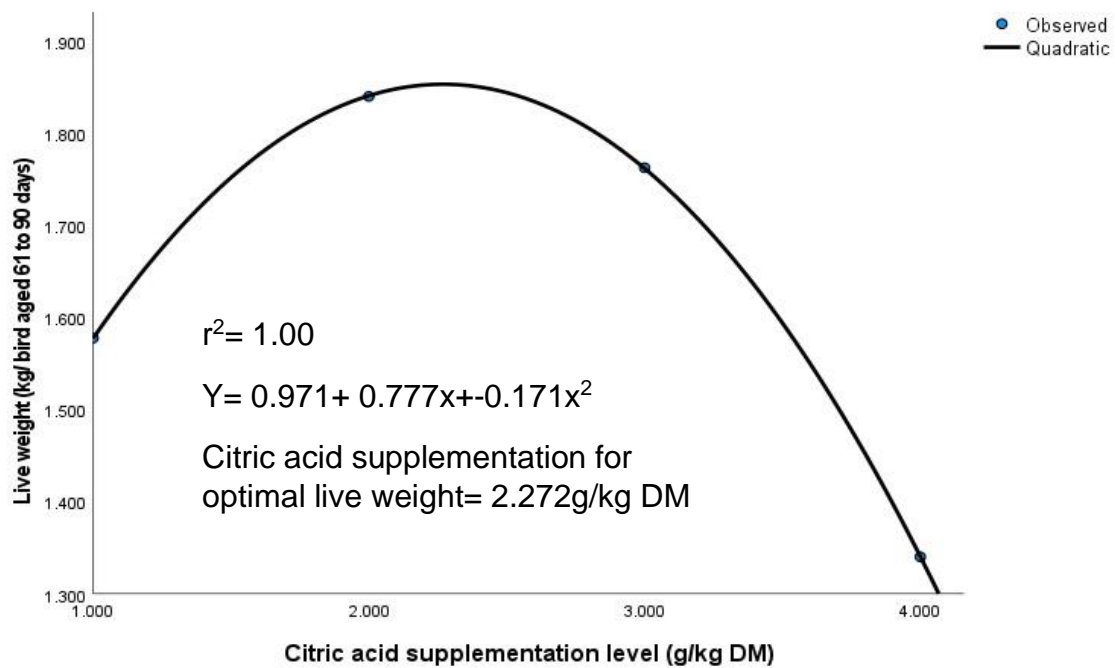


Figure 4.10 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged 61 to 90 days

Table 4.09 Citric acid supplementation level for optimal feed intake, growth rate, feed conversion ratio, and live weight of male Venda chickens aged 61 to 90 days

Variable	Formula	X	Y	r ²	Probability
Feed intake (kg/bird/day)	$Y=1.413+0.119x-0.038x^2$	1.566	1.506	0.965	0.186
Growth rate (kg/bird/day)	$Y=0.016+0.013x-0.003x^2$	2.167	0.031	1.000	0.002
FCR (kg DM feed/kg live weight gain)	$Y=2.050-0.737x+0.158x^2$	2.332	1.191	0.995	0.068
Live weight (kg/bird aged 61 to 90 days)	$Y=0.971+0.777x-0.171x^2$	2.272	1.854	1.000	0.003

r² : Coefficient of determination

4.5 Effect of citric acid supplementation on carcass characteristics of male Venda chickens aged 90 days

Results of the effect of citric acid supplementation on carcass characteristics of Venda chickens aged 90 days are presented in Table 4.10. Citric acid supplementation affected ($P<0.05$) live weight, carcass weight, dressing percentage and breast, wing, drumstick, and wing weights of Venda chickens aged 90 days. All the carcass characteristic attributes measure in the current study were significant different $P<0.05$ across the treatments. Venda chickens supplemented with 25g of citric acid per kg DM had heavier ($P<0.05$) live weight than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had heavier ($P<0.05$) live weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had the same ($P>0.05$) live weights. A 2.216g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal live weight of Venda aged 90 days (Figure 4.11 and Table 4.11).

Results of the current study indicate that Venda chickens supplemented with 25g of citric acid per kg DM had heavier ($P<0.05$) carcass weight than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had heavier ($P<0.05$) carcass weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had the same ($P>0.05$) carcass weights. A 2.199g of citric acid supplementation level per kg DM of the diet was

calculated using a quadratic equation to result in the optimal carcass weight of Venda aged 90 days (Figure 4.12 and Table 4.11).

Venda chickens supplemented with 25g of citric acid per kg DM had a higher ($P<0.05$) dressing percentage than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0, 12.5 or 50g of citric acid per kg DM had the same ($P>0.05$) dressing percentage. A 2.422g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal carcass weight of Venda aged 90 days (Figure 4.13 and Table 4.11).

The results of the current study indicate that Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had heavier ($P<0.05$) breast weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$) breast weights. A 1.925g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal breast weight of Venda aged 90 days (Figure 4.14 and Table 4.11).

Venda chickens supplemented with 12.5g of citric acid per kg DM had heavier ($P<0.05$) wing weight than those supplemented with 0, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 25g of citric acid per kg DM had heavier ($P<0.05$) wing weight than those supplemented with 0 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0g of citric acid per kg DM had heavier ($P<0.05$) wing weight than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$) wing weight. Similarly, Venda chickens supplemented with 0 or 25g of citric acid per kg DM had the same ($P>0.05$) wing weight. A 2.115g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal wing weight of Venda aged 90 days (Figure 4.15 and Table 4.11).

The results of the current study indicate that Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had heavier ($P<0.05$) drumstick weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0, 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$)

drumstick weights. A 2.129g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal drumstick weight of Venda aged 90 days (Figure 4.15 and Table 4.11).

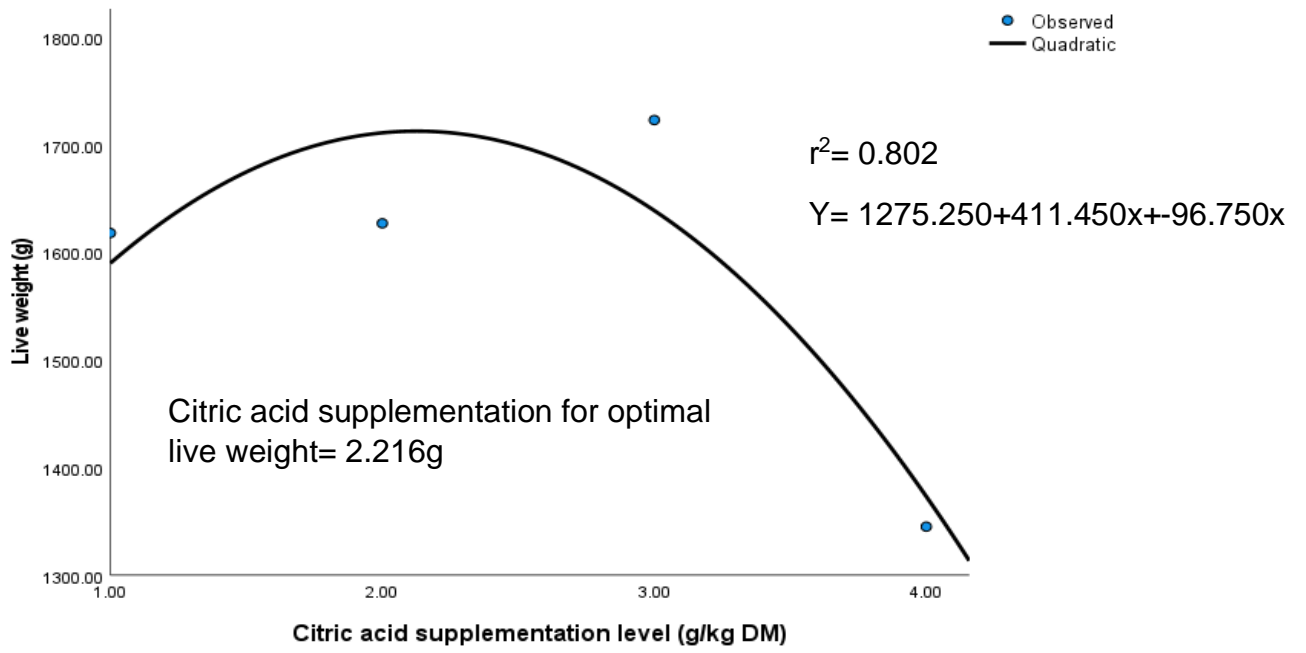
Venda chickens supplemented with 25g of citric acid per kg DM had heavier ($P < 0.05$) thigh weight than those supplemented with 0, 12.5 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 0 or 12g of citric acid per kg DM had heavier ($P < 0.05$) thigh weights than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had the same ($P > 0.05$) thigh weights. A 2.129g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal thigh weight of Venda aged 90 days (Figure 4.16 and Table 4.11).

Table 4.10 Effect of citric acid supplementation on carcass weight, live weight, meat parts weight and dressing percentage of male Venda chickens aged 90 days

Variable*	Diet#			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
Live weight (g)	1618.0 ^b ±82.97	1627.0 ^b ±81.93	1723.0 ^a ±74.84	1345.0 ^c ±59.86
Carcass weight (g)	1068.0 ^b ±61.79	1077.0 ^b ±65.50	1184.0 ^a ±66.03	871.0 ^c ±52.59
Dressing percentage (%)	66.0 ^b ±1.60	66.2 ^b ±1.08	68.7 ^a ±1.49	64.7 ^b ±2.18
Breast weight (g)	254.9 ^a ±15.24	261.7 ^a ±26.31	253.4 ^a ±20.60	201.6 ^b ±9.69
Wing weight (g)	76.6 ^b ±4.54	82.6 ^a ±6.7266	78.6 ^{ab} ±4.7947	66.0 ^c ±2.45
Drumstick weight (g)	78.9 ^a ±5.72	80.8 ^a ±8.33	83.1 ^a ±4.53	67.0 ^b ±5.99
Thigh weight (g)	89.0 ^b ±4.00	85.4 ^b ±5.92	99.4 ^a ±2.65	74.2 ^c ±3.00

* : Values presented as mean ± standard deviation a, b, c, : Means in the same row not sharing a same superscript are significantly different ($p > 0.05$)

: Diet codes are described in Chapter 3, Table 3.01



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Figure 4.11 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged 90 days

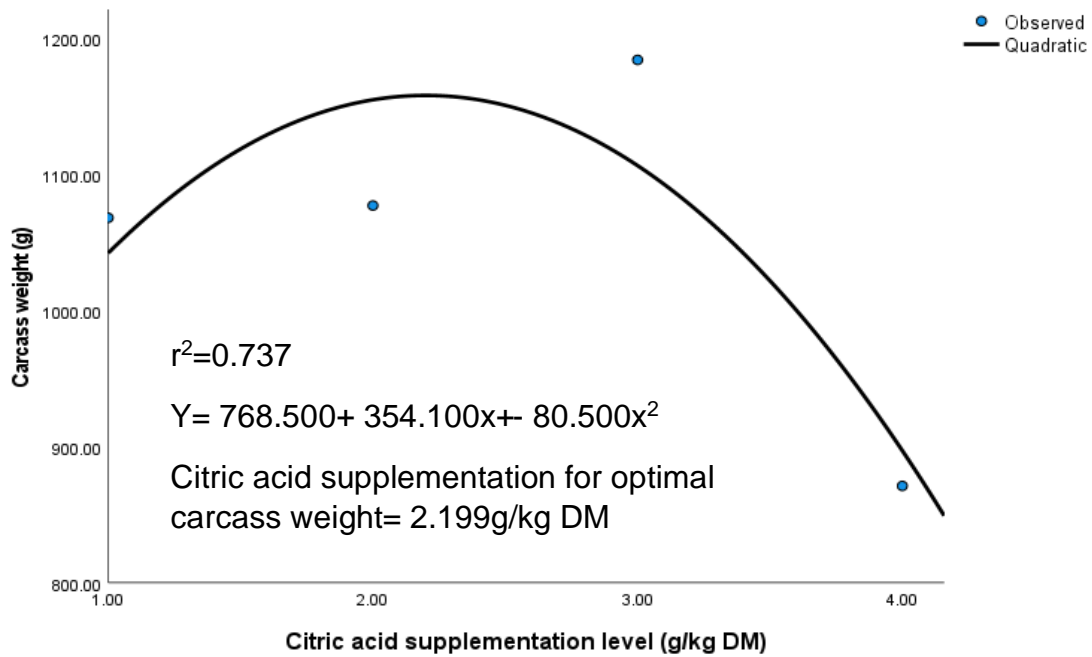


Figure 4.12 Effect of citric acid supplementation in a diet on carcass characteristics of male Venda chickens aged 90 days

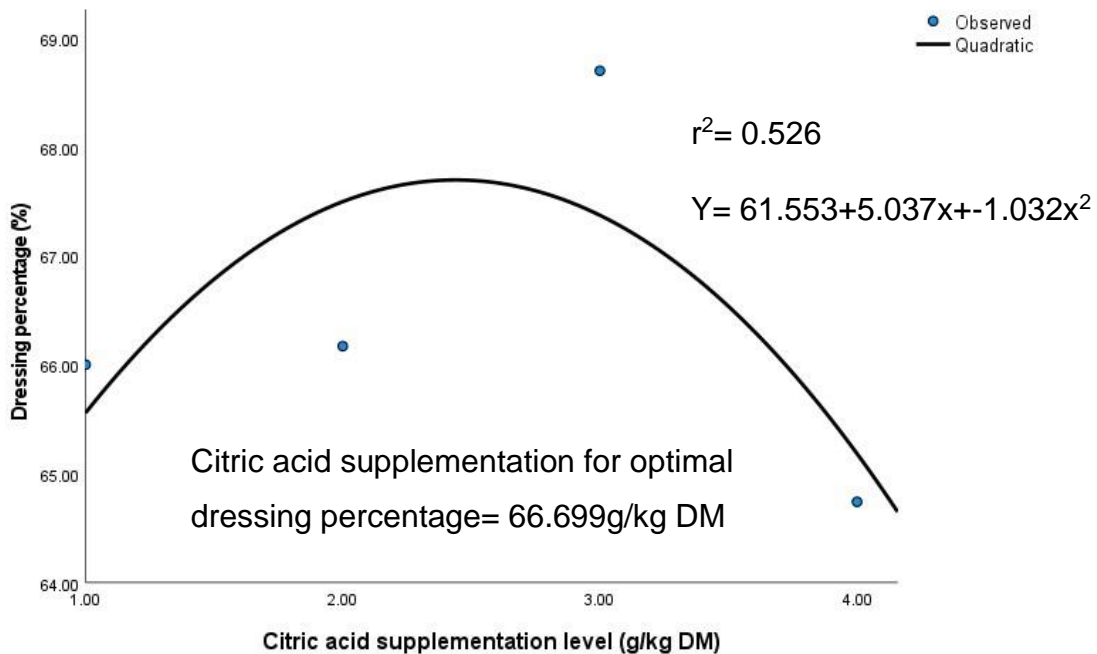


Figure 4.13 Effect of citric acid supplementation in a diet on dressing percentage of male Venda chickens aged 90 days

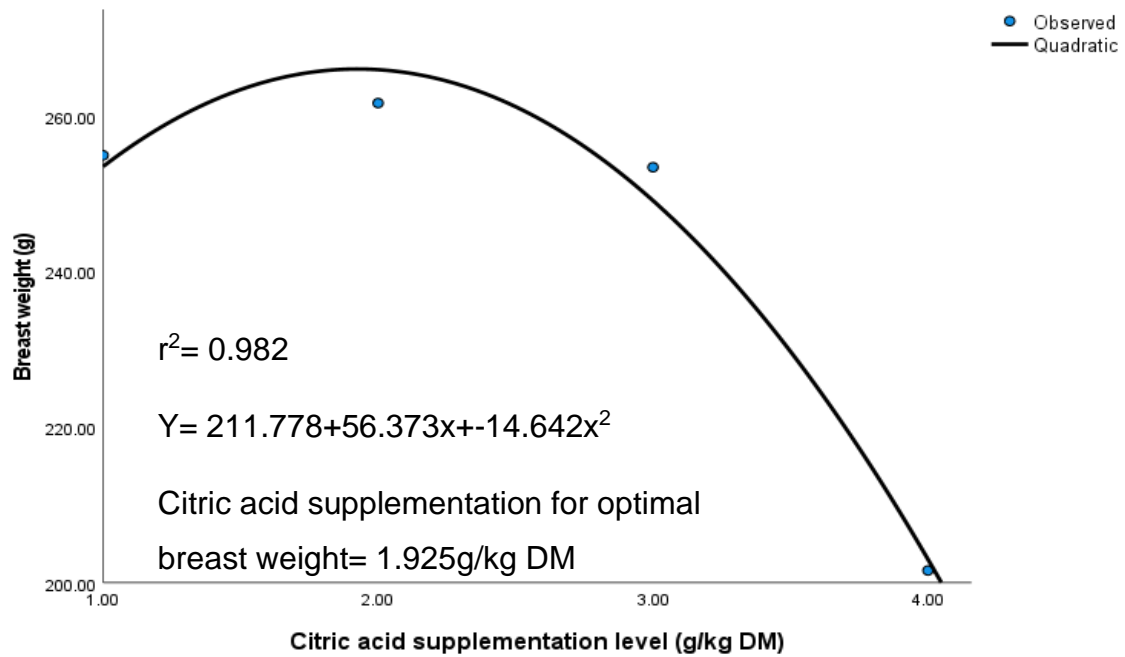


Figure 4.14 Effect of citric acid supplementation in a diet on breast weight of male Venda chickens aged 90 days

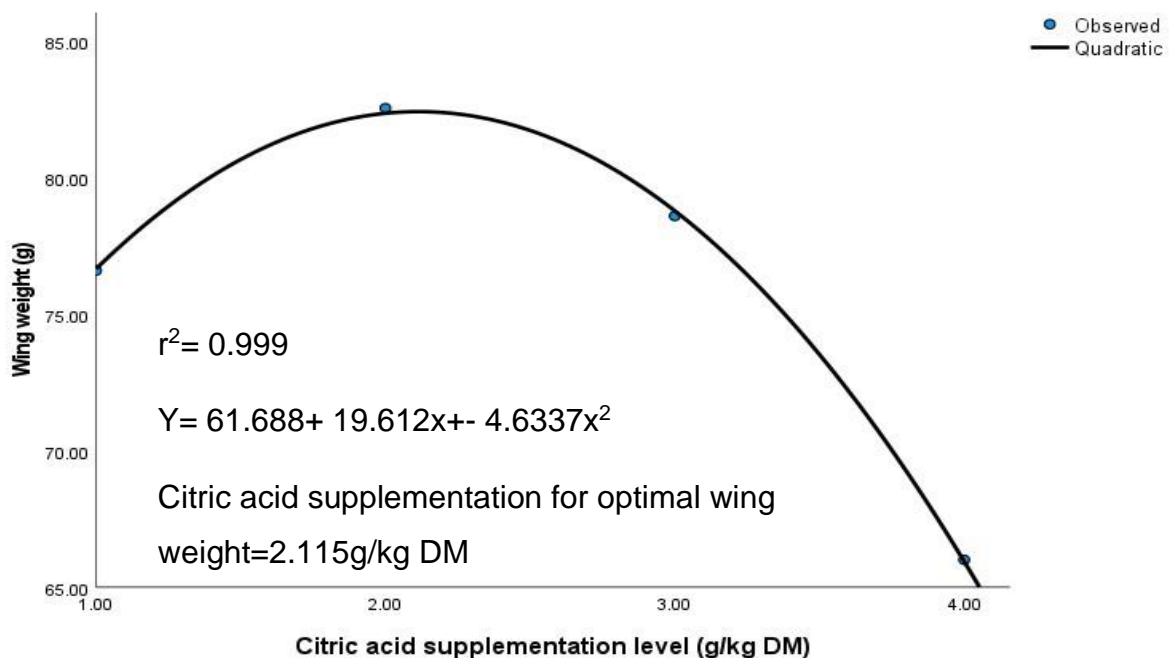


Figure 4.15 Effect of citric acid supplementation on a diet on wing weight of male Venda chickens aged 90 days

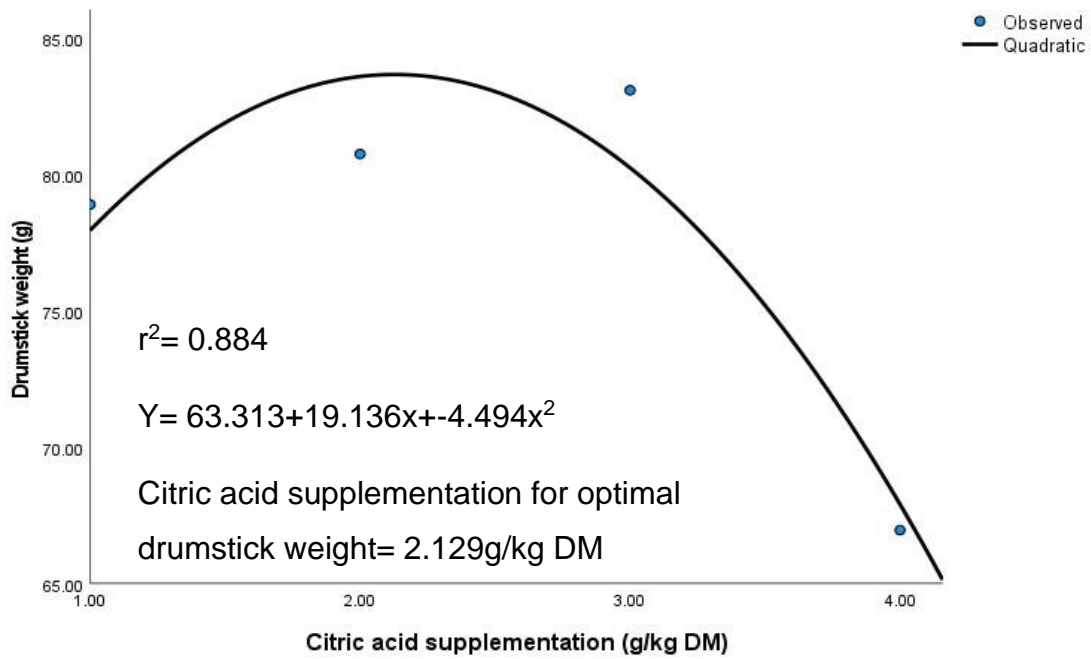


Figure 4.16 Effect of citric acid supplementation in a diet on drumstick weight of male Venda chickens aged 90 days

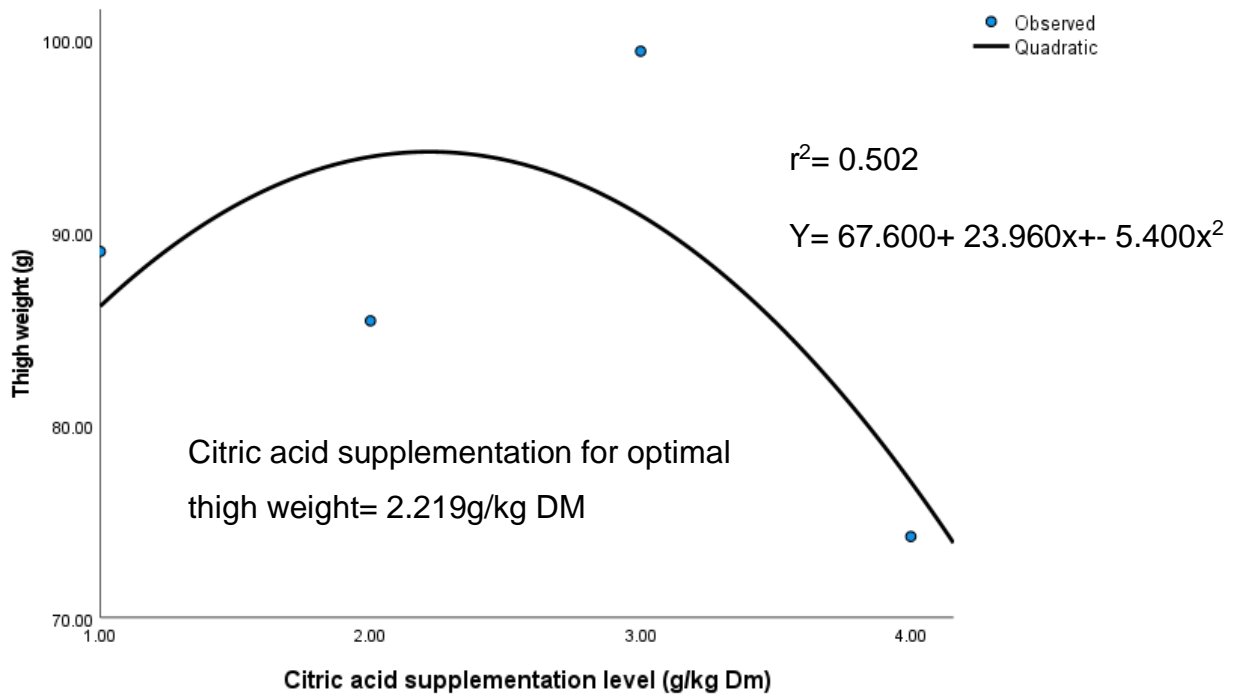


Figure 4.17 Effect of citric acid supplementation in a diet on thigh weight of male Venda chickens aged 90 days

Table 4.11 Citric acid supplementation level for optimal carcass weight, live weight, meat parts weight, and dressing percentage of male Venda chickens aged 90 days

Variable	Formula	X	Y	r ²	Probability
Live weight (g)	$Y=1275.250+411.450x+-96.750x^2$	2.216	1711.917	0.802	0.445
Carcass weight (g)	$Y=768.500+354.100x+-80.500x^2$	2.199	1157.900	0.737	0.513
Dressing percentage (%)	$Y=61.553+5.037x+-1.032x^2$	2.433	67.699	0.538	0.680
Breast weight (g)	$Y=211.778+56.373x+-14.642x^2$	1.925	266.038	0.983	0.132
Wing weight (g)	$Y=61.688+19.612x+-4.637x^2$	2.115	82.425	0.999	0.026
Drumstick weight (g)	$Y=63.313+19.136x+-4.494x^2$	2.129	83.684	0.886	0.338
Thigh weight (g)	$Y=67.600+23.960x+-5.400x^2$	2.219	94.178	0.502	0.705

r² : Coefficient of determination

4.6 Effect of citric acid supplementation on physico chemical attributes of male Venda chickens aged 90 days

Results of the effect of citric acid supplementation on pH values of Venda chickens aged 90 days are presented in Table 4.12. Citric acid supplementation affected ($P<0.05$) pH values of Venda chickens aged 90 days. Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) pH value at 24 hours post mortem than those supplemented with 12.5, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 12.5g of citric acid per kg DM had higher ($P<0.05$) pH values at 24 hours post mortem than those supplemented with 25 or 50g of citric acid per kg DM. However, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$) 24 hours pH values. Similarly, Venda chickens supplemented with 25 or 50g of citric acid per kg DM had the same ($P>0.05$) 24 hours pH values.

Results of the current study indicate that Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) pH value at 48 hour post mortem than those supplemented with 12.5, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 12.5, 25 or 50g of citric acid per kg DM had the same ($P>0.05$) 48 hours pH values.

Results of the current study indicate that Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) 72 hours pH value than those supplemented with 12.5, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented

with 12.5, 25 or 50g of citric acid per kg DM had the same ($P>0.05$) pH values at 72 hours post mortem.

Table 4.12 Effect of citric acid supplementation on meat pH values of male Venda chickens

Variable*	Diet#			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
24hours pH	5.9 ^a ±0.08	5.8 ^b ±0.05	5.8 ^{bc} ±0.02	5.7 ^c ±0.01
48hours pH	6.0 ^a ±0.13	5.9 ^b ±0.05	5.9 ^b ±0.05	5.8 ^b ±0.06
72hours pH	6.2 ^a ±0.12	6.0 ^b ±0.04	6.0 ^b ±0.05	5.9 ^b ±0.04

* : Values presented as mean ± standard deviation a, b, c,

: Means in the same row not sharing a same superscript are significantly different ($p > 0.05$)

: Diet codes are described in Chapter 3, Table 3.01

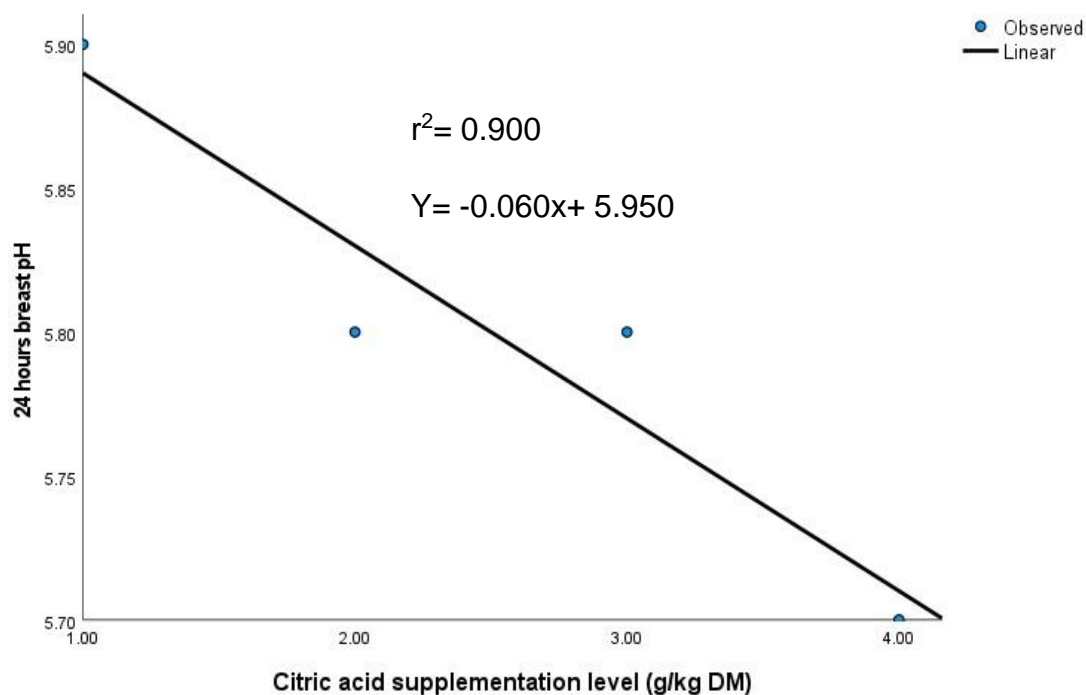


Figure 4.18 Relationship between citric acid supplementation level in a diet and 24 hours breast pH of male Venda chickens aged 90 days

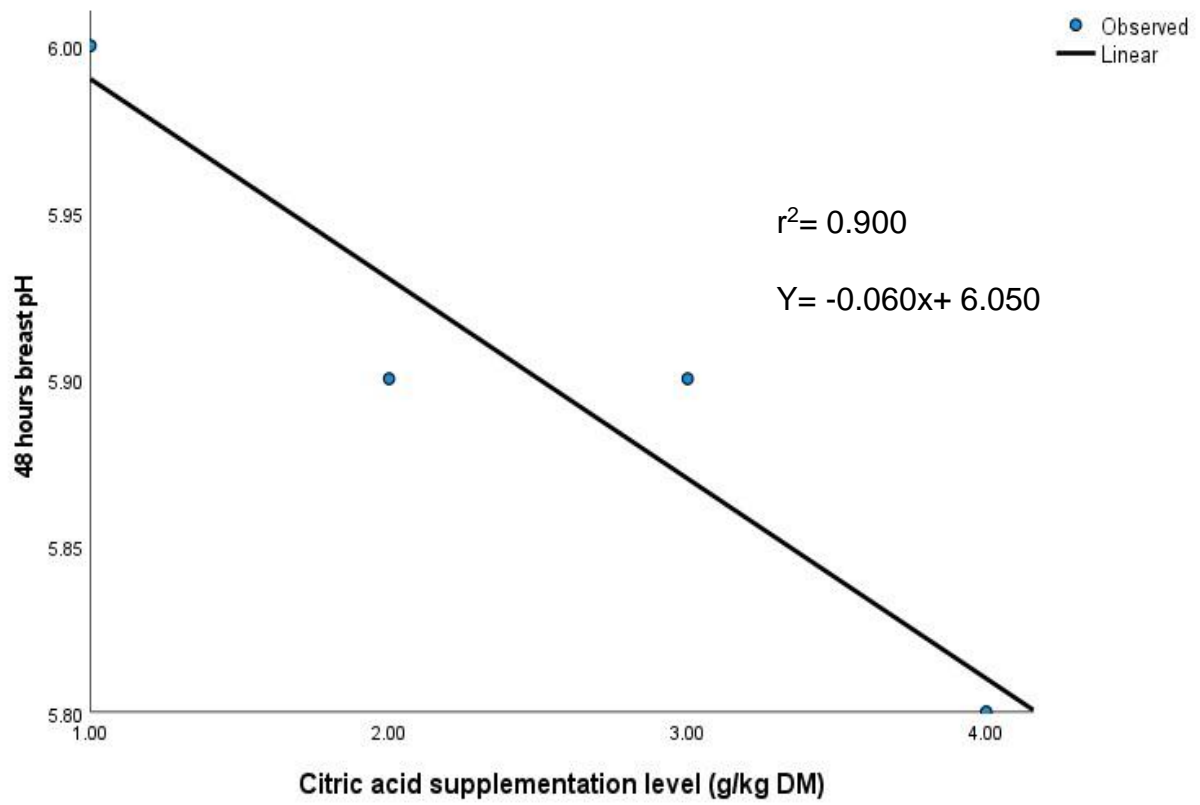


Figure 4.19 Relationship between citric acid supplementation level in a diet and 48 hours breast pH of male Venda chickens aged 90 days

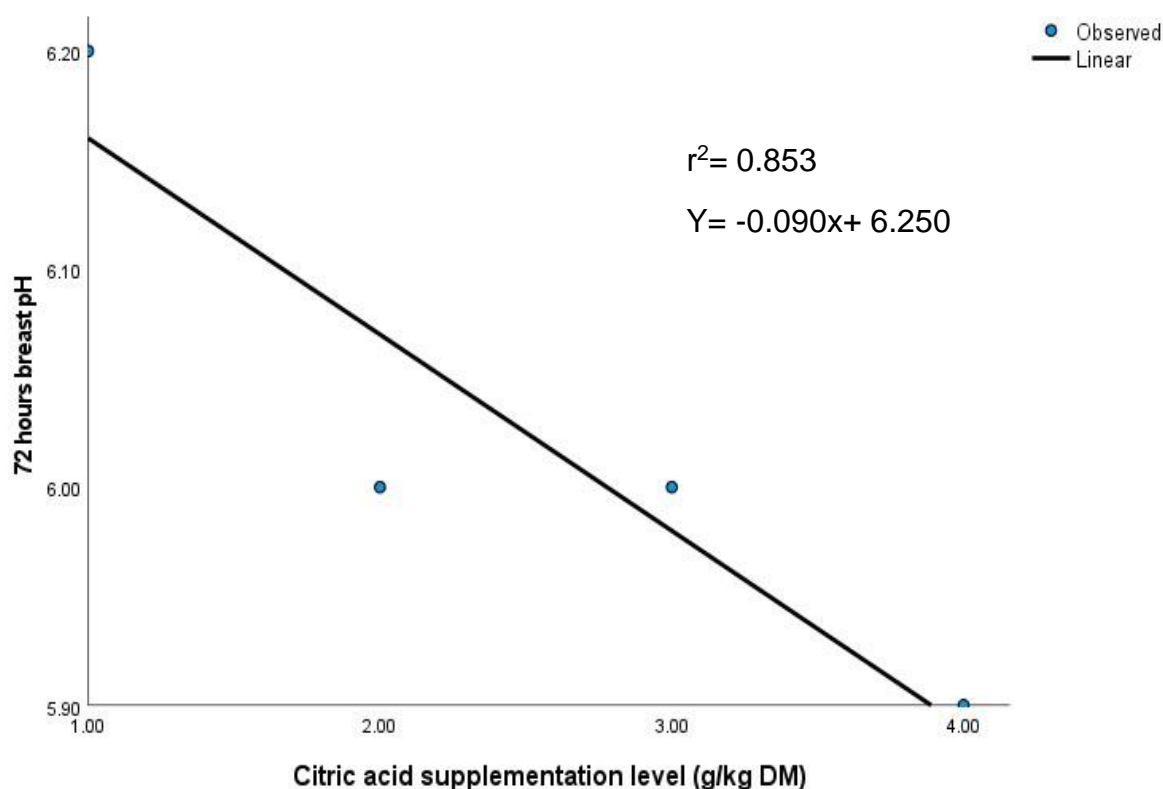


Figure 4.20 Relationship between citric acid supplementation level in a diet and 72 hours breast pH of male Venda chickens aged 90 days

4.7 Effect of citric acid supplementation on physico chemical attributes of male Venda chickens aged 90 days

Results of the effect of citric acid supplementation on physico chemical attributes of Venda chickens aged 90 days are presented in Table 4.13. Citric acid supplementation affected ($P < 0.05$) thawing loss, cooking loss and shear force values of Venda chickens aged 90 days. Venda chickens supplemented with 12,g or 25g of citric acid per kg DM had higher ($P < 0.05$) thawing loss than those supplemented with 0 or 50g of citric acid per kg DM. However, Venda chickens supplemented with 12,5 or 25g of citric acid per kg DM had similar ($P > 0.05$) thawing loss. Similarly, Venda chickens supplemented with 0 or 50g of citric acid per kg DM had the same ($P > 0.05$) thawing loss. A 2.576g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in the optimal thawing loss of Venda aged 90 days (Figure 4.21 and Table 4.14).

Results of the current study indicate that Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had higher ($P<0.05$) cooking loss than those supplemented with 25 or 50g of citric acid per kg DM. However, Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had similar ($P>0.05$) cooking loss. Similarly, Venda chickens supplemented with 25 or 50g of citric acid per kg DM had the same ($P>0.05$) cooking loss.

Venda chickens supplemented with 0g of citric acid per kg DM had higher ($P<0.05$) shear force value than those supplemented with 12.5, 25 or 50g of citric acid per kg DM. Similarly, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had higher ($P<0.05$) shear force values than those supplemented with 50g of citric acid per kg DM. However, Venda chickens supplemented with 12.5 or 25g of citric acid per kg DM had the same ($P>0.05$) shear force values.

Table 4.13 Effect of citric acid supplementation on meat thawing loss, cooking loss, and shear force of male Venda chickens aged 90 days

Variable*	Diet [#]			
	MCA ₀	MCA _{12.5}	MCA _{25.0}	MCA _{50.0}
Thawing loss (%)	3.1 ^b ±0.06	3.6 ^a ±0.11	3.6 ^a ±0.12	3.2 ^b ±0.12
Cooking loss (%)	35.1 ^a ±0.65	34.2 ^a ±2.04	30.0 ^b ±0.61	29.2 ^b ±1.24
Shear force	21.3 ^a ±1.43	17.8 ^b ±0.60	17.4 ^b ±0.54	15.1 ^c ±1.12

* : Values presented as mean ± standard deviation a, b, c, : Means in the same row not sharing a same superscript are significantly different ($p > 0.05$)

: Diet codes are described in Chapter 3, Table 3.01

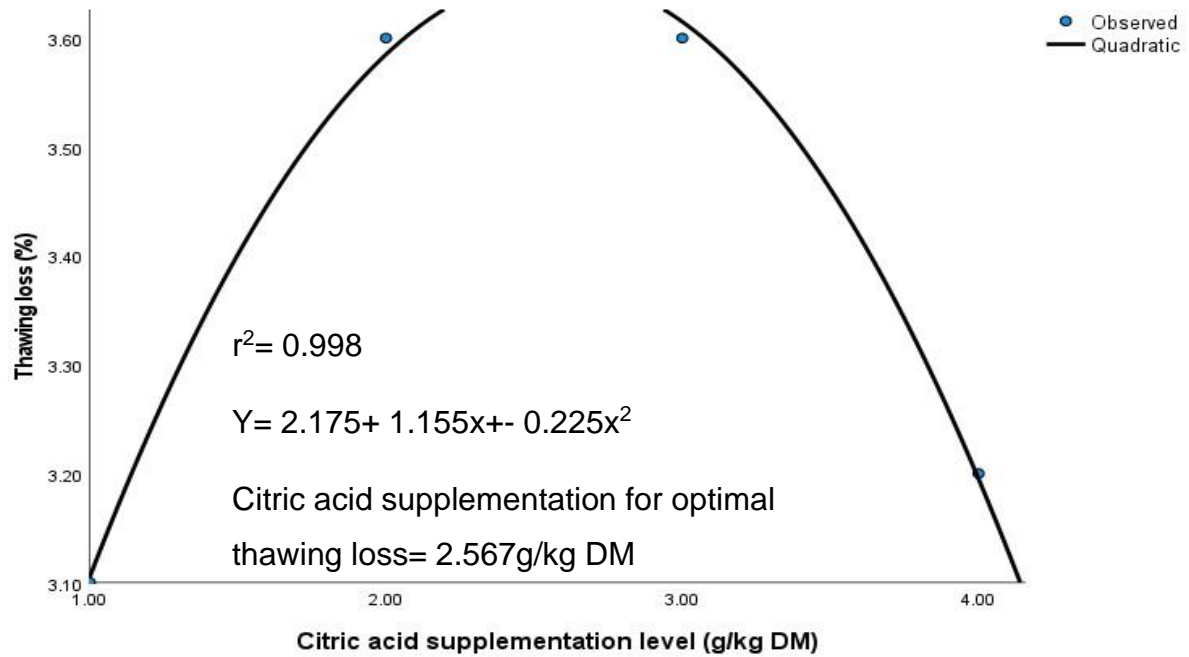


Figure 4.21 Effect of citric acid supplementation in a diet on thawing loss of male Venda chickens aged 90 days

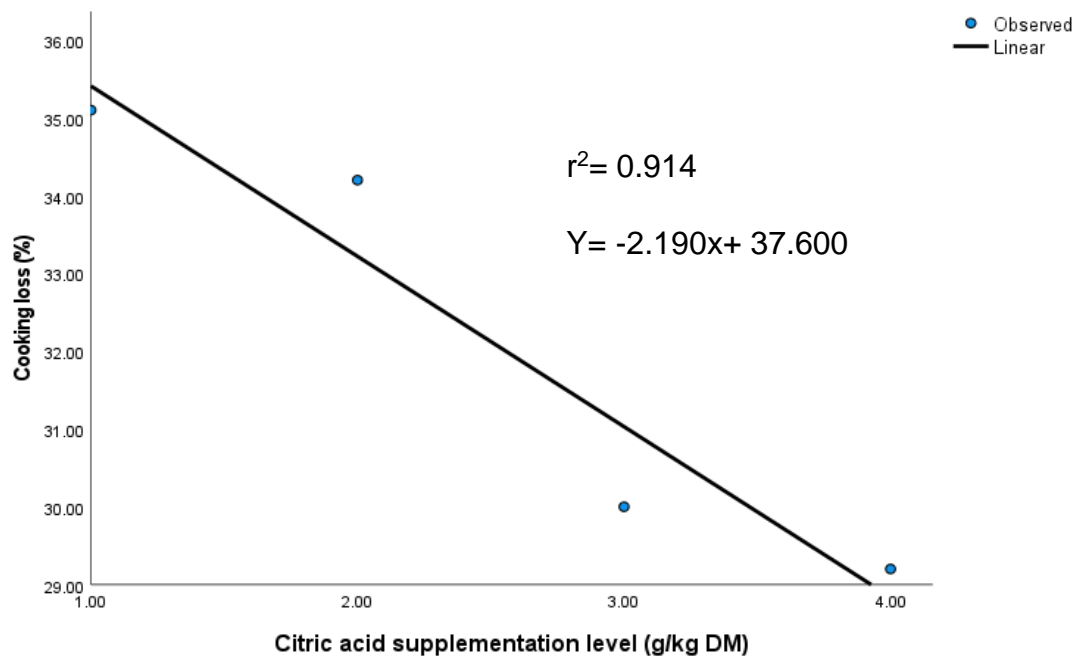


Figure 4.22 Relationship between citric acid supplementation level in a diet and cooking loss of male Venda chickens aged 90 days

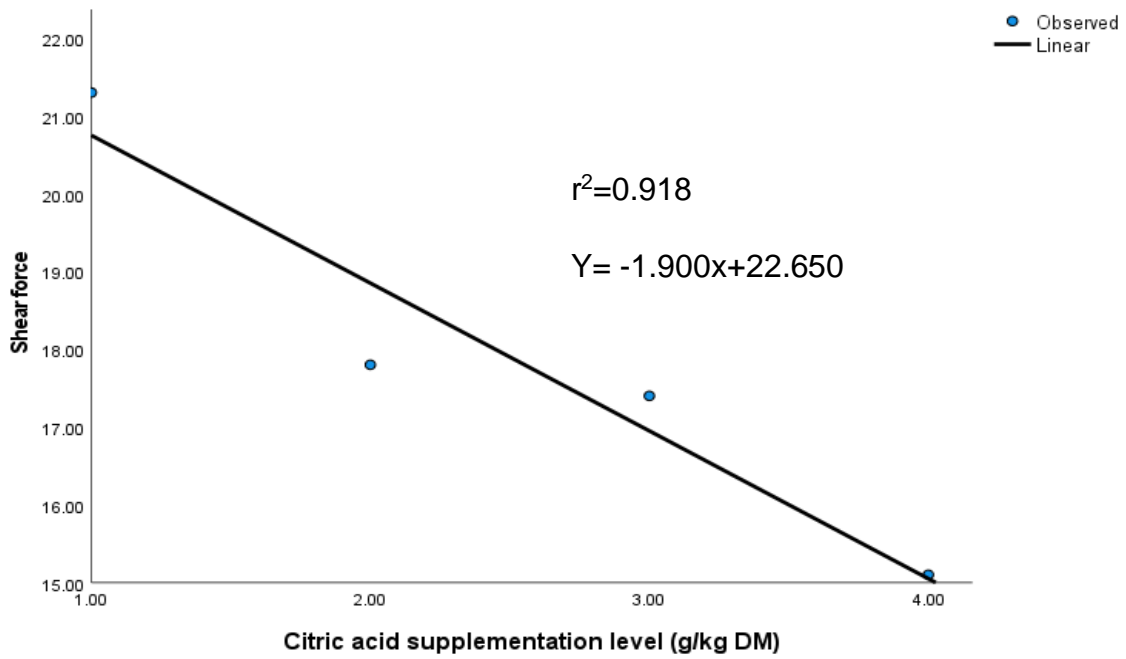


Figure 4.23 Relationship between citric acid supplementation level in a diet and shear force of male Venda chickens aged 90 days

Table 4.14 Citric acid supplementation level for optimal thawing loss, cooking loss, and shear force of male Venda chickens aged 90 days

Variable	Formula	X	Y	r ²	Probability
Thawing loss (%)	$Y=2.175+1.155x+-0.225x^2$	2.567	3.657	0.998	0.049

r² : Coefficient of determination

CHAPTER 5
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

According to Menconi *et al.* (2013), organic acids such as citric acid may improve animal welfare and economic problems in the chicken business by lowering body weight loss and enhancing meat quality qualities. Analysis of variance (ANOVA) was used to meet the study's objectives of identifying the influence of citric acid supplementation on live weight, carcass weight, dressing pieces, meat pH, shear force, and cooking loss. The current study found that citric acid supplementation had no effect on feed intake or feed conversion ratio in male Venda chickens aged one to 30 days, implying that Venda chickens can be fed a starter diet without citric acid supplementation and have no negative effect on feed intake or FCR. Citric acid supplementation, on the other hand, had an effect on the growth rate and live weights of male Venda chickens aged one to 30 days. Using quadratic equations, citric acid supplementation doses of 2.393 and 2.819g per kg DM of the food were calculated to result in optimal growth rate and live weight of male Venda chickens aged one to 30 days. The results of the present study are consistent with the observations made by Abd-El-Hlim *et al.* (2018) who reported that broiler chicks fed the recommended amount of protein + 1.5% citric acid diets, had improved body weights during the starter phase. Similarly, Snow *et al.* (2004) showed that adding phytase and citric acid to low available phosphorus diet (0.13% available P) tended to increase body weight in broiler chickens. Boling *et al.* (2000) indicated that supplementing dietary sodium citrate and citric acid together without inorganic P increased the body weight of broilers. Also, Rafacz-Livingston *et al.* (2005) found an increase in BWG of chickens fed with citric acid without dietary supplementation of P (0.13% available P).

In the current investigation, citric acid boosted feed consumption. Boling *et al.* (2000) obtained similar results. They hypothesized that this was due to the dilution of energy in the food caused by the addition of citric acid. Citric acid supplementation to a low accessible phosphorus diet boosted feed intake in broiler chickens, according to Rafacz-Livingston *et al.*(2005) and Snow *et al.* (2004). Chowdhury *et al.* (2009) and Haque *et al.* (2010) also found that adding citric acid to the diet boosted feed consumption by up to 35 days. The current findings, however, contradict the findings of Wickramasinghe *et al.* (2014), who found that 2% citric acid in broiler diets did not significantly inhibit weight gain from day 21 to day 42. Similarly, Ao *et al.* (2009) reported that citric acid significantly decreased the feed intake and weight gain of

broiler chicks. This suggests that the effects of citric acid on weight gain are mediated through its effects on feed intake.

Citric acid supplementation had an effect on male Venda chickens aged 31 to 60 days in terms of DM meal intake, growth rate, FCR, and live weights. Citric acid has a strong flavor, and high doses of supplementation may influence feed palatability and weight gain, according to Salgado-Tránsito (2011). The chickens tolerated increasing levels of citric acid supplementation from 12.5 to 25g per kg DM as their intake increased, but showed a significant decrease in feed consumption at 50g per kg DM feed acidification. Weight gain and daily feed intake were significantly improved in broiler chicks supplemented with 30 g citric acid/kg but repressed when citric acid was increased to 60 g/kg, according to Nourmohammadi and Khosravinia (2015). Islam (2012) and Nourmohammadi *et al.* (2016) reported that supplementing broiler diets with 0.5% citric acid significantly boosts live weight, weight gain, feed intake, and FCR when compared to control birds. The improved growth performance could be attributed to citric acid's beneficial effect on gut morphometry and size. According to Asgar *et al.* (2013), the 0.5% citric acid-fed group had the highest live weight, average weight gain, cumulative feed intake, and FCR when compared to the non-supplemented prebiotic, antibiotic, and control groups. Lower levels of citric acid (0.5%-3%) boost productivity, but greater levels of citric acid (6%) increase sourness and, as a result, feed consumption (Islam, 2012; Hosseini *et al.*, 2017), resulting in the above results. Rafacz-livingston *et al.* (2005) found that adding citric acid to meals having a low level of non-phytate phosphorus (NPP) (0.18%) improved growth performance. As a result, the absence of favorable impacts of citric on growth performance could be attributed to the usage of insufficient dietary NPP levels. Feeding citric acid, acetic acid, or a mixture of the two greatly boosted body weight gain and FCR in broiler chicks (Islam *et al.*, 2008). The improvement in growth performance observed in this study was most likely related to a drop in intestinal pH as well as improved nutrient digestion. Celik *et al.* (2003) discovered improved feed conversion in broilers fed an acidifier diet, while Hassan *et al.* (2010) discovered that organic acids improved feed conversion ratio considerably. Celik *et al.* (2003) discovered improved feed conversion in broilers fed an acidifier diet. According to Hassan *et al.* (2010), organic acids considerably enhanced feed conversion ratio.

However, the current study's findings contradict those of Ao *et al.* (2009), who found that broilers fed citric acid supplemented diets with appropriate quantities of NPP (0.45%) had lower growth performance. The lower growth performance could be attributed to the utilization of insufficient dietary non-phytate P (NPP). In another study, 1.5% commercial acetic acid improved broiler performance, but increasing citric acid levels to 3% yielded no additional benefits (Abdel Fattah *et al.*, 2008). , PinWu and Chen (2016), and Abd-El-Hlim *et al.* (2018) found that 1.0% citric acid supplemented meals had no effect on turkey development performance. The addition of 2% CA to broiler diets did not significantly inhibit weight gain from day 21 to day 42. (Wickramasinghe *et al.*, 2014).

The results of the present study indicate that citric acid supplementation affected DM feed intake, growth rate, FCR, live weight of male Venda chickens aged 61 to 90 days. Citric acid supplementation levels of 1.566, 2.167, 2.332 and 2.727g per kg DM of the diet was calculated using quadratic equations to result in optimal DM feed intake, growth rate, FCR and live weight of male Venda chickens aged 61 to 90 days. The results of the present study are consistent with the observations made by ELnaggar and Abo EL-Maaty, (2017) showed that ducklings fed 2 or 3% CA-supplemented diets had significantly greater LBW, BWG, and FCR compared to the control. The improvements in growth performance seen may be likely due to the decrease in the pH of gut content. Similarly, Atapattu and Nelligaswatta (2005) found that citric acid could have positive effects on growth performance and feed intake only when diets are low in a P and high Ca:P ratio. When Ca:P ratio is low, the release of additional P causes Ca deficiency by creating an unfavorable Ca:P ratio. The results of the research by Pirgozliev *et al.* (2008) and Ao *et al.* (2009) showed that the combination of cinnamon oil and citric acid has a positive effect on the production performance of poultry as it lowers the pH of the intestinal contents and increases intolerance of bacterial growth to pH changes (Pirgozliev *et al.*, 2008; Ao *et al.*, 2009). The result is better gut health, better intestinal villi integrity, and maximum nutrient absorption (Dibner and Buttin, 2002). Taherpour *et al.* (2009) observed that the birds fed diets supplemented with organic acids showed significantly higher body weight gains and feed conversion ratio (Adil *et al.*, 2011). Rafacz-livingston *et al.* (2005) reported a linear increase in weight gain and feed intake in chicks fed diets supplemented with up to

3% of citric acid. The above findings suggest that the effects of citric acid on weight gain are mediated through its effects on feed intake. Isabel and Santos (2009) discovered that organic acid has a substantial effect on feed conversion ratio (FCR). The increase in feed conversion due to citric acid supplementation was consistent with the findings of Afsharmanesh *et al.* (2005), who discovered higher feed conversion with citric acid administration in chicken. In contrast, Brenes *et al.* (2003) reported a decline in feed consumption as a result of citric acid. Lower FCR has been recorded for a variety of organic acids, including citric and ascorbic acid (Afsharmanesh and Pourreza, 2005) and a mixture of lactic, citric, and formic acid (Alçiçek *et al.*, 2004). The varying results reported in the preceding experiments imply that FCR may be modified by the type, shape, and inclusion levels of the various organic acids. These findings are consistent with those of previous researchers, Stipkovits *et al.* (1992), who discovered that higher levels of citric acid treatment reduced feed consumption. According to Alçiçek *et al.* (2004), a mixture of formic-, lactic-, and citric acid had no influence on broiler feed consumption at any age. The usage of organic acid may explain the reported findings. Nourmohammadi *et al.* (2010) found no significant effects on feed intake in broiler chicks on a citric acid-supplemented diet. Similarly, Nezhad *et al.* (2007) observed a non-significant effect on feed intake in broilers fed on corn-soybean meal diet supplemented with the 3 levels (0.0, 2.5 and 5%) of citric acid.

The current study found that supplementing with citric acid affected the carcass weight and dressing component weight (breast, wing, drumstick, and thigh weight) of 90days-old male Venda chickens. Citric acid supplementation amounts of 2.199, 2.125, 2.115, 2.128, and 2.219g per kg DM of the food were determined using quadratic equations to produce optimal carcass weight and dressing pieces in male Venda chickens aged one to 30 days. The current findings are comparable to those of Aksu *et al.* (2007), who found a significant improvement in carcass weight and some carcass parameters such as thigh, breast, and neck with 4 g/kg feed citric acid addition. Similarly, Fascina *et al.* (2012) found that food supplementation with citric acids improved carcass features. The results agree with Saki and Eftekhari (2012) who is of the view that the addition of citric acid increased the carcass weight, and breast weight, at 21 days of age. Similarly, (Odetola and Adetola, 2022) obtained significant differences in drumsticks and wings in their study. Jha *et al.* (2009) reported that the inclusion of OAs (formic + propionic acid, formic + citric acid, formic + sorbic, and formic+ lac-tic

acid) enhanced the meat thigh weight (29.03%), back weight (53.4%), wings weight (31.27%), and breast weight (34.57%) compared to the control group. Other researchers indicated numerical improvements in carcass yield in broilers fed 3% CA but not at a 6% level (Nourmohammadi *et al.*, 2010). According to ELnaggar and Abo EL-Maaty (2017), broiler chick fed 2 or 3% CA had considerably improved dressing and relative weight of total edible portions. Nonetheless, Islam *et al.* (2008) produced conflicting results, finding that dietary regimens with citric acid supplementation had no effect on carcass parameters. The aforementioned unmatched results could be attributed to different supplemented meals containing different percentages of nutrients. These findings are consistent with the findings of Kopecky *et al.* (2012), who found no significant impact of citric acid supplementation on carcass characteristics. Brzóska *et al.* (2013) found that adding OAs to a broiler diet had no significant effect on breast muscle content or leg muscle weight. This could be due to the degree of inclusion or, more likely, the type of organic acid utilized.

The current study found that citric acid supplementation affected the dressing percentage of male Venda chickens aged 90 days. Citric acid supplementation amounts of 2.433g per kg DM of meal were calculated using quadratic equations to achieve the optimal dressing percentage of male Venda chickens aged one to 90 days. These findings are consistent with those of Chowdhury *et al.* (2009), Hassan *et al.* (2010), and Hudha *et al.* (2010), who discovered a considerable increase in dressing percentage by using citric acid in the feed. The findings are consistent with those of Alcicek *et al.* (2004), who found that using an organic acid mixture containing citric acid increased dressing percentage relative to controls. Acidification may have boosted cell proliferation and, as a result, muscular size. According to Ahsan-ul-Haq *et al.* (2014), nutritional supplementation of different doses of citric acid in broilers increased dressing percentage (without skin), and analysis of variance demonstrated that dressing percentage was considerably enhanced. In the study done by Ahsan-ul-Haq *et al.* (2014), the addition of citric acid in the feed resulted in an average improvement in dressing percentage ranging from 0.66 to 2.18%. In contrast to the preceding findings, Denli *et al.* (2003) found that organic acid supplementation had no influence on dressing percentage. The disparity in these results could be attributed to the various durations of feed withdrawals before to slaughter. The findings are consistent with those of Islam *et al.* (2008), Abdel-Fattah *et al.* (2008), and Adil *et al.*

(2010), who supplied citric acid to broilers and found no significant effect on broiler dressing percentage. Different doses of citric acid supplementation had no influence on the relative breast meat of broilers in different treatment groups (Ahsan-ul-Haq *et al.*, 2014). Hudha *et al.* (2010) found that dietary acidification had a substantial influence on thigh meat but had no effect on breast meat.

Citric acid supplementation reduced cooking loss in male Venda chickens aged 90 days. The current study's findings are consistent with those of Onenc *et al.* (2004), who found that citric acid marinated beef samples had fewer cooking losses than the control group. This was most likely owing to the pH levels lowering the protein isoelectric point, resulting in excess water (Kahraman *et al.*, 2012). Yusop *et al.*, (2010) found no change in the cooking loss of chicken breast marinated in various pH-containing citric acid solutions. These findings could be attributed to a lack of change in the core pH of meats. Similarly, Cho *et al.* (2014) found that administering a microencapsulated OAs combination of citric and sorbic acids had no effect on cooking loss. Cooking loss values were not significantly different between citric acid-marinated samples and control samples (Fu-Yi He *et al.*, 2015). In contrast, Sammel and Claus (2003, 2006) discovered that increasing citric acid caused an increase in cooking loss percentages due to higher acidity (lower pH), which lowered the ability of meat to bind water. These findings contradict those of Hosseini and Esfahani Mehr (2015), who found that samples marinated with the highest quantity of citric acid had the lowest cooking loss. The reduction in loss caused by cooking acid treatments may be explained by the swelling impact on muscle proteins, which may hold more water. Serdaroglu *et al.* (2007) also found that samples treated with 0.1M citric acid had the highest cooking loss values. When samples are marinated in acidic solutions, the fibers enlarge and some muscle proteins can hold more water, reducing cooking loss (Klinhom *et al.*, 2011).

Shear force determination is a reliable method for evaluating meat tenderness, and the extent of myofibrillar protein proteolysis is dependent on it (Marcinkow-ska-Lesiak, *et al.*, 2016). The current study found that citric acid supplementation affected the shear force of male Venda chicken breasts aged 90 days. The current findings are consistent with those of Ji-Han Kim *et al.* (2015), who discovered that the shear forces of the citric acid supplemented groups were much lower than those of the control

groups. During storage, shear force decreased in all groups. Lipid oxidation influences the tenderness of meat products, which can be attributed to the interaction between lipid oxidation and protein cross-linking (Lund *et al.*, 2007; Xiong *et al.*, 2009). The drop in shear force of meat products as citric acid content increased was attributable to a fall in pH, which effects the water-binding capacity (Burke and Monahan, 2003). When the pH falls below the isoelectric point of the major myofibrillar proteins, the waterholding capacity and softness of muscle increase (Rao *et al.*, 1989a). The greater water-holding capacity of the muscle at lower pH values could be attributed to an increase in the net positive charges on the protein molecules, as well as the osmotic pressure produced by the presence of significant amounts of organic acids, which causes pH to decrease. Okeudo and Moss (2005) discovered a negative relationship between shear force and cooking yield and moisture content. The shear force of 5% citric acid, in particular, was much lower than that of the other groups. The denaturation of muscle protein caused by acidic solution treatment is known to cause tissue disintegration and diminish the tenderness of meat (Aktas and Kaya, 2001; Ke *et al.*, 2009). As a result, protein denaturation and moisture content may influence the reduction in shear force caused by citric acid-treated samples. Citric acid supplementation is known to reduce the shear force values of the meat since shear force is regulated by the pH of the meat, and as pH decreases, shear force decreases as well, hence no findings contradict the current study.

The current study found that citric acid supplementation impacted the 24 hour pH, 48 hour pH, and 72 hour pH of male Venda chicken breasts aged 90 days. This is consistent with the findings of Del Rio *et al.* (2007), who found that dipping chicken legs in a 2 mL citric acid/100 mL solution dramatically decreased pH immediately after treatment. Such variations may be attributable to the acid concentrations used. Because of their organic acid content, the samples' pH decreased after being marinated with citric acid (Unal *et al.*, 2022). Similarly, Serdaroglu *et al.* (2007) showed that citric acid marination reduced the pH of turkey flesh samples. Kumar *et al.* (2015) reported similar pH drops as a result of marination with an acidic solution (i.e. citric acid). Citric acid marinade had a linear effect on the final pH of marinated fillets; higher citric acid levels decreased pH. (Yang and Chen, 1993). The duck breast muscles marinated with citric acid had significantly lower pH values than those without citric acid marination (Hyun *et al.*, 2015). The lower pH in breast marinated with citric acid

might be mainly due to the low pH of citric acid (Ke *et al.*, 2009). Citric acid added to intact turkey breasts at either 0.2% or 0.3% reduced pH compared with the control regardless of pinking agent Sammel and Claus, (2003). Yang and Chen (1993) conducted a trial evaluating the effects of refrigerated storage, pH adjustment, and marinade on the color of raw and microwave cooked chicken meat and reported a decreased pH, because of marination in citric acid. The low pH could be owing to the presence of citric acid in the coating solution. According to Rio *et al.* (2007), immersing chicken meat in citric acid considerably reduced the pH following marination. The low pH value of meat products is known to affect various characteristics during storage, including redness loss, storage lengthening, water binding capacity stability, and texture (Sammel and Claus, 2003) According to Meltem *et al.* (2007), increasing citric acid concentration resulted in a noticeable reduction in muscle pH. Kim *et al.* (2015) also noted that immersing chicken meat in citric acid considerably reduced the pH. Yusop *et al.*, (2010) discovered that acidic marinade solutions lower pH. There were no contradictory findings because citric acid is known to alter the pH of the meat, thus more research is needed to confirm these findings.

5.2 Conclusion and recommendation

Citric acid addition in the starter meal had no effect on male Venda chickens aged one to 30 days' DM feed intake or feed conversion ratio. Citric acid supplementation, on the other hand, had an effect on the growth rate and live weight of male Venda chickens aged one to 30 days. Citric acid supplementation amounts of 2.939 and 2.536g per kg DM of the food were calculated to achieve the best growth rate and live weight in male Venda chickens aged one to 30 days. Male Venda chickens aged one to 30 days can be fed a diet without citric acid addition and have no adverse effect on growth rate or live weight since citric acid supplementation had no effect on them.

Citric acid addition in the grower diet increased male Venda chickens aged 31 to 60 days' DM meal intake, growth rate, FCR, and live weights. The chickens tolerated increasing doses of citric acid supplementation from 12.5 to 25g per kg DM as DM feed intake increased, but showed a decrease in DM feed intake at 50g per kg DM feed acidification. Citric acid supplementation amounts of 2.250, 2.373, and 2.308g per kg DM of the meal were determined to achieve the best growth rate, FCR, and live

weights in male Venda chickens aged 31 to 60 days. Increased feed consumption led in faster growth and larger live weights, resulting in improved growth performance.

Citric acid addition in the finisher diet increased male Venda chickens aged 61 to 90 days' DM meal intake, growth rate, FCR, and live weights. Citric acid supplementation amounts of 1.566, 2.167, 2.332, and 2.727g per kg DM of diet were determined to achieve optimal DM feed intake, growth rate, FCR, and live weights in male Venda chickens aged 61 to days. The lower DM feed intake in treatment 4 appeared to be a result of a high citric acid supplementation in the diet, so the chickens reduced their feed intake based on the unpalatable taste of citric acid in the diet, which had previously been reported to be rejected by chickens when offered in a two feed choices trial (Niknafs and Roura, 2018).

Citric acid supplementation affected live weight, carcass weight, dressing percentage, breast, wing, drumstick and thigh weigh of male Venda chickens aged 90 days. Citric acid supplementation levels of 2.216, 2.199, 2.433, 1.925, 2.115, 2.129 and 2.219g per kg DM of the diet were calculated to result in optimal live weight, carcass weight, dressing percentage, breast, wing, drumstick and thigh n weight of male Venda chickens aged 90 days. With citric acid supplementation in feed, microscopic organisms diminish within the gastrointestinal tract and consequently enhance feed intake and digestion, therefore enhancing the growth rate and carcass characteristics of chickens (Papatsiros *et al.*, 2013; Dittoe *et al.*, 2018). Citric acid supplementation level of 12.5 and 25g per kg DM are recommended for the improvement of production performance and carcass characteristics of male Venda chickens.

Citric acid supplementation influenced cooking loss, shear force, and meat pH in 90day-old male Venda chickens. The amount of moisture produced during cooking is determined by the pH of male Venda chickens. High pH meat loses less water during cooking than low pH meat (Honikel, 2004; Lawrie and Ledward, 2006). Meat pH also influences the shear force value of the meat. Shear force levels are low when the pH is low. In the current investigation, increasing citric acid levels resulted in lower cooking loss, lower shear force values, and lower pH values of meat from male Venda chickens aged 90 days. Citric acid supplementation at a rate of 50g per kg DM is advised for improving the physicochemical characteristics of male Venda chickens.

CHAPTER 6
REFERENCES

- Abd-El-Hlim, H.S., Attia, F.A.M., Saber, H.S. and Hermes, H.I. 2018. Effects of dietary levels of crude protein and specific organic acids on broilers performance. *Egyptian Journal of Animal Production*, 55: 15-27.
- Abdelrazek, H.M.A., Abuzead, S.M.M., Ali, S.A., El-Genaidy, H.M.A. and Abdel-Hafez, S.A. 2016. Effect of citric acid and acetic acid water acidification on broiler's performance with respect to thyroid hormones level. *Advances in Animal and Veterinary Science* 4(5): 271-278.
- Abu, O. Olaleru, L. and Omojola, A. 2015. Carcass characteristics and meat quality of broilers fed cassava peel and leaf meals as replacements for maize and soyabean meal. *Journal of Agriculture and Veterinary Science*, 8: 41-46.
- Abdel-Fattah, S.A., El-Sanhoury, M.H., El-Mednay, N.M. and Abdel-Azeem, F. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *International Journal of Poultry Science*, 7: 215-222.
- Adham, N.Z. 2002. Attempts at improving citric acid fermentation by *Aspergillus niger* in beet-molasses medium. *Bioresource Technology*, 84(1): 97-100.
- Adil, S, Banday, M.T., Salahuddin, M., Bhat, G.A. and Shanaz, S. 2011. Effect of organic acids on carcass characteristics of broiler chicken. *International Journal of Animal Nutrition*, 23: 218-221.
- Adil, S.T., Banday, G.A., Bhat, M., Mir, S. and Rehman, M. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology and serum biochemistry of broiler chicken. *Veterinary Medicine-International Journal*, 2010: 479-485.
- Afsharmanesh, M. and Pourreza, J. 2005. Effects of calcium, citric acid, ascorbic acid and vitamin D3 on the efficacy of microbial phytase in broiler starters fed wheat based diets. *International Journal of Poultry Science*, 4: 418-420.
- Agbede, J.O. and Aletor, V.A. 2003. Evaluation of fish meal replaced with leaf protein concentrate from *Glyricidia* in diets for broiler chicks: Effect on performance, muscle growth, haematology and serum metabolites. *International Journal of Poultry Science*, 2(4): 242-250.

Ahsan-ul-Haq., Tabassum, M., Ahmad, F., Shafi, J., Ashraf, M., Javed, M. and Shahidur-Rehman. 2014. Effect of dietary acidification with citric acid on carcass characteristics, haemogram and serum metabolite values of broiler chicken. *Pakistan Journal of Life and Social Sciences*, 12(1): 36-41.

Ajayi, F.O. 2010. Nigerian indigenous chicken: A valuable genetic resource for meat and egg production. *Asian Journal of Poultry Science*, 4(4): 164-172.

Aksu, T., Ates, C. T., Erdogan, Z. and Baytok, E. 2007. The response of broilers to dietary organic acid mixture. *Indian Veterinary Journal*, 84(4): 385-387.

Aktas, N., Aksu, M. I. and Kaya, M. 2016. The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. *Journal of Muscle Foods* 14:181-194.

Aktus, N. and Kaya, M. 2001. The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *European Food Research and Technology*, 213: 88-94.

Alabi, O.J. 2013. Effect of lysine to energy ratio on the productivity and carcass characteristics of indigenous Venda chickens aged one to thirteen weeks and raised in closed confinement. (Doctoral dissertation, University of Limpopo).

Alcicek, A., Bozkurt, M. and Cabuk, M. 2004. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. *South African Journal of Animal Science*, 34: 217-222.

Ali, S., Haq, I., Qadeer, M.A. and Iqbal, J. 2002. Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermenter. *Electronic Journal of Biotechnology*, 5: 258-271.

Angumeenal, A.R. and Venkappayya, D. 2013. An overview of citric acid production. *LWT-Food Science and Technology*, 50(2): 367-370.

Anonymous 2010a. Indigenous poultry breeds in South Africa. [WWW document] URL <http://www.wickedfoodearth.co.za/indigenous-poultry-breeds-in-south-africa/>. 1
December 2010.

- Ao, T., Cantor, A. H., Pescatore, A. J., Ford, M. J., Pierce, J. L. and Dawson, K. A. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poultry Science*. 88(1), 111-117.
- Atapattu, N.S.B.M. and Nelligaswatt, C.J. 2005. Effect of citric acid the performance and utilization of phosphorus and crude protein in broiler chicken fed rice by products based diets. *International Journal of Poultry Science*, 4: 990-993.
- Becker, T. 2000. Consumer perception of fresh meat quality: a framework for analysis. *British Food Journal*, 102(3), 158-176.
- Berri, C., Debut, M., Sante-Lhoutellier, V., Arnould, C., Boutten, B., Sellier, N., Baéza, E., Jehl, N., Jégo, Y., Duclos, M.J. and Le Bihan-Duval, E. 2005. Variations in chicken breast meat quality: implications of struggle and muscle glycogen content at death. *British Poultry Science*, 46(5): 572-579.
- Bihan-Duval, L., Debut, M., Berri, C.M., Sellier, N., Santé-Lhoutellier, V., Jégo, Y. and Beaumont, C. 2008. Chicken meat quality: genetic variability and relationship with growth and muscle characteristics. *BMC genetics*, 9(1): 1-6.
- Bogosavljevic-Boskovic, S., Mitrovic, S., Djokovic, R., Doskovic, V. and Djermanovic, V. 2010. Chemical composition of chicken meat produced in extensive indoor and free range rearing systems. *African Journal of Biotechnology*, 9(53): 9069-9075.
- Bogosavljević-Bošković, S., Rakonjac, S., Dosković, V. and Petrović, M.D. 2012. Broiler rearing systems: a review of major fattening results and meat quality traits. *World's Poultry Science Journal*, 68(2): 217-228.
- Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. and Baker, D.H. 2000. The effect of citric acid on phytate P utilization in young chicks and pigs. *Journal of Animal Science*, 78: 682-689.
- Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. and Carmen, B. 2003. The effects of citric acid and microbial phytase on mineral utilization in broiler chicks. *Animal Feed Science and Technology*, 110: 210-219.
- Brewer-Gunsaulis, V.B. and Owens, C.M. 2013. Physical and biochemical properties associated with broiler breast fillet texture at two market ages. *Poultry Science*, 9: 147.

- Brzóška, F., Sliwiński, B. and Michalik-Rutkowska, O. 2013. Effect of Dietary Acidifier on Growth, Mortality, Post-Slaughter Parameters and Meat Composition of Broiler Chickens/Wpływ zakwaszacza diety na masę ciała, śmiertelność, wydajność rzeźniczą i skład mięsa kurcząt rzeźnych. *Annals of Animal Science*, 13: 85–96.
- Burke, R.M. and Monahan, F.J. 2003. The tenderization of shin beef using a citrus juice marinade. *Meat Science*, 63: 161- 168.
- Castellini, C., Mugnai, C. and Dal Bosco, A. 2002a. Effect of organic production system on broiler carcass and meat quality. *Meat Science*, 60, 219-225.
- Castellini, C., Mugnai, C.A.N.D. and Dal Bosco, A. 2002. Effect of organic production system on broiler carcass and meat quality. *Meat Science*, 60(3): 219-225.
- Celik, K., Denil, M. and Okan, F. 2003. Effect of dietary probiotic, organic acids and antibiotic supplementation of diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition*, 2: 89-91.
- Chartrin, P., Météau, K., Juin, H., Bernadet, M.D., Guy, G., Larzul, C., Rémygnon, H., Mourot, J., Duclos, M. J. and Baéza, E. 2006. Effects of intramuscular fat levels on sensory characteristics of duck breast meat. *Poultry Science*, 85(5): 914-922.
- ChemBioDraw. 2014, version12, CambridgeSoft.com.
- Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L. and Yuan, Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry. *Frontiers Microbiology*, 5: 217.
- Cho, J.H., Song, M.H. and Kim, I.H. 2014. Effect of microencapsulated blends of organic acids and essential oils supplementation on growth performance and nutrient digestibility in finishing pigs. *Revista Colombiana de Ciencias Pecuarias*, 27: 264–272.
- Choo, Y.K., Kwon, H.J., Oh, S.T., Um, J.S., Kim, B.G., Kang, C.W., Lee, S.K. and An, B.K. 2014. Comparison of growth performance, carcass characteristics and meat quality of Korean local chickens and silky fowl. *Asian-Australian Journal of Animal Science*, 27: 398-405.
- Chowdhury, R., Islam, K. M., Khan, M. J., Karim, M., Haque, M. N., Khatun, M. and Pesti, G. 2009. Effect of citric acid, avilamycin, and their combination on the

performance, tibia ash, and immune status of broilers. *Poultry Science*, 88 (8): 16161622.

Combes, S., Lepetit, J., Darche, B. and Lebas, F. 2003. Effect of cooking temperature and cooking time on Warner–Bratzler tenderness measurement and collagen content in rabbit meat. *Meat Science*, 66: 91-96.

Commission Directive. Laying down specific purity criteria on food additives other than colours and sweeteners. 2008. *Official Journal of the European Union*, 253, 1.

Conroy, C., Sparks, N., Chandrasekaran, D., Sharma, A., Shindey, D., Singh, L.R., Natarajan, A. and Anitha, K. 2005. The significance of predation as a constraint in scavenging poultry systems: some findings from India. *Livestock Research for Rural Development*, 17(6): 70.

Costa, L.B., Luciano, F.B., Miyada, V.S. and Gois, F.D., 2013. Herbal extracts and organic acids as natural feed additives in pig diets. *South African Journal of Animal Science*, 43(2): 181-193.

Dal Bosco, A., Mugnai, C., Ruggeri, S., Mattioli, S. and Castellini, C. 2012. Fatty acid composition of meat and estimated indices of lipid metabolism in different poultry genotypes reared under organic system. *Poultry Science*, 91(8): 2039-2045.

Dana, N., Van der Waaij, L.H., Dessie, T. and Van Arendonk, J.A. 2010. Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources. *Tropical Animal Health and Production*, 42(7): 1519-1529.

Debut, M., Berri, C., Arnould, C., Guemene, D., Sante-Lhoutellier, V. Sellier, N., Baéza, E., Jehl, N., Jégo, Y., Beaumont, C. and Le Bihan-Duval, E., 2005. Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute heat stress. *British Poultry Science*, 46(5): 527-535.

Dehghani-Tafti, N. and Jahanian, R. 2016. Effect of supplemental organic acids on performance, carcass characteristics, and serum biochemical metabolites in broilers fed diets containing different crude protein levels. *Animal Feed Science and Technology* 211: 109-116.

Del R'io, E., M. Panizo-Mor'an, M., Prieto, C., Alonso-Calleja. and Capita, R. 2007. Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. *International Journal of Food Microbiology*, 115: 268-280.

Demographia World Urban Areas. 2022. 18th Annual Edition. New York: Brooklyn, Lower Manhattan and Jersey City.

Denli, M., Okan, F. and Celik, K. 2003. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition*, 2: 89-91.

Dittoe, D.K., Ricke, S.C. and Kiess, A.S. 2018. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. *Frontiers in Veterinary Science* 5: 216.

Economou, V. and Gousia, P. 2015. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, 8: 49–61.

ELnaggar, A. As. and Abo EL-Maaty H. M. A. 2017. Impact of using organic acids on growth performance, blood biochemical and hematological traits and immune response of ducks (*Cairina moschata*). *Egyptian Poultry Science*, 37: 907-925.

Evaris, E.F., Franco, L.S. and Castro, C.S., 2019. Slow-growing male chickens fit poultry production systems with outdoor access. *World's Poultry Science Journal*, 75(3): 429-444.

Fanatico, A. C., Owens, C. M. and Emmert, J. L. 2009. Organic poultry production in the United States: Broilers. *Journal of Applied Poultry Research*, 18(2): 355-366.

Fanatico, A.C., Cavitt, L.C., Pillai, P.B., Emmert, J.L. and Owens, C.M. 2005 a. Evaluation of slower-growing broiler genotypes grown with and without outdoor access: meat quality. *Poultry Science*, 84: 1785-1790.

FAO, 2010. Chicken genetic resources used in smallholder production systems and opportunities for their development by Sorensen, P. FAO Smallholder Poultry Production Paper NO 4. Rome.

FAO. 2015. World Agriculture: Towards 2015/2030-An FAO Perspective. <http://www.fao.org/docrep/005/y4252/y4252e05b.htm> Accessed 15 September 2017.

Fascina, V.B., Sartori, J.R., Gonzales, E., De Carvalho, F.B., De Souza, I.M.G.P., Polycarpo, G.D.V. Stradiotti, A.C. and Pelícia, V.C. 2012. Phytogetic additives and organic acids in broiler chicken diets. *Revista Brasileira de Zootecnia*, 41: 2189-2197.

Fazayeli-Rad, A.R., Nazarizadeh, H., Vakili, M., Afzali, N. and Nourmohammadi, R. 2014. Effect of citric acid performance, nutrient retention and tissue biogenic amine contents in breast and thigh meat from broiler chickens. *European Poultry Science* 78: 1-9.

Femi-Ola, T.O. and Atere, V.A. 2013. Citric acid production from brewers spent grain by *Aspergillus niger* and *Saccharomyces cerevisiae*. *International Journal of Research in BioSciences*, 2(1): 30-36.

Fletcher, D. L. 2002. Poultry meat quality. *Worlds Poultry Science Journal*, 58 (2), 131-145.

Gadzirayi, C.T., Masamha, B., Mupangwa, J.F. and Washaya, S. 2012. Performance of broiler chickens fed on mature *Moringa oleifera* leaf meal as a protein supplement to soyabean meal. *International Journal of Poultry Science*, 11(1): 5-10.

Gaya, L.D.G., Mourão, G.B., Ferraz, J.B.S., Mattos, E.C.D., Costa, A.M.M.A.D., Michelan Filho, T., Rosa, A.F., Felício, A.M. and Eler, J.P. 2011. Estimates of heritability and genetic correlations for meat quality traits in broilers. *Scientia Agricola*, 68: 620-625.

Goforth, R. L. and Goforth, C. R. 2000. Appropriate regulation of antibiotics in livestock feed. *Boston College Environmental Affairs Law Review*, 28:39.

Gollnisch, K., Wald, C. and Berk, A. 2001. The use of different essential oils in the breeding of piglets. In *6th German Society Quality Research Conference. Jena, Germany*. 259-262.

Gondwe, T.N. and Wollny, C.B.A. 2007. Local chicken production system in Malawi: Household flock structure, dynamics, management and health. *Tropical Animal Health and Production*, 39(2): 103-113.

Goraga, Z., Caron, L., Wilbert, C. and Brockmann, G.A. 2016. Characterization of village chicken production systems and challenges across agro-climatic zones in Ethiopia. *International Journal of Livestock Production*, 7: 94-105.

- Guerrero-Legarreta, I. ed. 2010. Handbook of Poultry Science and Technology. John Wiley & Sons, Inc. Hoboken, New jersey. (5): 293-327.
- Hagan, J.K. and Adjei, A.I. 2012. Evaluation of the growth and carcass yield characteristics of crossbred naked-neck and frizzle cockerel phenotypes reared under hot and humid environments. *Journal of Agricultural and Biological Science*, 7(8): 576582.
- Haider, M.M. 2014. Citric acid production from carob pod extract by *Aspergillus niger*. *Journal of Pharmacy and Biological Sciences*, 9: 112-116.
- Hamid, A. 1992. The developmental cycle of a drug epidemic: The cocaine smoking epidemic of 1981–1991. *Journal of Psychopharmacology Drugs*, 24(4): 337-48.
- Haque, M.N., Islam, K.M., Akbar, M., Chowdhury, R., Khatun, M., Karim, M. and Kempainen, B. 2010. Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. *Canadian Journal of Animal Science*, 90 (1): 57-63.
- Hassan, H.M.A., Mohamed, M.A., Youssef, A.W. and Hassan, E.R. 2010. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. *Asian-Australas Journal of Animal Science*, 10(23): 1348-1353
- Honikel, K. O. 2004. Chemical and physical characteristics of meat/pH measurement. In: *Encyclopedia of Meat Sciences*, 238-242.
- Hosseini, S.M., Milad Manafi, M. and Nazarizadeh, H. 2017. Effects of xylanase supplementation and citric acid on performance, ileal nutrients digestibility, and gene expression of intestinal nutrient transporters in broilers challenged with *Clostridium perfringens*. *Journal Poultry Science*, 54(2): 149–56
- Hudha, M.N., Ali, M.S., Azad, M.A.A., Hossain, M.M., Tanjim, M., Bormon, S.C., Rahman, M.S., Rahman, M.M. and Paul, A. K. 2010. Effect of acetic acid on growth and meat yield in broilers. *International Journal of Biological Research*, 1: 31-35.
- Hughes, J.M., Oiseth, S.K., Purslow, P.P. and Warner, R.D. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat science*, 98(3)520-532.

Husak, R.L., Sebranek, J.G. and Bregendahl, K.A. 2008. Survey of Commercially Available Broilers Marketed as Organic, Free-Range, and Conventional Broilers for Cooked Meat Yields, Meat Composition, and Relative Value. *Poultry Science*, 87: 2367-2376.

Hyun., Fu-Yi He., -Wook Kim., Ko-Eun Hwang., Dong-Heon Song., Yong-Jae Kim., Youn-Kyung Ham., Si-Young Kim., In-Jun Yeo., Tae-Jun Jung. and Cheon-Jei Kim. 2015. Effect of ginger extract and citric acid on tenderness of duck breast muscles. *Korean Journal for Food Science of Animal Resources*, 6: 35.

Isabel, B. and Santos, Y. 2009. Effect of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *Journal of Applied Poultry Research*, 18(3): 472-476.

Islam, K. M. S. 2012. Use of citric acid in broiler diets. *World's Poultry Science Journal* 68: 104-118.

Islam, M.Z, Khandaker, Z.H. and Chowdhury, S.D. 2008. Effect of citric acid and acetic acid on the performance of broilers. *Journal of Bangladesh Agricultural University*, 6(2): 315-320.

Jahanian, R. and Golshadi, M. 2015. Effect of dietary supplementation of butyric acid glycerides on performance, immunological responses, ileal microflora, and nutrient digestibility in laying hens fed different basal diets. *Livestock Science*, 178: 228-236.

Jaturasitha, S., Kayan, A. and Wicke, M. 2008a. Carcass and meat characteristics of male chickens between Thai indigenous compared with improved layer breeds and their crossbred. *Archives Animal Breeding*, 51(3): 283-294.

Jaturasitha, S., Srikanchai, T., Kreuzer, M. and Wicke, M. 2008b. Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red). *Poultry Science*, 87(1): 160-169.

Jha, A.K., Azad, Md.H., Ali, Md.S.N., Alam, P., Sheikh, N., Ali, H. and Ansari, K. 2019. Evaluation of Growth and Carcass 590 Characteristics of Broiler Chickens (Cobb 500) Feed on Different Level of Organic Acids Inclusion in Diet. *Parwanipur Nepalese Veterinary Journal*, 3: 137–147,

- Ji-Han Kim., Go-Eun Hong., Ki-Won Lim., Woojoon Park. and Chi-Ho Lee. 2015. Influence of Citric Acid on the Pink Color and Characteristics of Sous Vide Processed Chicken Breasts During Chill Storage. *Korean Journal of Food Science* 35(5): 585-596.
- Joo, S. T.; Kim, G. D.; Hwang, Y. H.; Ryu, Y. C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science*, 95, 828-836.
- Kahraman, T., Bayraktaroglu, A.G., Issa, G., Ertugrul, T., Bingol, E.B. and Ergun, L. 2012. Effects of Temperature Conditioning and Citrus Juice Marinade on Quality and Microstructure of Aged Beef. *Journal of Food Agriculture and Environment*, 10 (1): 117–122.
- Kaminski, N.A. and Wong, E.A. 2017. Differential mRNA expression of nutrient transporters in male and female chickens. *Journal of Poultry Science* 97: 313-318.
- Ke, S., Huang, Y., Decker, E. A. and Hultin, H. O. 2017. Impact of citric acid on the tenderness, microstructure, and oxidative stability of beef muscle. *Meat Science* 82:113-118.
- Ke, S., Hung, Y., Decker, E.A. and Hultin, H.O. 2009. Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science*, 82: 113-118.
- Kerth, C.R., 2013a. *The science of meat quality*. Blackwell Publishing. 99-118.
- Kim, Y. H. B.; Warner, R. D.; Rosenvold, K. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. *Animal Production Science*, 54, 375-395.
- Klinhom, P., Klinhom, J., Senapa, J. and Methawiwat, S. 2011. "Influence of Citric Acid, Lemon and Porcupine Fruit Juice on the Tenderness and Sensory Properties of Bovine Meat." In Proceedings of the 49th Kasetsart University Annual Conference, Kasetsart University, Kasetsart, Thailand, February 1–4. 4. Subject: Agricultural Extension and Home Economics, 1–10.
- Kopecky, J., Hrnčar, C. and Weis, J. 2012. Effect of organic acids supplement on performance of broiler chickens. *Animal Science Biotechnology*, 45(1): 51-54.

- Kumar, S. and Aalbersberg, B. 2006. Nutrient retention in foods after earth-oven cooking compared to other forms of domestic cooking 1. Proximates carbohydrates and dietary fibre. *Journal of Food Composition and Analysis*, 19(4): 302-310.
- Kumar, Y., Singh, P., Tanwar, V.K., Ponnusamy, P., Singh, P.K and Shukla, P. 2015. "Augmentation of Quality Attributes of Chicken Tikka Prepared from Spent Hen Meat with Lemon Juice and Ginger Extract Marination." *Nutrition and Food Science*, 45 (4): 606-615.
- Lang hout, P. 2000. New additives for broiler chickens. *World's Poultry*, 16(3): 22-27.
- Lawrie, R.A. and Ledward, D.A., 2014. *Lawrie's meat science*. Woodhead Publishing.
- Le Bihan-Duval, E., Debut, M., Berri, C.M., Sellier, N., Santé-Lhoutellier, V., Jégo, Y. and Beaumont, C. 2008. Chicken meat quality: Genetic variability and relationship with growth and muscle characteristics. *BMC Genetics*, 9: 53.
- Lonergan, S.M., Deeb, N., Fedlet, C.A. and Lamont, S.J. 2003. Breast meat quality and composition in unique chicken populations. *Poultry Science*, 82: 1990-1994.
- Long, S.F., Xu, Y.T., Pan, L., Wang, Q.Q., Wang, C.L., Wu, J.Y., Wu, Y.Y., Han, Y.M., Yun, C.H. and Piao, X.S. 2018. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Animal Feed Science and Technology* 235: 23-32.
- Lund, M.N., Lametsch, R., Hviid, M.S., Jensen, O.N. and Skibsted, L.H. 2007. High oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science*, 77: 295-303.
- Lyon, B. G.; Lyon, C. E.; Meullenet, J. F.; Lee, Y. S. 2010. Meat quality: Sensory and instrumental evaluations. *Poultry Meat Processing*, 125-155.
- Madruga, M. S.; Torres, T. S.; Carvalh, F. F.; Queiroga, R. C.; Narain, N.; Garruti, D. M.; Souza Neto, M. A.; Mattos Carla, W.; Costa, R. G. 2009. Meat quality of Moxoto and Canide goats as affected by two levels of feeding. *Meat Science*. 30, 149-154.
- Magothe, T.M., Okeno, T.O., Muhuyi, W.B. and Kahi, A.K. 2012. Indigenous chicken production in Kenya: I. Current status. *World's Poultry Science Journal*, 68(1): 119-132.

- Majumder, L., Khalil, I., Munshi, M.K., Alam, K., Rashid, H.O., Begum, R. and Alam, N. 2010. Citric acid production by *Aspergillus niger* using molasses and pumpkin as substrates. *European Journal of Biological Sciences*, 2(1): 01-08.
- Makut, M.D. and Ekeleme, I.K. 2018. Citric acid production by *Aspergillus niger* and *Trichoderma viride* using hydrolysed potato peels substrate. *Asian Journal of Advances in Agricultural Research*, 5(1): 1-7.
- Manyelo, T.G., Selaledi, L., Hassan, Z.M. and Mabelebele, M. 2020. Local chicken breed of Africa: Their description, uses and conservation methods. *Animals*, 10(12): 2257.
- Marcinkowska-Lesiak, M., Zdanowska-Sąsiadek, Ż., Stelmasiak, A., Damaziak, K., Michalczyk, M., Poławska, E., Wyrwicz, J. and Wierzbicka, A. 2016. Effect of packaging method and cold-storage time on chicken meat quality. *CyTA-Journal of Food*, 14(1): 41-46.
- Marić, M., Stajčić, I., Prodanović, R., Nikolova, N., Lika, E. and Puvača, N. 2021. Chili Pepper and its Influence on Productive Results and Health Parameters of Broiler Chickens. *Journal of Agronomy, Technology and Engineering Management*, 4(1): 540546.
- Martin, H., Frands, D. and Robyn, A. 2012. Products and profit from poultry 3. FAO Diversification. Rome.
- Martin, J.E. 2015. Humane mechanical methods to kill poultry on-farm. (Doctoral dissertation, University of Glasgow).
- Martin, J.E., McKeegan, D.E.F., Sparrey, J. and Sandilands, V. 2016. Comparison of novel mechanical cervical dislocation and a modified captive bolt for on-farm killing of poultry on behavioural reflex responses and anatomical pathology. *Animal Welfare*, 25576(2): 227-241.
- Mason, C., Spence, J., Bilbe, L., Hughes, T. and Kirkwood, J. 2009. Methods for dispatching backyard poultry. *Veterinary Record*, 164:220.
- Max, B., Salgado, J.M., Rodríguez, N., Cortés, S., Converti, A. and Domínguez, J.M., 2010. Biotechnological production of citric acid. *Brazilian journal of Microbiology*, 41: 862-875.

- McCormick, R. J. ed., 2009. *Applied muscle biology and meat science*. CRC Press.
- McDonald, P., Edwards, R.A, Greenhalgh, J.F.D. and Morgan, C.A. 2010. *Animal Nutrition*. Pearson Education Limited, sixth edition, London.
- McMahon, S.J. 2018. The linear quadratic model: usage, interpretation, and challenges. *Physics in Medicine and Biology*, 64(1): 1.
- Mehaffey, J.M., Pradhan, S.P., Meullenet, J.F., Emmert, J.L. McKee, S.R. and Owens, C. M. 2006. Meat quality evaluation of minimally aged broiler breast fillets from five commercial genetic strains. *Poultry Science*, 85: 902-908.
- Melesse, A. 2014. Significance of scavenging chicken production in the rural community of Africa for enhanced food security. *World's Poultry Science Journal*, 70(3): 593-606.
- Mellor, S. 2000. Nutraceuticals-alternatives to antibiotics. *World's Poultry Science*, 16(2): 30-33.
- Meltem, S., Kyialbek, A. and Alper, O. 2007. The effects of marinating with citric acid solutions and grape fruit juice on cooking and eating quality of Turkey breast. *Journal of Muscle Foods*, 18: 162- 172.
- Meltem, S., Kyialbek, A. and Alper, O. 2017. The effects of marinating with citric acid solutions and grape fruit juice on cooking and eating quality of Turkey breast. *Journal of Muscle Foods*, 18: 162-172.
- Menconi, A., Kuttappan, V.A., Hernandez-Velasco, X., Urbano, T., Matté, F., Layton, S., Kallapura, G., Latorre, J., Morales, B.E., Prado, O., Vicente, J.L., Barton, J., Andreatti Filho, R.L., Lovato, M., Hargis, B.M. and Tellez, G. 2013. Evaluation of a commercially available organic acid product on body weight loss, carcass yield, and meat quality during preslaughter feed withdrawal in broiler chickens: A poultry welfare and economic perspective. *Journal of Poultry Science* 2: 448-455.
- Mengesha, M. 2006. Survey on village chicken production under traditional management system in Jimmy Woreda, South Wollo, Ethiopia (Doctoral dissertation, Haramaya University).
- Missohou, A., Dieye, P.N. and Talaki, E. 2002. Rural poultry production and productivity in Southern Senegal. *Livestock Research for Rural Development*, 14(2).

Mngonyama, M.B.A., 2012. Morphometric characteristics and consumer acceptability of meat from Potchefstroom Koekoek, Black Australorp, Venda and Ovambo chickens (Doctoral dissertation, University of KwaZulu-Natal).

Mogesse, H.H., 2007a. Phenotypic and genetic characterization of indigenous chicken populations in Northwest Ethiopia (Doctoral dissertation, University of the Free State).

Mtileni

Mtileni, B.J., Muchadeyi, F.C., Maiwashe, A., Groeneveld, E., Groeneveld, L.F., Dzama, K. and Weigend, S., 2011. Genetic diversity and conservation of South African indigenous chicken populations. *Journal of Animal Breeding and Genetics*, 128(3): 209-218.

Mtileni, B.J., Muchadeyi, F.C., Maiwashe, A., Phitsane, P.M., Halimani, T.E., Chimonyo, M. and Dzama, K. 2009. Characterisation of production systems for indigenous chicken genetic resources of South Africa. *Applied Animal Husbandry and Rural Development*, 2(1): 18-22.

Muaz, K., Riaz, M., Akhtar, S., Park, S. and Ismail, A. 2018. Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: a review. *Journal of Food Protection*, 81: 619-627.

Muchadeyi, F.C., Sibanda, S., Kusina, N.T., Kusina, J. and Makuza, S. 2004. The village chicken production system in Rushinga District of Zimbabwe. *Livestock Research for Rural Development*, 16(6).

Muchadeyi, F.C., Wollny, C.B.A., Eding, H., Weigend, S., Makuza, S.M. and Simianer, H. 2007a. Variation in village chicken production systems among agro-ecological zones of Zimbabwe. *Tropical Animal Health and Production*, 39(6): 453-461.

Muchenje, V., Dzama K., Chimonyo, M., Strydom, P.E., Hugo, A. and Raats, J.G. 2009. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry*, 112: 279 - 289.

Nadeem, A.; Syed, Q.; Baig, S.; Irfan, M.; Nadeem, M. 2010. Enhanced production of citric acid by *Aspergillus niger* M-101 using lower alcohols. *Turkish Journal of Biochemistry*, 35: 7-13.

- Ncube, S., Halimani, T.E., Chikosi, E.V.I. and Saidi, P.T. 2018. Effect of *Acacia angustissima* leaf meal on performance, yield of carcass components and meat quality of broilers. *South African Journal of Animal Science*, 48(2): 271-283.
- Nezhad, Y.E., Shivazad, M., Nazeeradi, M. and Babak, M.M.S. 2007. Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. *Journal of the Faculty of Veterinary Medicine*, 61: 407-413.
- Niknafs, S. and Roura, E., 2018. Nutrient sensing, taste and feed intake in avian species. *Nutrition Research Reviews*, 31(2), pp.256-266.
- Nissen, P.M. and Young, J.F. 2006. Creatine monohydrate and glucose supplementation to slow- and fast-growing chickens changes the post mortem pH in *pectoralis major*. *Poultry Science*, 85, 1038–1044.
- Norris, D., Ngambi, J.W., Benyi, K., Makgahlele, M.L., Shimelis, H.A. and Nesamvuni, E.A. 2007. Analysis of growth curves of indigenous male Venda and Naked Neck chickens. *South African Journal of Animal Science*, 37(1): 21-26.
- Nourmohammadi, R and Khosravinia, H. 2015. Acidic stress caused by dietary administration of citric acid in broiler chickens. *Archives Animal Breeding*, 58: 309-315.
- Nourmohammadi, R., Hosseini, S.M. and Farhangfar, H. 2010. Effect of Dietary acidification on some blood parameters and weekly performance of broiler chickens. *Journal Animal and Veterinary Advance*, 9: 3092-3097.
- Nournohammadi, R. and Afzali, N. 2013. Effect of citric acid and microbial phytase on small intestinal morphology in broiler chickens. *Italian Journal of Animal Science*, 12: 44-47.
- Novakovi, S. and Tomaševi, I. 2017. A comparison between Warner-Bratzler shear force measurement and texture profile analysis of meat and meat products: a review. *IOP Conference Series: Earth and Environmental Science* 85: 1-7.
- NRC. 1994. Nutrient Requirements of Poultry. 9th Revised Edition. National Academy Press: Washington, DC.

Obuz, E. and Dikeman, M.E. 2003. Effects of cooking beef muscles from frozen of thawed states on cooking traits and palatability. *Meat Science*, 65: 993-997.

Odetola, O.M. and Adetola, O. O. 2022. Growth response, carcass analysis and sensory evaluation of meat from broiler chicken served acidified water. *Nigerian Journal of Animal Production*, 49(1): 284-292.

Official Methods of Analysis. 2005.17th edition. AOAC International, Rockville, MD, Method 977-1.

Okeudo, N. J. and Moss, B. W. 2005. Interrelationships amongst carcass and meat quality characteristics of sheep. *Meat Science*, 69: 1-8.

Olwande, P.O., Ogara, W.O., Okuthe, S.O., Muchemi, G., Okothi, E., Odindo, M.O. and Adhiambo, R.F. 2010. Assessing the productivity of indigenous chickens in an extensive management system in Southern Nyanza. *Kenya Tropical Animal Health and Production*, 42: 283-288.

Önenç, A., Serdaroglu, M. and Abdraimov, K. .2004. Effect of various additives to marinating baths on some properties of cattle meat. *European Food Research and Technology*, 218: 114–117.

Owens, C.M., Alvarado, C.Z. and Sams, A.R. eds. 2010. Poultry Meat Processing. CRC Press. Boca Raton.

Owens, C.M., Hirschler, E.M., McKee, S.R., Martinez-Dawson, R. and Sams, A.R. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. *Poultry Science*, 79(4): 553-558.

Papagianni, M. 2007. Advances in citric acid fermentation by *Aspergillus niger*: biochemical aspects, membrane transport and modeling. *Biotechnology Advances*, 25(3): 244-263.

Papatsiros, V.G., Katsoulos, P.D., Koutoulis, K.C., Karatzia, M., Dedousi, A. and Christodouloupoulos, G. 2013. Alternatives to antibiotics for farm animals. *CAB Reviews*, 8: 1-15.

Papatsiros, V.G., Katsoulos, P.D., Koutoulis, K.C., Karatzia, M., Dedousi, A. and Christodouloupoulos, G. 2013. Alternatives to antibiotics for farm animals. *CAB Reviews* 8: 1-15.

- Pawar, V.A. and Pawar, P.R. 2014. Effect of various parameters on citric acid production using different substrates. *Indian Journal of Research*, 3(7): 13-15.
- PinWu, C. and Chen, L. K. 2016. Effect of dietary citric acid, fumaric acid or sodium bicarbonate on growth performance and bone characteristics in male turkey poultry. *Journal of the Chinese Society of Animal Science*, 45: 187-196.
- Pirgozliev, V., Muohy, T.C., Owens, B., George, J. and McCann, M.E.E. 2008. Fumaric and sorbic acids as additives in broiler feed. *Research in Veterinary Science*, 84: 387–394.
- Poltowicz, K. and Doktor, J., 2011. Effect of free-range raising on performance, carcass attributes and meat quality of broiler chicken. *Animal Science Papers & Reports*, 29(2).
- Ponte, P. I. P.; Alves, S. P.; Bessa, R. J. B.; Ferreira, L. M. A.; Gama, L.T.; Bra`s, J. L. A.; Fontes, C. M.; Prates J.A.M. 2008. Influence of pasture intake on the fatty acid composition, and cholesterol, tocopherols, and tocotrienols content in meat from freerange broilers. *Poultry Science*, 87: 80–88.
- Powell, T.H., Dikeman, M.E. and Hunt, M.C. 2000. Tenderness and collagen composition of beef semitendinosus roasts cooked by conventional convective cooking and modelled, multistage, convective cooking. *Meat Science*, 55: 421-425.
- Prasad, M.P.D., Sridevi, V., Surendra babu, N.V., Reddy, O.V.S., Harsha, N. 2013. Studies on fermentative production of citric acid by *Aspergillus niger* isolate using sorghum malt and its optimization. *International Journal of Innovative Research in Technology, Science and Engineering*, 2: 2961-2968.
- Puchala, M., Krawczyk, J. Sokolowicz, Z. and Utnik-Banas, K. 2015. Effect of breed and production system on physicochemical characteristics of meat from multi-purpose hens. *Annals of Animal Science*, 15(1): 247.
- Rafacz-Livingston, K., Martinez-Amezcuca, C., Parsons, C., Baker, D. and Snow, J. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chicks. *Poultry Science*, 84: 1370-1375.
- Rao, M.V., Gault, N.F. and Kennedy, S. 1989a. Variations in water-holding capacity due to changes in the fibre diameter, sarco-mere length and connective tissue

morphology of some beef muscles underacidic conditions below the ultimate pH. *Meat Science*, (26): 19-37.

Raphulu, T., Jansen van Rensburg, C. and Van Ryssen, J.B.J. 2015. Assessing nutrient adequacy from the crop contents of free-ranging indigenous chickens in rural villages of the Venda region of South Africa. *South African Journal of Animal Science*, 45(2): 143-152.

Rio D. E., Muriente R., Prieto M., Alonso-calleja C., Capite R. 2007. Effectiveness of trisodiumphosphate, acidified sodium chlorite, citric acid and peroxy acids against pathogenic bacteria on poultry during refrigerated storage. *Journal of Food Protection*, 70: 2063–2071.

Robinson, T. P.; Wint, G. R. W.; Conchedda, G.; Van Boeckel, T.; Mcleod, M.; Bett, B.; Grace, D.; Gilbert, M. The global livestock sector: Trends, drivers, and implications for society, health and the environment. In proceedings of Science with Impact Annual Conference, University of Chester, Chester, United Kingdom. 4-15.

Saki, A. and Eftekhari, M., 2012. Effect of organic acids mixture and two methionine sources on performance, carcass characteristics and gastrointestinal tract in broiler. *Iranian Journal of Animal Science*, 43(1): 41-49.

Salgado-Tránsito, L., Del Río-García, J. C., Arjona-Román, J. L., Moreno-Martínez, E. and Méndez-Albores, A. 2011. Effect of citric acid supplemented diets on aflatoxin degradation, growth performance and serum parameters in broiler chickens. *Archivos De Medicina Veterinaria*, 43, 215-222.

Salgado-Tránsito, L., Del Río-García, J.C., Arjona-Román, J.L., Moreno-Martínez, E. and Méndez-Albores, A. 2012. Effect of citric acid supplemented diets on aflatoxin degradation, growth performance and serum parameters in broiler chickens. *Archivos de Medicina Veterinaria*, 43 (3): 215-222.

Salum, M. R., A. Mtambuki, and R. C. T. Mulangila. 2002. "Designing a vaccination regime to control Newcastle disease in village chickens in the Southern zone of Tanzania." In *Proceedings of the joint 17th Scientific Conference of the Tanzania Society for Animal Production and the 20th Scientific Conference of the Tanzania Veterinary Association held in Arusha, Tanzania on 3rd to 5th December*, 299-305.

- Sammel, L.M. and Claus, J.R. 2003. Citric acid and Sodium Citrate Effects on Reducing Pink Colour Defect of Cooked Intact Turkey Breast and Ground Turkey Rolls. *Journal of Food Science*, 68: 874-878.
- Sammel, L.M. and Claus, J.R. 2006. Citric acid and sodium citrate effects on pink colour development of cooked turkey irradiated pre and post-cooking. *Meat Science*, 72: 567-573.
- Sams, A.R. and Owens, C.M. 2010. Second processing; Parts, deboning, and portion control. *Poultry Meat Processing*, 51-65. CRC Press.
- Sanka, Y.D. and Mbagi, S.H. 2014. Evaluation of Tanzanian local chicken reared under intensive and semi-intensive systems: I. Growth performance and carcass characteristics. *Livestock Research for Rural Development*, 26(7): 127.
- SAS. 2008. SAS User's Guide Version 9.2 2nd Edition. SAS Institute, Inc. Raleigh, North
- Serdaroglu, M., Abdraimov, K. and Onenc, A. 2007. The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of turkey breast. *Journal of Muscle Foods*, 18: 162-172.
- Sharma, B. 2010. Poultry production, management and bio-security measures. *Journal of Agriculture and Environment*, 11: 120-125.
- Sheng, Z., Pettersson, M.E., Hu, X., Luo, C., Qu, H., Shu, D., Shen, X., Carlborg, Ö., and Li, N. 2013. Genetic dissection of growth traits in a Chinese indigenousxcommercial broiler chicken cross. *Biomed central Genome*, 14(1): 151.
- Shung, C.C., Hsin, K.Y., Tan, F.J. and Chen, S.E. 2022. Effects of hand and soft scalding on defeathering and carcass quality of different breeds of chickens. *Animals*, 12: 3145.
- Sirri, F., Castellini, C., Bianchi, M., Petracci, M., Meluzzi, A. and Franchini, A. 2011. Effect of fast-, medium-and slow-growing strains on meat quality of chickens reared under the organic farming method. *Animal*, 5(2): 312-319.
- Snow, J., Baker, D. and Parsons, C. 2004. Phytase, citric acid, and 1 α hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a cornsoybean meal diet. *Poultry Science*, 83: 1187-1192.

Sorvari, R., Kiviranta, I. and Luoma, H. 2010. Erosive effect of a sport drink mixture with and without addition of fluoride and magnesium on the molar teeth of rats, *European Journal of Oral Sciences*, 96(3): 226-231.

Sossidou, E.N., Dal Bosco, A., Castellini, C. and Grashorn, M.A. 2015. Effects of pasture management on poultry welfare and meat quality in organic poultry production systems. *World's Poultry Science Journal*, 71(2): 375-384.

Stipkovits L, Csiba E, Laber G and Bruch DGS (1992), Simultaneous treatment of chickens with salinomycin and tiamutin in feed. *Avian Diseases*, 36, 11-16.

Swain, M.R., Ray, R.C. and Patra, J.K., 2011. Citric acid: microbial production and applications in food and pharmaceutical industries. *Citric Acid: Synthesis, Properties and Applications, Edition, 1*: 97-118.

Swatson, H.K. 2003. The small holder poultry development workshop: The Potential of smallholder indigenous poultry production in improving the livelihoods and food security of rural households. Proceedings of Nature and Development Group of Africa in Action for Sustainable Rural Development, 28-31.

Taherpour, K., Moravej, H., Shivazad, M., Adibmoradi, M. and Yakhchali, B. 2009. Effects of dietary probiotic, prebiotic and butyric acid glycerides on performance and serum composition in broiler chickens. *African Journal of Biotechnology*, 8(10).

Thornton, P.K. 2010. Livestock production: Recent trends future prospects, *Philosophical Transactions of the Royal Society*, 365: 2853-2867.

Tong, H.B., Cai, J., Lu, J., Wang, Q., Shao, D. and Zou, J.M. 2015. Effects of outdoor access days on growth performance, carcass yield, meat quality, and lymphoid organ index of a local chicken breed. *Poultry Science*, 94(6): 1115-1121.

Torrado, A.M., Cortés, S., Salgado, J.M., Max, B., Rodríguez, N., Bibbins, B.P., Converti, A. and Domínguez, J.M. 2011. Citric acid production from orange peel wastes by solid-state fermentation. *Brazilian Journal of Microbiology*, 42: 394-409.

Tsiloyiannis, V.K., Kyriakis, S.C., Vlemmas, J. and Sarris, K. 2001a. The effect of organic acids on the control of post-weaning oedema disease of piglets. *Research in Veterinary Science*, 70(3): 281-285.

- Unal, K., Alagöz, E., Çelik, İ. and Sarıçoban, C. 2022. Marination with citric acid, lemon, and grapefruit affects the sensory, textural, and microstructure characteristics of poultry meat. *British Poultry Science*, 63: 31-38.
- Van Marle-Koster, E. and Nel, L.H. 2000. Genetic characterization of native southern African chicken populations: evaluation and selection of polymorphic microsatellite markers. *South African Journal of Animal Science*, 30(1): 1-6.
- Van Marle-Köster, E., Hefer, C.A., Nel, L.H. and Groenen, M.A.M. 2008. Genetic diversity and population structure of locally adapted South African chicken lines: Implications for conservation. *South African Journal of Animal Science*, 38(4): 271-281.
- Van Marle-Köster, E.; Webb, E. C. 2006. Carcass characteristics of South African native chicken lines. *South African Journal of Animal Science*, 30(1), 53-56.
- Walley, K., Parrot, P., Custance, P., Meledo-Abraham, P. and Bourdin, A. 2015. A review of French consumers purchasing patterns, perceptions and decision factors for poultry meat. *World Poultry Science Journal*, 71: 5-14.
- Wang, K. H.; Shi, S. R., Dou, T. C.; Sun, H. J. 2009, Effect of a free-raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. *Poultry Science*, 88: 2219-2223.
- Warriss, P.D., 2010. Meat Science—An Introductory Text. 2nd ed CABI. *Cambridge, UK*.
- Wattanachant, S. 2008. Factors affecting the quality characteristics of Thai indigenous chicken meat. Suranaree, *Journal of Science and Technology*, 15(4): 317-322.
- Weather Service. 2015. South African.
- Weaver, A.D., Bowker, B.C. and Gerrard, D.E. 2009. Sarcomere length influences ucalpain mediated proteolysis of bovine myofibrils. *Journal of Animal Science*, 87: 2096-2103.
- Wickramasinghe, K. P., Atapatta, N. S. B. N. and Seresinhe, R. T. 2014. Effects of citric acid on growth performance and nutrient retention of broiler chicken fed diets having two levels of non-phytate phosphorus and rice bran. *Iranian Journal of Applied Animal Science*, 809-815.

- Xiong, Y. L., Park, D. and Ooizumi, T. 2009. Variation in the cross-linking pattern of porcine myofibrillar protein exposed to three oxidative environments. *Journal of Agriculture and Food Chemistry*, 57: 153- 159.
- Yadegary, M., Hamidi, A., Alavi, S.A., Khodaverdi, E., Yahaghi, H., Sattari, S., Bagherpour, G. and Yahaghi, E. 2013. Citric acid production from sugarcane bagasse through solid state fermentation method using *Aspergillus niger* mold and optimization of citric acid production by Taguchi method. *Jundishapur Journal of Microbiology*, 6(9).
- Yamane, T. 1967. *Statistics, An Introductory Analysis*, 2nd Edition. New York: Harper and Row.
- Yang, C.C. and Chen, T.C. 1993. Effects of refrigerated storage, pH adjustment, and marinade on color of raw and microwave cooked chicken meat. *Poultry Science*, 72: 355-362.
- Yang, X., Zhang B., Guo Y. Jiao P. and Long, F. 2010. Effects of dietary lipids and clostridium butyricum on fat deposition and meat quality of broiler chickens. *Poultry Science*, 89(2): 254-260.
- Young, L.L., Northcutt, J.K., Buhr, R.J., Lyon, C.E. and Ware, G.O. 2001. Effects of age, sex, and duration of postmortem aging on percentage yield of parts from broiler chicken carcasses. *Poultry Science*, 80(3): 76-379.
- Yu, L.H., Lee, E.S., Jeong, J.,Y., Paik, H.D., Choi, J.H. and Kim, C.J. 2005. Effects of thawing temperature on the physicochemical properties of prerigor frozen chicken breast and leg muscles. *Meat Science*, 71: 375–382.
- Yusop, S. M., Kerry, J. F. and Kerry, J. P. 2010. Effect of marinating time and low pH on marinade performance and sensory acceptability of poultry meat. *Meat Science*, 85: 657-663.
- Zhao, J.P., Zhao, G.P., Jiang, R.R., Zheng, M.Q., Chen, J.L., Liu, R.R. and Wen, J.,2012. Effects of diet-induced differences in growth rate on metabolic, histological, and meat-quality properties of 2 muscles in male chickens of 2 distinct broiler breeds. *Poultry Science*, 91(1): 237-247.