# EFFECTS OF CITRIC ACID SUPPLEMENTATION IN DIET ON GROWTH PERFORMANCE AND GUT MORPHOLOGY OF MALE VENDA CHICKENS

by

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**THESIS**

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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 previously been submitted by me for a degree at this or any other university, this is my own work in design and executive, and that all materials contained herein has been duly acknowledged.

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To my family, thank you for your encouragement and prayers.
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## DEDICATIONS

This study is dedicated to my twin brother (Lehumo), Mama and Ntata, and I extend my dedications to my nieces (Botshelo, Bohlale, Bonolo, Boikgancho, and Boikanyo) and nephews (Oarabile, and Morero), may the good Lord protect and bless you. I love you.

## ABSTRACT

Two trials were carried out to see how citric acid supplementation affected the growth performance and gut morphology of male Venda chickens aged one to 90 days. The first trial looked at how citric acid supplementation affected feed intake and growth rate, feed conversion ratio, and live weight in male Venda chickens. A total of 200 male Venda chickens, each with ten chicks, were randomly separated into 20 equal pens. Diets were formulated to contain four different levels of citric acid (CA), $CA_1$ (0 g of CA /kg DM), $CA_2$ (12.5 g of CA/kg of DM), $CA_3$ (25 g of CA/kg DM) and $CA_4$ (50 g of CA/kg DM) and were allocated to the chicks. Each treatment catered for five constant pens for a period of 90 days. A maize-soya bean meal-based diet was formulated to meet the nutritional requirements of male Venda chickens. The diets were iso-energetic and iso-nitrogenous, 12.14 MJ ME/kg DM and 180 g CP/kg DM, respectively. The second experiment commenced from the first experiment at day 90, with the same 200 chickens as no mortality recorded. The second experiment the effect of citric acid supplementation on gut digesta organ weights, crop and gizzard pH, and intestine length. Data on male Venda chickens' live weight, feed intake, average daily body weight gain, feed conversion ratio, gut digesta weights, pH of crops, and gizzards were analysed using one-way ANOVA and the General Linear Model procedure of the Statistical Analysis System (version 9.2). Tukey HSD test was used to separate the significant differences between treatment means at $P \leq 0.05$. Citric acid supplementation in a starter diet did not significantly improve ($P$ >0.05) feed intake, feed conversion ratio, live weight, and growth rate of male Venda chicks. Citric acid supplementation levels of 0, 12.5, 25, and 50g of CA per kg DM of the diet were calculated, using quadratic equations, to result in optimal feed intake, feed conversion ratio, live weight at 30 days and growth rate, respectively, of male Venda chickens aged one to 30 days. In conclusion, the lack of impact on feed intake, feed conversion ratio, live weight, and growth rate could be caused by a sour taste diet brought on by citric acid supplementation levels because the chicks during this were still young and more sensitive to sourness, resulting in poor feed intake and poor growth performance.

Feed intake, FCR, live weight was much improved ($P$ <0.05) with the supplementation of 12.5 or 25g of CA per kg DM compared to control and 50g of CA per kg DM during grower and finisher phase (31 to 90 days). The pH values of crop and gizzard greatly decreased ($P$ <0.05) with the increasing levels of citric acid supplementation. The lower pH in both crop and gizzard observed in diet supplemented with 50g of CA/kg DM and higher pH value in crop and gizzard observed in control diet, 25g of CA/kg DM in diet recorded to have second lowest value of pH in crop and gizzard and 12.5g of CA/kg DM in diet recorded to have second highest pH value in crop and gizzard. Citric acid supplementation at all levels had has no effect impact (*P* >0.05) on intestinal length, weight of small intestine segments, crop, pro-gizzard, and ceca of male Venda chickens aged 90 days old. 25g of CA/kg DM supplementation improved (*P* <0.05) weight of gizzard and crop compared to others supplementation levels in male Venda chickens aged 90 days old.

The use of citric acid in the diet of male Venda chickens aged 31 to 90 days improves growth performance and lowers gut digesta pH values, rendering it hazardous to dangerous bacteria and therefore improving gut health and gut functioning. More research is needed, however, to confirm these conclusions.

**Keywords:** Gizzard, growth rate, gut digesta pH, indigenous chicken, organic acid.

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# ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| $^0C$ | Degree centigrade |
| ANOVA | Analysis of variance |
| AOAC | Association of Analytical Chemists |
| Ca | Calcium |
| CA | Citric acid |
| Cm | Centimetre |
| CP | Crude protein |
| DM | Dry matter |
| LWG | Live weight gain |
| FCR | Feed conversion ratio |
| G | Gram |
| GIT | Gastrointestinal tract |
| Kg | Kilograms |
| ME Kcal | Metabolizable energy |
| GI | Gastrointestinal |
| MJ | Mega joule |
| NRC | National Research Council |
| P | Phosphorous |
| pH | Potential of Hydrogen |
| $r^2$ | Coefficient of determination |
| SAS | Statistical Analysis System |
| SE | Standard error |

| | |
|---|---|
| FI | Feed Intake |
| Mm | Milli metre |
| P | Chlorine |
| Na | Sodium |
| SS | Sum of Squares |
| MS | Mean of Squares |
| DF | Degree of Freedom |
| GLM | General Linear Model |
| Meth | Methionine |
| *E. coli* | *Escherichia coli* |
| $^{a,b,c,d}$ | Superscripts |

# CHAPTER ONE

# INTRODUCTION

# INTRODUCTION

## 1.1 Background

Indigenous chickens account for approximately 85% of overall poultry output (Alabi, 2013). The rural areas of Venda, Vhembe District, Limpopo Province, have a large population of chickens that forage for food (Raphulu and Jansen van Rensburg, 2018). According to Mosisi (2009), indigenous chickens are practically raised by every rural household in low-income, food-deficit regions of the world, but their contribution to food security at both the household and national levels is unknown. Furthermore, Nduthu (2013) concluded that these chickens are regularly kept among other domestic animals. Despite their enormous population in rural areas, their output is low due to a high mortality rate, particularly among chicks (Bett et al., 2013).

Every animal's growing performance is given a lot of attention, thus is carefully planned because it is viewed as an economically important attribute (Gong et al., 2022). Animals with good growth performance are typically viewed as healthy, suitable for breeding, and in great demand in the meat sector (Ajayi et al., 2009). In comparison to other commercial chickens, indigenous chickens are reported to have stunted growth (Deng et al., 2022). Venda chickens are also known for their slow growth rate and late maturity (Norris et al., 2007).

According to Nourmohammadi et al. (2011), a contemporary problem in poultry production is the use of specialized dietary supplements in indigenous chicken to increase bird performance. After animal nutritionists banned the use of antibiotics such as a growth enhancer in livestock production, researchers discovered a substitute that boosts bird performance (Oluwafemi et al., 2020). Citric acid (CA) is an organic acid that is extensively used in the poultry industry to stimulate growth by acidulating the gastrointestinal tract (GIT) (Niaz et al., 2022). Citric acid is a recently discovered organic acid that appears to be regulated by diet makeup (Nourmohammadi and Khosravinia 2015 and AL-Harthi and Attia, 2016). Citric acid, according to Islam et al. (2008), alters intestinal pH and increases the activity of several enzymes that require acidic conditions, such as pepsin and phytase. Citric acid is also used in poultry diets due to its positive influence on intestinal health through modulating internal bacteria. The use of organic acids such as citric acid in poultry production improves nutrient use, growth, and feed efficiency (Denli et al., 2003). According to Chowdhury et al. (2009), the usage of citric acid creates an acidic environment (pH 3.5 to 4.0) in the gut that is favourable for the presentation of appropriate bacteria and the repression of unwanted bacteria, as well as protecting the young chicks through competitive exclusion (La Ragione, and Woodward, 2003).

Improved Venda chicken production in Venda's rural areas can boost the supply of quality protein in the form of meat and eggs to communities, resulting in the generation of family income and jobs (Manyelo et al. 2020). Because there is little knowledge on the dietary requirements of Venda chickens, nutrient supplementation solutions to help the indigenous chicken sector are needed.

As a result, the goal of this study is to investigate how citric acid supplementation in the diet affects growth performance (feed intake and growth rate, feed conversion ratio, and live weight) and gut morphology (weight and length of intestines, crop and gizzard weight, and crop and gizzard pH) in male Venda chickens.

## 1.2. PROBLEM STATEMENT

Indigenous chickens provide cash and protein to rural households all around the world (Chuma, 2016). Indigenous chickens, on the other hand, are known for their delayed growth. They do not meet the required amounts in Limpopo's rural areas (Manyelo et al., 2020). These chickens have poor reproductive success, high predation, slow development rates, mortality, and illnesses, mortality (Conroy et al., 2005). With Limpopo province's predicted significant population expansion, this will become even more crucial in the future (Delport et al., 2017). However, one method for increasing chicken growth is to provide better nutrition (Beski et al., 2015). As a result, there is a need to improve productivity of this breed.

## 1.3. RATIONALE

Citric acid supplementation in meals regulates intestinal microbial and fungal development, improve digestibility and absorption of nutrients in broiler chickens (Abdelrazek et al., 2016; Islam et al., 2018). Citric acid supplementation in feeds, according to Dittoe et al. (2018), lowers non-beneficial bacteria and fungi in the gastrointestinal tract, enhancing feed intake and digestion, and thereby improving broiler chicken’s growth performance. Citric acid addition in broiler chicken diets increased the length of the villus and the weights of the gizzard, ileum, and proventriculus, enhancing nutrient digestion and absorption, according to Nourmohammadi and Khosravinia (2015). Citric acid inclusion in broiler chicken feeds lowers the pH of the gastrointestinal tract, which improves nutritional absorption. Improved nutrient digestion and absorption lead to increased broiler chicken growth rates and carcass quality (Rahmani et al., 2005; Abdelrazek et al., 2016). Citric acid supplementation in broiler chicken diets increased the release of gastrin and cholecystokinin hormones, which aid in the digestion and absorption of protein and lipids, therefore boosting growth rates and carcass quality.

However, no information is available on the effect of citric acid supplementation on feed intake, growth rate, feed conversation ratio (FCR), live weight, and gut morphology in male Venda chickens. As a result, there is a need to evaluate the effect of citric acid supplementation on male Venda chicken development performance and gut morphology.

## 1.4 Purpose of the study

### 1.4.1 Aim

The aim of the study is to improve growth performance and gut morphology of male Venda chickens through citric acid supplementation in the diet.

### 1.4.2 Objectives

The objectives of the study were:

i. To determine the effect citric acid supplementation in the diet on feed intake and growth rate, feed conversion ratio, and live weight of male Venda chickens.
ii. To determine the effect of citric acid supplementation in diet on gut morphology (weight and length of intestines, the weight of crop and gizzard and pH of gizzard and crop) of male Venda chickens.

### 1.4.3 Hypotheses

The hypotheses to be tested were:

i. Citric acid supplementation has no effect on feed intake and, growth rate, feed conversion ratio, and live weight of Venda chickens.
ii. Citric acid supplementation in diet has no effect on gut morphology (weight and length of intestines, weight of crop and gizzard and pH of gizzard and crop) of Venda chickens.

# CHAPTER TWO

# LITERATURE REVIEW

## LITERATURE REVIEW

### 2.1 Introduction

Venda chickens are indigenous chickens that are commonly raised in rural parts of South Africa and are frequently subjected to external control, according to Manyelo et al. (2020). Venda chickens are raised on a free-range scavenging system (Gueye, 1998) with minimal or no inputs for housing, feeding, and health care (Moges, 2010). According to Norris et al. (2007), indigenous chickens mature slowly but have a greater live weight at maturity than native bare necked chickens, which have a lower live body weight at maturity but mature early. They are a slow-growing breed that can withstand extreme climatic conditions (Desta and Wakeyo, 2012). These chickens are scavengers, and kitchen garbage is their primary source of nutrition (Aini, 1990). Venda chickens are indigenous chickens from Venda, South Africa, and are called after the Venda community.

Indigenous Venda chicken production has a significant economic and social impact on households, particularly in disadvantaged areas (Nyoni et al., 2022), and contributes significantly to household food security (Van Marle-Köster et al., 2008). The production of indigenous village chickens could be increased by changing management practices, particularly diet (Desta, 2021). Nhlane et al. (2020) observed that keeping indigenous chickens in an intensive production system with the provision of a ration with growth boosting qualities can increase the chickens' growth (Nhlane et al., 2020). Organic acids have long been used in the broiler business because of their "harmlessness," which allows them to maintain the appropriate functioning of the gut (Sugiharto, 2020).

However, in the broiler sector, there is a continuing interest in using organic acid as growth enhancers (Sugiharto, 2020). According to SA et al. (2008), the proper use of organic acid in conjunction with "nutrition, management, and biosecurity measures" may be the best instrument for preserving the good health of the gastrointestinal tract. Citric acid is a weak organic acid that regulates the pH of the intestine (Upadhaya et al., 2014). It is the most commonly utilized organic acid in poultry diets. It is thought to be a growth-promoting chemical that acidifies the gastrointestinal (GI) tract (Cave, 1984). Furthermore, citric acid in bird feed can change the pH of the gut, promoting the operation of some enzymes that require acidic conditions, such as pepsin and

phytase, and therefore improving protein and mineral absorption (Wickramasinghe et al.,2014).
Citric acid aids in reducing the pH of gut morphology, with the exception of feces (Islam, 2012). Distinct amounts of citric acid inclusion in a rice by-product-based diet of broiler chickens have a different influence on crop pH, with 0% citric acid inclusion resulting in a crop pH of 5.1,1% and 2% citric acid inclusion resulting in crop pH of 4.8 and 4, respectively (Atapattu and Nelligaswatta, 2005). This demonstrates that high levels of citric acid in broiler chicken diets lowers crop pH. According to Nourmohammadi et al., (2011), including citric acid in the food lowers the intestinal pH of broiler chickens. Pathogenic microorganisms are reduced by the low pH of the intestines since they require high pH to live. Furthermore, SA et al. (2008) discovered that citric acid supplementation in a basal diet of Hubbard broiler chickens reduced pH values of gastrointestinal tract (GIT) segments and increased the density of the small intestine but has no effect on liver functions. The use of 0.5g of CA/kg DM as a supplement in broiler chickens affects intestinal homeostasis and thereby increases chicken performance (Elbaz et al., 2021). There is currently no information on the effect of citric acid on the growth performance and gut morphology of male Venda chickens. As a result, further research on this study is required in order to produce relevant knowledge that can be utilized as a tool to fill the gap. As a result, increase the productivity of Venda chickens. As a result, the purpose of this review is to determine the effects of citric acid supplementation in the food on the gut morphology of Venda chickens.

This research will look at the significance and production methods of indigenous chickens. The study goes on to discuss the use of organic acids as antibiotic replacements, citric acid description, biochemical functions of citric acid, the use of citric acid as growth promoters and its effects on growth performance, gut morphology, profitability, and a comparison of GIT of Venda and broiler chickens. Furthermore, this study reviews “the effect of other acids on Venda chickens.”

## 2.2 Importance of indigenous Venda chickens to households

According to Alabi (2013), indigenous chickens account for around 85% of overall poultry output. Indigenous Venda chickens have a vital role in rural communities as a source of income, an important supply of animal nutrition, and in traditional religions for spiritual purification and gift giving (Mngonyama, 2012). They are also used as a natural clock, allowing them to forecast the time of day, particularly in rural settings (Njenga, 2005). Because of the ever-increasing human population and their vital functions in rural families, indigenous chickens serve as a source of wealth and a method for alleviating food insecurity in rural households (Khobondo et al., 2015).

## 2.3 Production system of Indigenous chickens

The sort of free-range chicken production system depends on the farmer's aims, such as becoming a commercial or subsistence farmer, input and output levels, and agricultural practices, according to Mujyambere et al. (2022). The chickens in extended farming are allowed to roam for feed, the level of input varies from low to medium, and the main goal of raising them is for household consumption (Gondwe and Wollny, 2007). If a farmer's goal is commercial farming, the chickens are raised intensively in a well-confined house and are provided with food, water, and medications; they are not permitted to roam, and the main goal of maintaining them is to generate a profit (Kingori et al., 2010).

### 2.3.1 The extensive system of indigenous chickens

According to Magothe et al. (2012), chicken rearing is subject to considerable management. These chickens provide meat and eggs for their own households, as well as a source of revenue and participation in other socio-cultural activities. Furthermore, Khobondo et al. (2015) stated that this system is often used in rural regions and is essentially a less input-less output type of system. The chickens leave their nests early in the morning to hunt for feeds at the field (Kingori et al., 2010), they consume anything they find edible, they typically eat grass insects, earthworms, and kitchen garbage (Mngonyama, 2012). Each flock typically has little more than 30 mature indigenous chickens, care is minimal, and no feed supplementation is provided (Kingori et al., 2010). Indigenous chic

kens are feed scavengers, there is significant rivalry for feeding because all ages are mixed, and water is rarely provided in broken clay pots or tins (King'ori, 2004). The lower the inputs, the lower the production of indigenous chicken. Figure 2.01 depicts indigenous Venda chickens.

**Figure 2.01** Indigenous Venda chickens (Manyelo et al., 2020).

### 2.3.2 The Intensive production system of Indigenous chickens

According to Kapella et al, (2022), an intense production system of indigenous chickens is difficult to come by. This is due to the fact that the majority of indigenous chickens are raised in rural regions, where the primary goal of raising chickens is for home use (Bwalya and Kalinda, 2014). This is a prevalent system in commercial farming with high inputs and high outputs. The chickens are being closely monitored and are receiving medication, nutrition, and water. This system is distinguished by increased egg production, rapid development rates, and fewer mortalities (Okeno et al., 2012). This approach, however, has not been properly studied for indigenous chicks such as Venda chickens in South Africa (Khobondo et al., 2015). This could be owing to a diet of local chickens that has yet to be discovered.

## 2.4 The use of organic acids as a replacement of antibiotics

Considering the well-being of both animals and humans, a few alternatives to antibiotic growth promoters have been proposed for use in an animal's diet, including organic acids, probiotics, prebiotics, and herbal items (Islam, 2012). Miles et al. (2006) discovered that antibiotic feeding increased body weight but usually decreased intestinal length and weight when compared to untreated chickensns. As a result, antibiotics differ in their potential to induce disease or improve development and feed efficacy. According to Islam (2012), customers are concerned about using antibiotics in feed since it would create resistant strains of undesired bacteria, posing a risk to consumers when eating meat or goods from impacted chickens. Furthermore, according to Metzler et al. (2005), the increasing frequency of antibiotic-resistant bacteria as a result of the use of antibiotics in feeds has led to a growing debate and concern about the proper use of antimicrobic components in animal feeds and antibiotic substitutes. As a result, the feed manufacturing businesses, farmers, and academics are eager to investigate antibiotic substitutes (Luise et al., 2020). Pearlin et al. (2020) discovered that organic acids and their salts outperformed all of the possible tactics that could be used as antibiotic substitutes because they demonstrated promising results in regulating intestine-related diseases and improving an animal's growth performance, particularly in monogastric animals. Organic acids such as citric acid, lactic acid, and propionic acid have predominantly been used to sanitise feeds and improve digestion, nutritional digestibility, gut health, growth performance Long et al. (2018), and prevent salmonella infections in chicken (Chowdhury et al., 2009).

### 2.5 Citric acid description

Citric acid was discovered by Scheele in 1784 as a component of "citrus" fruit and is distinguished by its pleasant flavor, low toxicity, and ease of absorption and digestion (Kubicek et al., 1985). It is classified as a weak organic acid because it is not harmful to animals or consumers. According to Islam (2012), citric acid preserves food and drinks naturally and adds an acidic or sour taste to them. The proper use of citric acid in feeds may be the most effective technique for sustaining gastrointestinal tract health (SA et al., 2008). Figure 2.02 depicts its molecular structure.

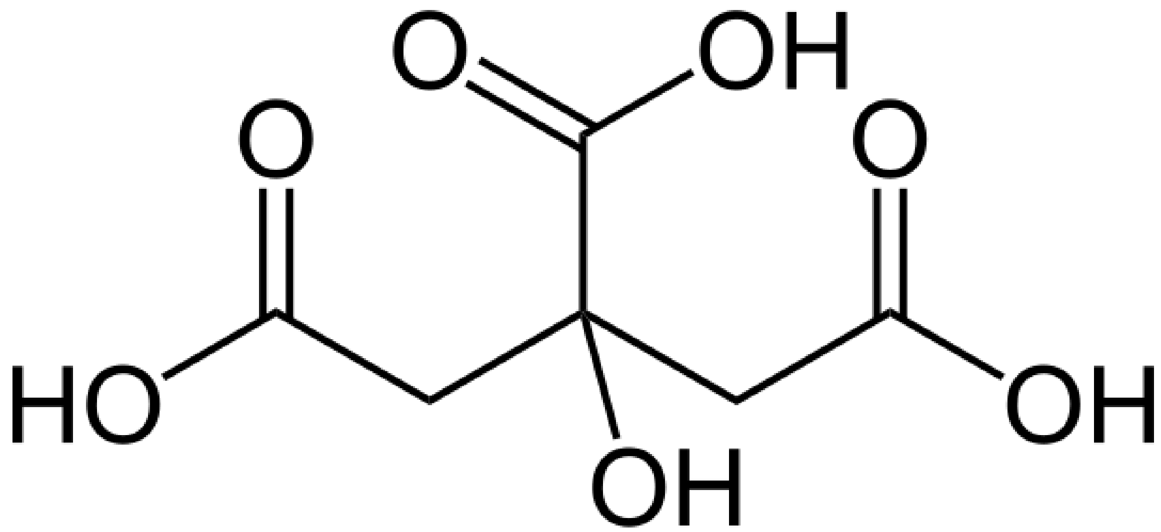


**Figure 2.02** molecular structure of citric acid (Islam, 2012).

Citric acid demonstrates adequate antimicrobic task to safeguard the feed from bacterial wastage, however at the same time lowers the levels of unwanted bacteria (e.g., *E. coli*.) in the gastrointestinal tract which then eventually enhance growth rate (Deepa et al., 2011). Citric acid supplementation at high levels may significantly impair the taste of the meal, whilst low levels boost feed consumption in birds (SA, 2008). Daskiran et al. (2004) confirmed that introducing an acid-supplemented meal to birds at an early stage could result in easier adaption and reduce the eventual medical task as acidifier. As a result, they planned to use acidifiers at the grower stage rather than the early stage to reduce financial failures caused by high temperature pressure.

**2.6 Biochemical functions of citric acid**

Citric acid that is fragrance-free and colourless is exceedingly water-soluble and poses no damage to human health or the environment (Ciriminna et al.,2017). When citric acid is broken down in water, it exhibits feeble acidity, however the acid taste is strong and impacts pleasantness, giving a fruit-like taste, which broadens its application as a supplement organic product flavour in the food and beverage sectors (Ciriminna et al.,2017). Citric acid in vaccinated broiler chickens boosts immunity, preventing Newcastle disease (Islam,2012). According to Koromyslova et al. (2015), citric acid is utilized as a disinfectant against human norovirus and may work as a treatment in people who are already infected by reducing the symptoms of norovirus sickness. Citric acid addition in broiler chicken feed improves intestinal health (Kishawy et al.,2018). Moreover, it has an influential effect on intestinal mucosa growth, which then upgrade nutrient digestion and animal performance (Romero et al., 2011).

**2.7 Effect of citric acid on growth performance**

Organic acid addition in the diet resulted in a considerable increase in body weight and feed conversion ratio of broiler chicks during the starter phase, but had no influence on body weight gain, feed intake, or FCR during the grower phase (Agboola et al., 2015). Citric acid at 1% and 2% levels, on the other hand, had no influence on growth performance or FCR while increasing feed intake and digestibility of "crude protein and crude fibre" in broiler chickens (Atapattu and Nelligaswatta, 2005). These findings are consistent with those of SA et al. (2008), who discovered that inclusion of citric acid at any level in the diet of broiler chickens improves feed digestion and absorption due to increased density of the small intestine, which indicates villi dimension. The use of citric acid as a feed supplement on broiler chickens resulted in a higher rise in feed intake and a slight improvement in feed conversion ratio during the starter phase aged one to 21 days, as well as a significant increase in body weight gain during the grower phase (Khan and Iqbal, 2016). This could be because chicks in the starter phase require more nutrients for body development, such as bone building, whereas chicks in the grower phase require nutrients for growth. As a result, broiler performance and morphological indices have improved.

According to Papatsiros et al. (2013), adding citric acid to broiler drinking water reduces mortality and total feed consumption but has no effect on body weight increase; this may be due to the presence of citric acid in the water alone. Citric acid supplementation at high levels could substantially reduce feed palatability by making feed taste excessively strong, resulting in reduced intake and growth, whereas a small quantity resulted in increased feed intake in avian species (Islam, 2012). According to Islam et al. (2008), using 0.5% citric acid in the diet enhances live body weight and feed conversion ratio in broiler chickens aged 0 to 5 weeks, and also improves feed intake in broiler chickens aged 2 to 3 weeks. There is a negligible difference in growth performance measures between 1.5% and 3% citric acid levels in a Hubbard broiler chicken diet, but the inclusion of both levels results in a superior increase in feed digestion and absorption, body weight gain, live body weight, and feed conversion ratio (SA et al.,2008). Citric acid in feed lowers the pH of digesta and enhances the integrity of the intestinal mucosa barrier, which improves digestion and absorption (Elbaz et al., 2021). According to Das et al. (2011), citric acid in low nutrient diets (low protein, low energy, and both low energy and protein) compensates for lost live weight gain and higher feed intake is obtained when citric acid is added in diet containing low energy compared to low protein and both low energy and protein, and FCR is greatly improved with addition of citric acid in diet containing low energy and low protein, followed by diet containing low protein (Table 2.01).

**Table 2. 0**1 Live weight gain, feed intake and feed conversion ratio of broilers fed low nutrient and supplemented 0.5% CA for 35 days. Source: Das et al. (2011).

| **Parameters** | **Treatments** | | | |
|---|---|---|---|---|
| | **Control** | **Low protein** | **Low energy** | **Low protein and low energy** |
| LWG(g) | $1561^{a} \pm 23.19$ | $1587^{a} \pm 26.08$ | $1591^{a} \pm 29.78$ | $1604^{a} \pm 24.61$ |
| FI(g) | $3532^{b} \pm 53.74$ | $3548^{b} \pm 16.37$ | $3615^{a} \pm 43.89$ | $3532^{b} \pm 47.85$ |
| FCR(g) | $2.26^{a} \pm 0.03$ | $2.24^{ab} \pm 0.03$ | $2.27^{a} \pm 0.06$ | $2.20^{b} \pm 0.04$ |

LWG: Live weight gain, FI: Feed intake, FCR: Feed conversion ratio, g: gram

$^{a,b,c}$: Means in the same column not sharing same subscript are significantly different ($P < 0.05$).

SE: standard error

## 2.8 Effect of citric acid on crop of chickens

The location of a crop in terms of pH and microbial comprise appears to be highly important when looking at its ability to resist pathogens (Corrier et al., 1999a). High population of *Lactobacilli* and little pH value in the crop have power to reduce the manifestation of *Salmonella* in the crop (Hinton et al., 2000). Avila et al. (2003) reported that organic acids in drinking water at pre-slaughter feed withdrawal period reduce *Salmonella* and *Campylobacter* adulteration of crops and carcasses at processing. The linking of citric acid with *d*-Limonen*e* reduce the number of pathogens in crop when starved before termination (Barnhart et al., 1999). Byrd et al. (2001), informed that crop pH is lowered by inclusion of 0.5% of other organic acids presented in water with feed restriction few hours before termination of the experiment. Additionally, Nourmohammadi et al. (2011) found that diets supplementation with citric acid lower the crop pH. The slide in pH of the gut has great affection on the colony of acid intolerant bacteria like Coliforms (Luckstadt, 2007). Opposingly, Acikgoz et al. (2010), stated that formic acid does not alter the microflora of chickens under high ambient temperature and that could be the result of little microbial load in the gut and Corrier et al. (1999b), found that feed restriction before slaughtering elevate pH of the crop and *Salmomella* contamination in acid supplemented commercial chickens. Avila et al. (2003), showed that citric acid inclusion in water reduce pH of the crop but cannot modulate the *salmonella* colonization in the crop of birds compared to untreated group.

**2.09 Citric acid effect on gizzard of chickens**

Citric acid at 4.5 and 6% concentrations in water significantly reduces the pH of the gizzard, ceca, and faeces in chickens when compared to untreated water (Alzawqari et al., 2013). Nonetheless, Watkins et al. (2004) observed that acidulated water has no effect on the gizzard pH of chickens. The disparities in these findings could be attributed to the different types and quantities of organic acids used in the research. Nourmohammadi et al. (2011), on the other hand, discovered lower pH values of gizzards when commercial chicken diets were supplemented with citric acid. The addition of citric acid to drinking water prior to slaughtering may diminish the colony of Salmonella enteritidis, and this may be employed as a remote control of Salmonella adulteration on poultry products during Agro processing (Avila et al., 2003). According to Alzawqari et al. (2013), chickens that are deprived of feeds a few hours before slaughter and given only drinking water that is amalgamated with citric acid, particularly at 4.5 and 6% insertion levels, have a significant reduction in bacillus, clostridium, coliform, facultative aerobic, and other bacteria in the gizzard when compared to untreated water. Moreover, Philipsen, (2006) discovered that the insertion of organic acid in drinking water lower the population of pathogens in the water and adjust the gut microflora.

According to Kim et al. (2015), organic acids such as citric acid and others are swiftly absorbed in the early segments (crop and gizzards) of the gastrointestinal tract, and only organic acids ingested can reach the end-parts of the gastrointestinal system. As a result, lowering the pH of the early segments of the GIT may expedite nutrient digestion, hence boosting nutrient absorption in meals via enzymes in the gut triggered by lower stomach pH (Nguyen et al., 2020).

### 2.10 Effect citric acid has on small intestine weight and length on chicken

Stamilla et al. (2020) demonstrated that diet combined with organic acid has a significant impact on small intestinal weights. Furthermore, in industrial chickens, organic acids (citric acid, acetic acid, and/or a combination of citric and acetic acids) thicken the muscularis of all compartments of the small intestine. However, Attia (2018) discovered that organic acids added to the diet have no effect on the muscular thickness of the small intestine in industrial chickens. The disparities in these outcomes could be attributed to the varying nutrient composition of the diets. Stamilla et al. (2020) demonstrated that diet combined with organic acid has a significant impact on small intestinal weights. Furthermore, in industrial chickens, organic acids (citric acid, acetic acid, and/or a combination of citric and acetic acids) thicken the muscularis of all compartments of the small intestine. However, Attia (2018) discovered that organic acids added to the diet have no effect on the muscular thickness of the small intestine in industrial chickens. The disparities in these outcomes could be attributed to the varying nutrient composition of the diets. The improved nutrient digestion and absorption could be due to the small intestine being a site of digestion and absorption. If the small intestine increases in “size,” particularly in length, feeds will have enough space and time to stay in the small intestine for digestion and absorption of nutrients to occur before defecation. Citric acid supplementation in chicken feed reduces bursa of Fabricius weight because the bursa of Fabricius participates in the establishment of immunity against Gumboro virus (Agboola et al., 2015). However, the decrease in weight of the Fabricius bursa is inversely related to body weight. This could be because there was no or very little disease outbreak or infection in the chickens during the trial, putting no strain on the bursa of Fabricius to release disease fighters. As a result, the decrease in weight of the Fabricius bursa had no effect on body weight.

### 2.11 Effect citric acid has on small intestines micro bacteria on avian species

Moharrery and Mahzonieh, (2005), detected that 0.1% organic acid like malic acid inclusion in drinking water worked bizarrely in decreasing *E. coli* counts in the small intestine of laying chickens. Similarly, Chaveerach et al. (2004) found that acidulated water decreases population of *Campylobacter* in the ceca contents of chickens. Moreover, Pelicano et al. (2005) reported that 0.5 to 5% level of organic acid in broiler chickens' diet primarily modulate the bacteria in the intestine and thus increase villi height and villi widths. Citric acid might suppress number of pathogens in small intestine of Japanese quail Ghosh et al. (2010) or reduce *Salmonella* in faeces of chickens Patten & Waldroup, (1988) and result in better immune response in chickens (Wickramasinghe et al., 2014).

### 2.12 Effect citric acid has on pH of small intestine, ceca, and other GIT part of birds

According to Jozefiak and Rutkowski (2005), incorporating citric acid into the diet reduces the pH of the intestine and caecal digesta. Citric acid supplementation in commercial chicken diets, according to Likeweise, Nourmohammadi et al. (2011), reduces pH levels in all three regions of the small intestine. According to Saleem et al. (2016), the use of organic acid in poultry feed has a significant impact on the adjustment of villi heights and width, as well as the pH of all three segments of the small intestine, resulting in a healthy bowel, better digestion, and nutrient absorption. According to Nourmohammadi and Khosravinia (2015), dietary citric acid improved proventriculus, gizzard, and ileum percentage as well as villus length, crypt depth, and goblet cell number in duodenum, jejunum, and ileum as well as ileal digestibility of crude protein, apparent metabolizable energy, and total phosphorus while decreasing the pH of contents in the gut segments in broiler chickens.

### 2.13 Effect of citric acid on profitability of chickens

According to Islam (2012), the use of citric acid in broiler industries has resulted in an increase in feed costs; however, the effect on feed costs has never affected the overall profit in the broiler industry because citric acid lowers the mortality rate and aids in the grading of the chickens' growth. Similarly, Islam et al. (2008) discovered that adding 0.5% citric acid to feed increases feed cost, but the profit gained is greater due to improved growth and feed efficacy. According to Salem and El-Garhy (2021), the introduction of citric acid resulted in an increase in broiler chicken profit when compared to the untreated group of broiler chickens. This could be due to growth boosters being used to manipulate growth. The inclusion of both 1.5% and 3% levels of citric acid in a diet of Hubbard broiler chickens improves economic efficiency and relative economic efficiency (SA et al., 2008). This may be due to the usage of growth promoters which result in chicken reaching market weight early.

### 2.14 Comparison of GIT weight and length of indigenous Venda chickens and broiler chickens

Mabelebele et al. (2017), studied "blood profiles and histo-morphometric analysis of the gastrointestinal tracts of Ross 308 broiler and indigenous Venda chickens fed the same diet" and found that Venda chickens had lower GIT, crop, gizzard, small and large intestines, weights than Ross 308 broiler chickens and the length of the whole GIT, small and large intestines in Ross 308 broiler chickens is longer than that of indigenous Venda chickens at both 42 and 90 days old. Borin et al. (2006) investigated "Digestibility and digestive organ development in indigenous and improved chickens and ducks fed diets with increasing inclusion levels of cassava leaf meal" and discovered that indigenous Cambodian chickens have larger weights of gizzard and proventriculus and longer length of small intestine than improved broiler chickens, but the weight of small intestine was the same in both breeds of chickens. The longer small intestine may be genetically advantageous, as the longer the small intestine, the lower the outflow rate and the higher the digestibility, followed by overall high body weight (gizzard and proventriculus included) and the same weight of small intestine in both breeds may be the result of shorter but thick small intestine in improved chickens and longer but narrow in Cambodian chickens.

### 2.15 Effects of other acid supplements on Venda chickens

Adesola et al. (2012) studied "Effect of ascorbic acid supplementation level to the diets of Venda chickens on live weight at 7 weeks of age (g/bird), feed intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g feed/g live weight gain), and mortality (%) of chicks from a day old up to 7 weeks of age". The results showed that adding 500g ascorbic acid to the diet of Venda chickens resulted in chicks with higher feed intake than controls and additions of 1000g and 1500g ascorbic acids. When compared to other groups, the addition of 200 and 500g of ascorbic acid to the feed of Venda chickens improves growth rate significantly. The addition of 1000g of ascorbic acid to the food results in considerably greater live weight than the control and group of chicken fed diet supplemented with 1500g of ascorbic acid. The introduction of 1000g and 1500g of ascorbic acid in the food significantly enhanced FCR, and mortality rate was only reported in the group of chickens fed diet supplemented with 200g of ascorbic acid, as shown in Table 2.02. Malebane et al. (2010), discovered that supplementation of ascorbic acid at all levels (0, 100, 400, 800, 1000, 1200, and 2000g) has no effect on DM intake, FCR, and mortality rate but greatly improves growth rate when supplemented. These unexpected results could be attributed to the observation of results from both direct (Venda chickens) and indirect (Chicks from Venda Chickens) recipients of boosted diets.

**Table 2.02.** Effect of ascorbic acid supplementation level to diets of indigenous Venda chickens on intake, growth rate, live weight FCR and mortality rate of chicks. Source: Adesola et al. (2012).

| Diet | Intake | Growth rate | Live weight | FCR | Mortality |
|---|---|---|---|---|---|
| $A_0$ | $40.0^c$ | $5.3^b$ | $498.0^b$ | $4.2^b$ | 0.0 |
| $A_{200}$ | $43.0^{ab}$ | $7.1^a$ | $530.0^{ab}$ | $4.3^a$ | 6.7 |
| $A_{500}$ | $44.0^a$ | $7.2^a$ | $501.0^b$ | $3.4^d$ | 0.0 |
| $A_{1000}$ | $41.0^{bc}$ | $5.4^b$ | $623.0^a$ | $4.1^c$ | 0.0 |
| $A_{1500}$ | $39.0^c$ | $5.5^b$ | $499.0^b$ | $4.0^c$ | 0.0 |
| SE | 0.7 | 0.2 | 33.8 | 0.00 | 3.0 |

$^{a,b,c}$: Means in the same column not sharing same superscript are significantly different ($P < 0.05$).

SE: standard error

## 2.16 Conclusion

Given its preservative properties in feed, enhancing intestinal health, growth, and feed conversion efficiency, citric acid could be a viable alternative to the use of growth-promoting antibiotics in native chicken feed. Its consumption lowers the pH of the gastrointestinal system and promotes gut health. Citric acid is most effective in enhancing broiler chicken development performance. Citric acid can be used in indigenous chickens such as Venda chickens, however further research is needed to understand its mechanism of action and appropriate application in indigenous chicken commercial production.

# CHAPTER 3

# METHODOLOGY AND ANALYTICAL PROCEDURE

## METHODOLOGY AND ANALYTICAL PROCEDURE

### 3.1 Study site

The current study was done at Animal Unit in the University of Limpopo, South Africa, with that ranges from 20 and 36 °C in summer and 10 and 25 °C in winter, and average annual precipitation of less than 400 mm (Kutu and Asiwe, 2010).

### 3.2 Acquisition of materials and chickens

The Agricultural Research Council (ARC) in Pretoria, South Africa, provided 200 male Venda chicks. Angel Feeds in Polokwane, South Africa, supplied the feeds, home disinfectant (Virokill), 250-watt infrared lamps, sawdust, feeders, and drinks. Prestige Laboratory Supplies South Africa supplied the citric acid.

### 3.3 Experimental house preparation

Water and disinfectants were used to clean the experimental dwelling (ViroKill). After cleaning, the house was rested for seven days to allow thorough drying and to destroy the organisms that cause the ailments. Following that, the house was divided into 20 equal-sized floor pens, and 7cm of fresh sawdust was spread on the floor. Throughout the experiment, the experimental equipment (feeders and drinkers) was carefully cleaned and disinfected.

### 3.4 Experimental design, treatments, and procedure

This study was divided into two experiments. The first experiment determined the effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens from a period of one to 90 days. Different citric acid supplementation levels (Table 3.01) in a completely randomized design were randomly allocated to 200 male Venda chicks, with five replicates and ten chicks in each replicate for a period of 90 days. A maize-soya bean meal-based diet was formulated based on NRC (1994) recommendations. Diets were formulated to contain 0 g/kg of citric acid, 12.5 g/kg of citric acid, 25 g/kg of citric acid and 50 g/kg of citric acid presented by partial ANOVA Table 3.03. The formulation of the experimental diets was done to meet the nutritional requirements of male Venda chickens (Raphulu and Jansen Van Rensburg, 2018) (Table 3.02). The diets were formulated to be iso-energetic and iso-nitrogenous, 12.14 MJ ME/kg DM and 180 g CP/kg DM, respectively. The chickens were offered feed and freshwater *ad libitum*. There was 23 hours of lights and one hour of no lights everyday throughout the experiments and ventilation was controlled using plastic curtains. The mortality was monitored daily.

The second experiment examined the effect of citric acid supplementation on gut morphology (intestine weight and length, crop and gizzard weight, and gizzard and crop pH) in male Venda chickens. According to the recommendations of the University of Limpopo Animal Research Ethics Committee, the 90-day-old chikens from the first stage of the experiment were slaughtered via cervical dislocation. The experimental design was the same.

**Table 3.01** Dietary treatments for experiment 1 and 2 (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 12.5 g of citric acid/kg of diet. |
| $MCA_{25}$ | Male Venda chickens fed a maize-soybean meal-based diet supplemented with 25 g of citric acid /kg of diet. |
| $MCA_{50}$ | Male Venda chickens fed a maize-soybean meal-based diet supplemented with 50 g of citric acid/kg of diet. |

**Table 3.02** Ingredients and nutrient composition of experiment 1 and 2

| | Starter | | | | Grower | | | | Finisher | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CA level** | **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 | 33.0 |
| 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 | 49.1 |
| 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 |
| Vit 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 |
| **Nutrients 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 | 21.81 | 21.75 | 21.00 | 20.21 | 20.07 | 20.00 | 20.4 |
| Fat (%) | 7.22 | 7.67 | 7.90 | 8.55 | 8.25 | 8.69 | 8.93 | 9.56 | 8.80 | 9.24 | 9.00 | 9.58 |
| Fibre (%) | 3.15 | 3.12 | 3.00 | 2.66 | 2.86 | 2.83 | 2.75 | 2.62 | 2.62 | 2.60 | 2.60 | 2.59 |
| 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 | 3129.59 | 3125 | 3100.10 | 3219.39 | 3210.87 | 3210.00 | 3110 |
| Lysine (%) | 1.40 | 1.40 | 1.43 | 1.44 | 1.33 | 1.33 | 1.33 | 1.32 | 1.22 | 1.216 | 1.24 | 1.26 |
| Methionine (%) | 0.65 | 0.65 | 0.64 | 0.63 | 0.631 | 0.63 | 0.62 | 0.613 | 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 | 0.133 | 0.133 | 0.133 | 0.13 |
| CL (%) | 0.28 | 0.28 | 0.25 | 0.19 | 0.22 | 0.22 | 0.22 | 0.22 | 0.194 | 0.19 | 0.19 | 0.19 |

**Table 3.03**. Partial ANOVA for the effects of different citric acid supplementation levels ($CA_0$; $CA_{12.5}$; $CA_{50}$; $CA_{50}$) in diet on growth performance and gut morphology of 200 male Venda chickens, replicated five times in each treatment and with 10 chickens per replicate.

| SOURCES OF VARIATION | DF | SS | MS | $F_{CALCULATED}$ |
|---|---|---|---|---|
| Treatments | t-1=4-1=3 | | | |
| Error | n-t=200-4 =196 | | | |
| Total | n-1=200-1= 199 | | | |

## 3.5. Data collection

### 3.5.1 Measurements of growth performance

The initial live weight of male chickens was measured using a weighing scale at the start of the experiment, and weekly live weight measurements were recorded after that. The daily feed intake (feed provided minus feed remaining) was calculated based on the number of chickens in the pen. The feed conversion ratio (FCR) (feed consumed/weight gain) was computed. Weekly growth rates (body weight gain/total number of days per period) were calculated.

### 3.5.2 Slaughtering procedure

At 90 days old, all the chickens were slaughtered. Slaughtering was done by cervical dislocation as recommended by the University of Limpopo Animal Research Ethics Committee.

### 3.5.3 Measurements of gut morphology

Before slaughtering the chickens were weighed. The offal (gizzards, crops, etc.) weights were determined according to McDonald et al. (2010). The length of intestines was measured using tape measure (cm). The gut digesta pH (gizzards and crops) was determined by means of a portable pH meter (Crison pH 25 CRISON Instruments SA, Spain).

### 3.5.4 Diet sample analysis

Dry matter contents of feeds were determined by oven-drying of the samples at 105 °C for 24 hours. Gross energy values for feeds and were determined using a bomb calorimeter according to the method previously described by Association of Analytical Chemists (AOAC) (2010) at the University of Limpopo Animal Nutrition Laboratory Table 3.04.

**Table 3.04** Nutrient composition of the diets

| Nutrients | Unit | Starter | | | | Grower | | | | Finisher | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Analysis | | | | Analysis | | | | Analysis | | | |
| Moisture | % | 9,973 | 9,77 | 9,5 | 9,34 | 9,988 | 9,78 | 9,65 | 9,264 | 10,04 | 9,832 | 9,35 | 9,3 |
| Protein | % | 23,025 | 23 | 23 | 22,8 | 21,949 | 21,805 | 21,75 | 21 | 20,21 | 20,067 | 20 | 20,49 |
| Fat | % | 7,224 | 7,67 | 7,9 | 8,55 | 8,25 | 8,686 | 8,93 | 9,56 | 8,797 | 9,239 | 9 | 9,58 |
| Fibre | % | 3,15 | 3,123 | 3 | 2,66 | 2,86 | 2,829 | 2,75 | 2,615 | 2,624 | 2,597 | 2,597 | 2,588 |
| Ash | % | 1,675 | 1,675 | 1,53 | 1,304 | 1,29 | 1,301 | 1,301 | 1,314 | 1,276 | 1,3 | 1,31 | 1,321 |
| AMEN | kcal/kg | 3017,5 | 3009,8 | 3008,5 | 3010,9 | 3137,8 | 3129,6 | 3125 | 3100,1 | 3219,4 | 3210,9 | 3210 | 3110 |
| Lysine | % | 1,402 | 1,4 | 1,43 | 1,44 | 1,33 | 1,33 | 1,33 | 1,32 | 1,22 | 1,216 | 1,24 | 1,26 |
| Meth | % | 0,65 | 0,65 | 0,64 | 0,63 | 0,631 | 0,63 | 0,62 | 0,613 | 0,56 | 0,56 | 0,56 | 0,56 |
| Ca | % | 0,809 | 0,9 | 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 | 0,6 | 0,6 | 0,6 | 0,6 | 0,6 | 0,6 | 0,6 | 0,6 |
| Na | % | 0,19 | 0,19 | 0,17 | 0,13 | 0,15 | 0,15 | 0,15 | 0,15 | 0,133 | 0,133 | 0,133 | 0,133 |
| Cl | % | 0,28 | 0,28 | 0,25 | 0,19 | 0,22 | 0,22 | 0,22 | 0,22 | 0,194 | 0,19 | 0,19 | 0,19 |

### 3.6 Statistical analysis

Data on live weight, feed intake, growth rate, feed conversion ratio, gut digesta weights, digesta content pH of the gizzards and crops of male Venda chickens were analysed using the General Linear Model (GLM) procedure of SAS (version 9.2) (SAS, 2008). Tukey HSD test was used to separate the significant differences between treatment means at $P \leq 0.05$. The following statistical model was used for analysing the data obtained:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: $Y_{ij}$ = response variables, $\mu$=overall mean, $T_i$ = effect of citric acid treatments and $e_{ij}$= random error.

The optimal responses in live weight, feed intake, growth rate, feed conversion ratio, gut digesta weights, gut digesta pH of male Venda chickens to the level of citric acid supplementation were modelled using the following quadratic equation:

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

Where Y = response variables; 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 quadratic model was used because it gives the best fit

# CHAPTER FOUR

# RESULTS

# RESULTS

## 4.1 Effect of citric acid supplementation on feed intake (FI), growth rate (GR), feed conversion ratio (FCR) and live weight of male Venda chickens aged one to 30 days

Results of the effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio (FCR) and live weight of male Venda chickens aged one to 30 days is presented by Table 4.01. Citric acid supplementation in diet at all levels ($CA_0$, $CA_{12.5}$, $CA_{25}$ & $CA_{50}$) had no influence (*P* > 0.05) on feed intake and FCR of male Venda chickens aged one to 30 days (starter phase). However, CA supplementation had a significantly influence (*P* <0.05) on growth rate and live weights of male Venda chickens among the dietary treatments.

Chickens fed control diet improved (*P* < 0.05) growth rate compared to those fed CA supplemented diet. However, chickens fed diet supplemented with $CA_{12.5}$, $CA_{25}$ and $CA_{50}$ had similar (*P* > 0.05) growth rate.

Male Venda chickens fed diet supplemented with $CA_{25}$ had improved (*P* < 0.05) live weight compared to those fed diet supplemented with $CA_{50}$. However, chickens fed diet supplemented with $CA_0$, $CA_{12.5}$ and $CA_{25}$ had similar (*P* > 0.05) live weights. Chickens fed diet supplemented with $CA_{50}$ showed lower (*P* < 0.05) live weight compared to other groups. However, the difference in live weight of chickens fed diet supplemented with $CA_0$, $CA_{12.5}$ and $CA_{50}$ was not significant (*P* > 0.05).

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 male Venda chickens aged one to 30 days (Table 4.02 and Figure 4.01) and a 2.536g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal live weight of male Venda chickens aged one to 30 days (Table 4.02 and Figure 4.02).

**Table 4.01** Effect of citric acid supplementation on DM feed intake, growth rate, feed conversion ratio (FCR) 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) | 0.54±0.044 | 0.50±0.058 | 0.51±0.020 | 0.47±0.029 |
| Growth rate (kg/bird/day) | $0.08^a$±0.001 | $0.03^b$±0.004 | $0.03^b$±0.001 | $0.03^b$±0.001 |
| FCR (kg DM feed/kg live weight gain) | 1.40±0.107 | 1.45±0.597 | 1.06±0.111 | 1.40±0.300 |
| Live weight (kg/bird aged 1 to 30 days) | $0.60^{ab}$±0.015 | $0.56^{ab}$±0.091 | $0.68^a$±0.030 | $0.53^b$±0.018 |

* : Values presented as mean ± standard deviation

a, b, c: Means in the same row not sharing a same superscript are significantly different ($p < 0.05$)

# : The treatments were citric acid supplmentation in the diet of ($MCA_0$), ($MCA_{12.5}$), ($MCA_{25.0}$) or 50g of CA/kg DM of feed.

Table 4.02 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) | $Y=0.021+0.0067x+-0.0014x^2$ (0.03;0.05) | 2.393 | 0.0290 | 0.401 | 0.774 |
| Live weight (kg/ bird aged 30 days) | $Y=0.433+0.142x+-0.028x^2$ (0.61;0.97) | 2.536 | 0.653 | 0.401 | 0.774 |

$r^2$ =Coefficient of determination,

X= Optimal citric acid level

Y= Optimal variable

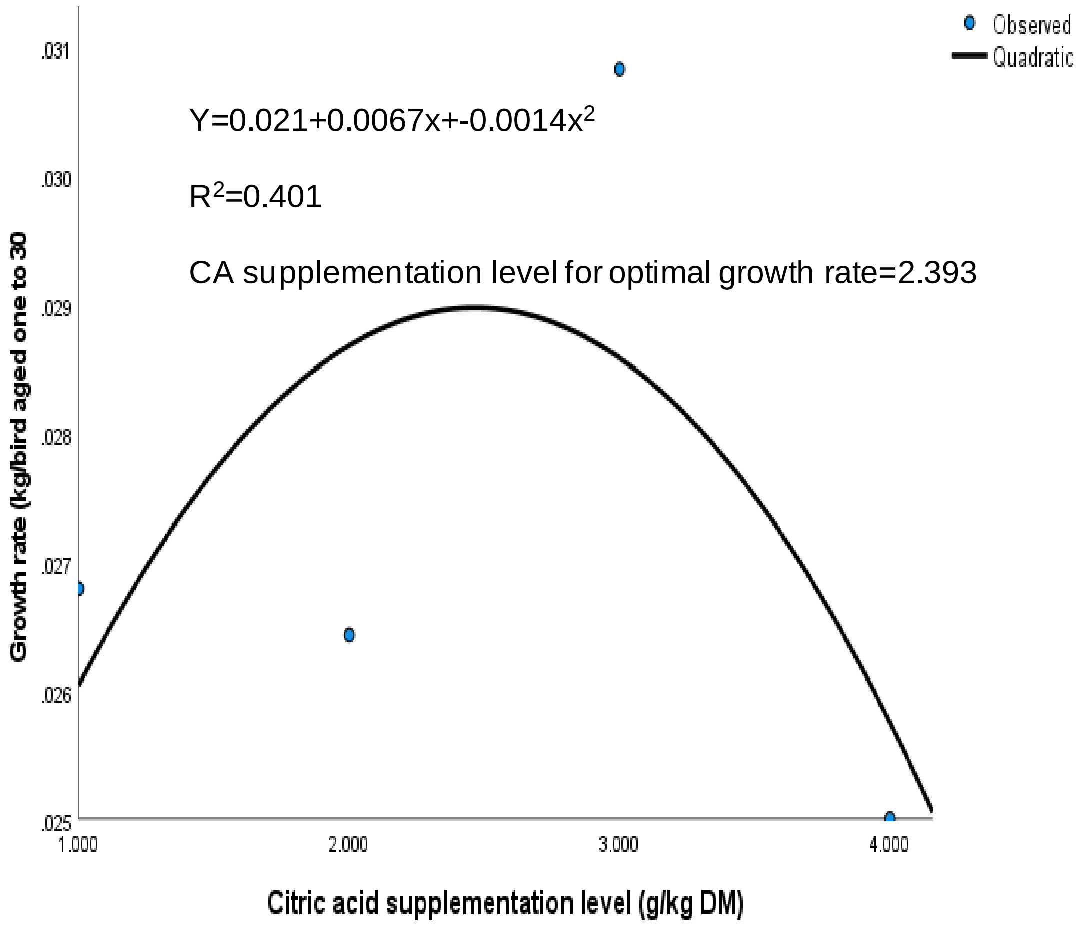


**Figure 4.01** Effect of citric acid supplementation in diet on growth rate of male Venda chicken aged one to 30 days

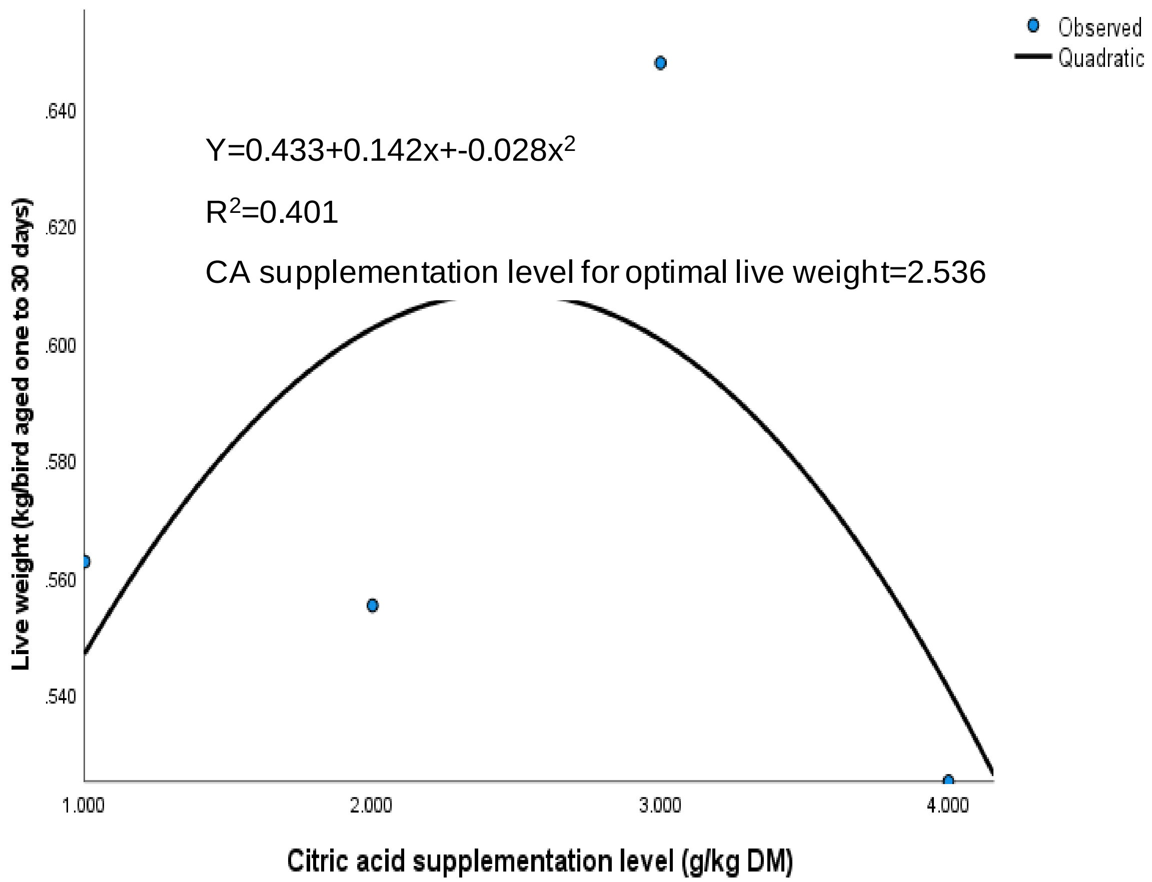


**Figure 4.02** Effect of citric acid supplementation in diet on live weight of male Venda chicken aged one to 30 days

## 4.2 Effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio (FCR) and live weight of male Venda chickens aged 31 to 60 days

Results on the effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio (FCR) and live weight of male Venda chickens aged 31 to 60 days is presented by Table 4.03. Citric acid supplementation had a significantly difference ( $P < 0.05$) on feed intake, growth perfomance, FCR and live wight of male Venda chickens across the treatments.

Chicken fed diet supplemented with $CA_{25}$ had better ($P < 0.05$) feed intake compared to those fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{50}$. However, chickens fed diet supplemented with $CA_{0}$, $CA_{25}$ and $CA_{50}$ had similar ($P > 0.05$) feed intake in male Venda chickens. Chicken fed diet supplemented with $CA_{12.5}$ had lower ($P < 0.05$) feed intake compared to those fed diet supplemented with $CA_{25}$. However, chickens fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{50}$ had feed intake that is not significantly different ($P > 0.05$).

Chickens fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{25}$ had the highest ($P < 0.05$) growth rate compared to those fed diet supplemented with $CA_{50}$. However, chickens fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{25}$ had similar ($P > 0.05$) growth rate. Chickens fed diet supplemented with $CA_{50}$ showed a decline ($P < 0.05$) in growth rate compared to all other treatments.

Chickens fed diet supplemented with $CA_{12.5}$ had better ($P < 0.05$) FCR compared to those fed diet supplemented with $CA_{0}$, $CA_{25}$ and $CA_{50}$. However, the differece in FCR between chickens fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{25}$ is not significant ($P > 0.05$). Therefore, chicken fed diet supplemented with $CA_{50}$ had poor FCR ($P < 0.05$) compared to those fed diet supplemented with $CA_{12.5}$ and there is no significant difference ($P > 0.05$) in FCR of chickens fed diet supplemented with $CA_{0}$, $CA_{25}$ and $CA_{50}$.

Chickens fed diet supplemented with $CA_{12.5}$ and $CA_{25}$ greatly increased ($P < 0.05$) live weight of male Venda chickens aged 31 to 60 days compared to those fed diet supplemented with $CA_{0}$ and $CA_{50}$. However, chickens from diet containing $CA_{12}$ and $CA_{25}$ had similar ($P > 0.05$) live weight. Chickens fed diet supplemented with $CA_{0}$ had better ($P < 0.05$) live weight compared to those fed diet supplemented with $CA_{50}$.

Chickens fed diet supplemented $CA_{50}$ showed reduced live weight in male Venda chicken aged 31 to 60 days.

A 2.250g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal growth rate of male Venda chickens aged 31 to 60 days (Table 4.04 and Figure 4.03) and a 2.373g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal FCR of male Venda chickens aged 31 to 60 days (Table 4.04 and Figure 4.04) and a 2.308g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal live weight of male Venda chickens aged 31 to 60 days (Table 4.04 and Figure 4.05).

**Table 4.03** Effect of citric acid supplementation on DM feed intake, growth rate, feed conversion ratio (FCR) 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) | $1.09^{ab}$±0.028 | $1.03^{b}$±0.030 | $1.12^{a}$±0.028 | $1.05^{ab}$±0.058 |
| Growth rate (kg/bird/day) | $0.03^{a}$±0.003 | $0.03^{a}$±0.001 | $0.03^{a}$±0.002 | $0.02^{b}$±0.002 |
| FCR (kg DM feed/kg live weight gain) | $2.49^{ab}$±1.024 | $1.32^{b}$±0.170 | $1.55^{ab}$±0.186 | $2.95^{a}$±0.925 |
| Live weight (kg/bird aged 31 to 60 day | $1.03^{b}$±0.126 | $1.29^{a}$±0.040 | $1.24^{a}$±0.063 | $0.84^{c}$±0.070 |

* : Values presented as a mean ± standard deviation

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

# :The treatments were citric acid supplmentation level in the diet ($MCA_0$ ),( $MCA_{12.5}$ ) ($MCA_{25}$), and ($MCA_{50}$) in 1kg of DM of feed.

**Table 4.04** 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

| Variables | Formula and answers | X | Y | $r^2$ | Probability |
|---|---|---|---|---|---|
| Growth rate (kg/bird/day) | $Y=0.010+0.018x+-0.004x^2$ (0.03; 0.07) | 2.250 | 0.031 | 0.999 | 0.35 |
| FCR (kg DM feed/kg live weight gain) | $Y=4.875+-3.042x+0.641x^2$ (1.27; 15.70) | 2.373 | 1.266 | 0.999 | 0.036 |
| Live weight (kg/bird aged 31 to 60 days) | $Y=0.436+0.757x+-0.164x^2$ (1.31;3.06) | 2.308 | 1.310 | 0.999 | 0.035 |

$r^2$=Coefficient of determination

X= Optimal citric acid level

Y=Optimal variable

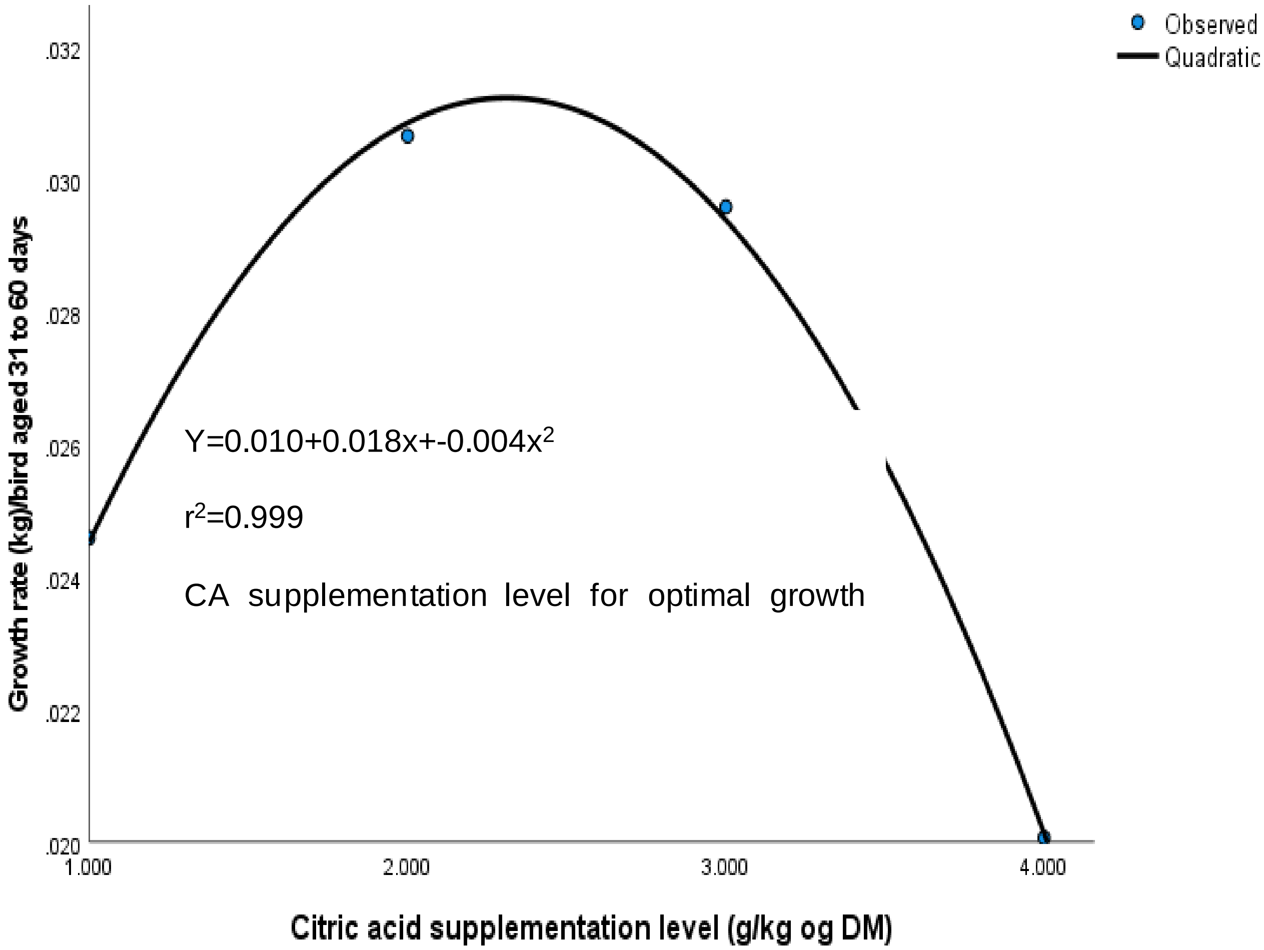


**Figure 4.03** Effect of CA supplementation in diet on growth rate of male Venda chickens aged 31 to 60 days old

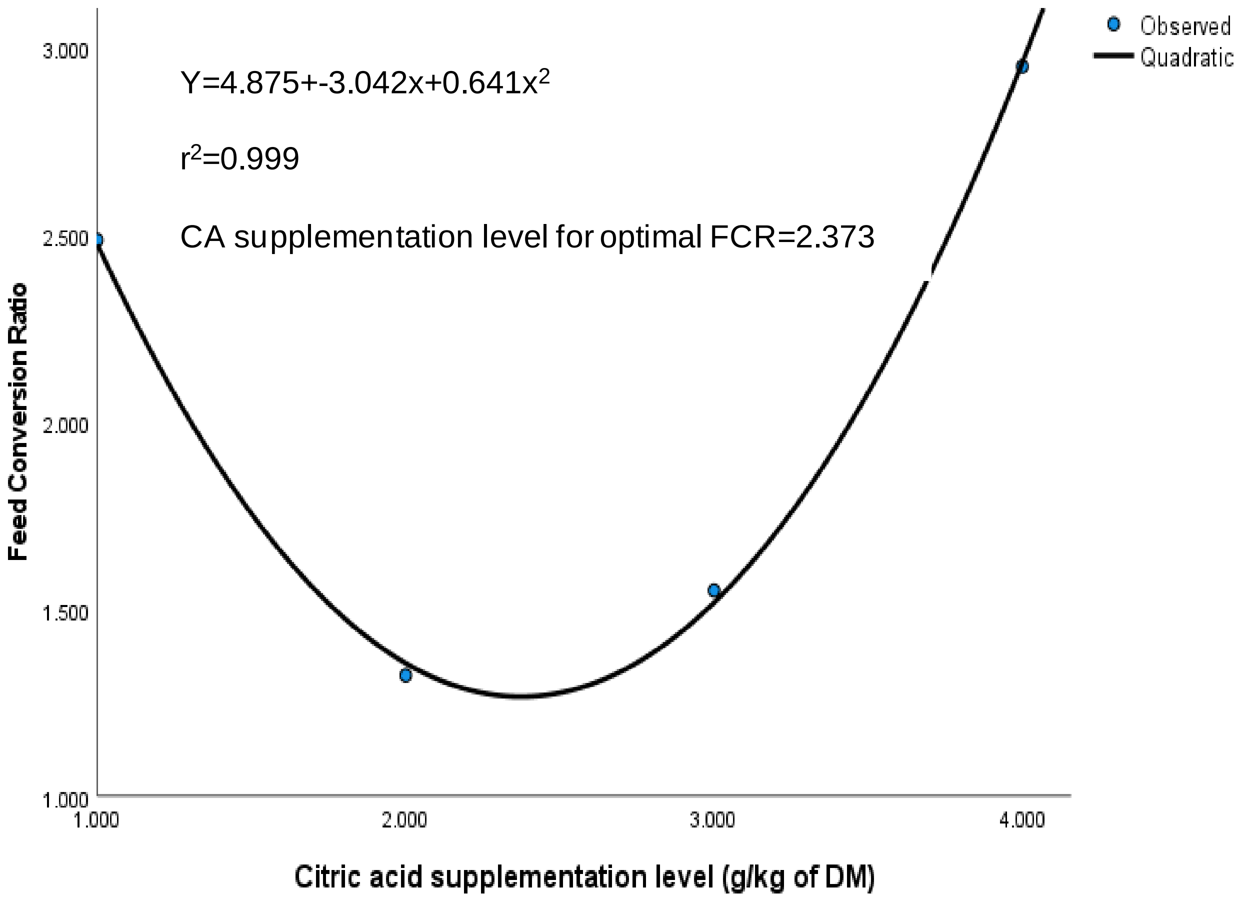


**Figure 4.04** Effect of CA supplementation on FCR of male Venda chicken aged 31 to 60 days old

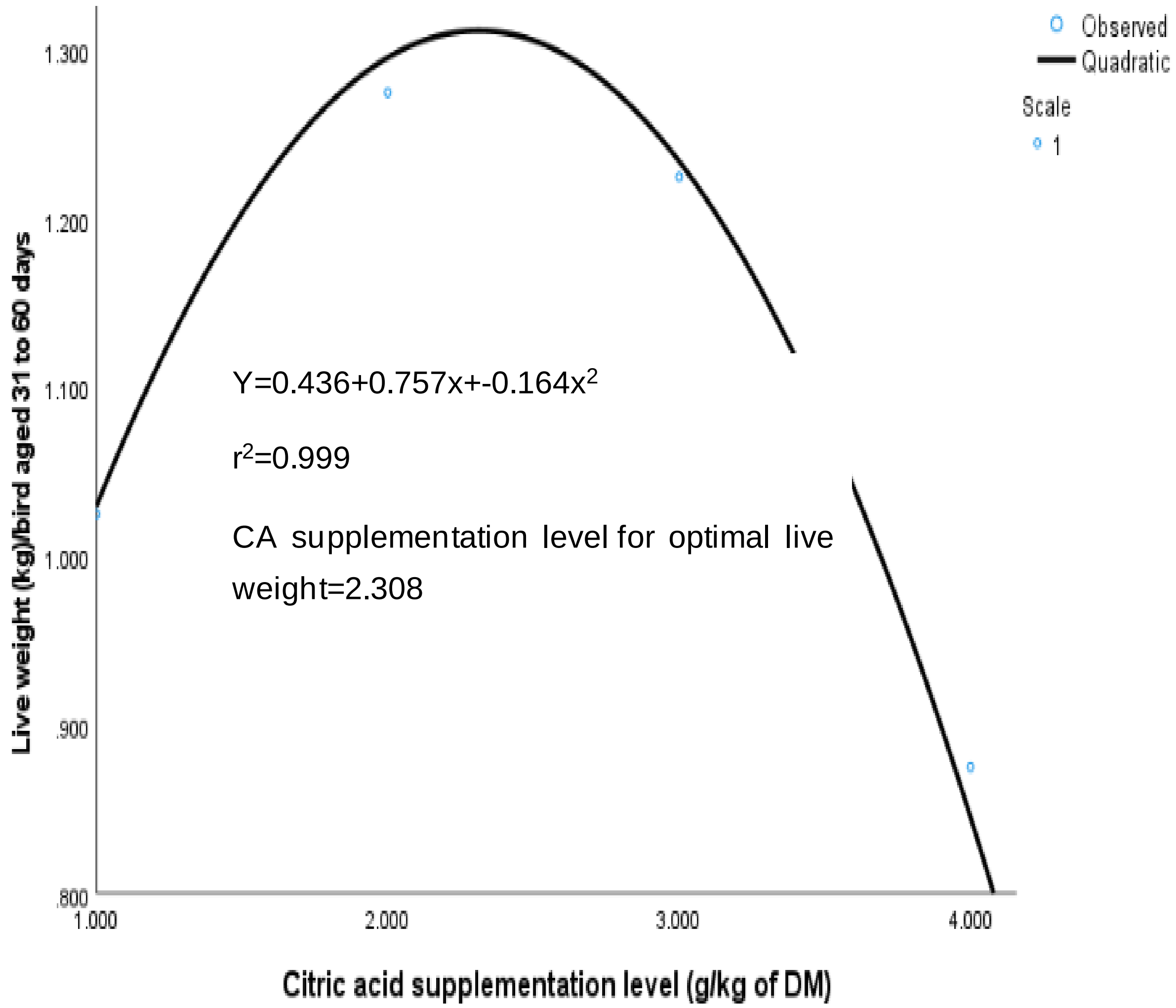


**Figure 4.05** Effect CA supplementation has on live weight of male Venda chicken aged 31 to 60 days

### 4.3 Effect of citric acid supplementation in diet on DM feed intake, growth rate, feed conversion ratio (FCR), and live weight of male Venda chickens aged 61 to 90 days

Results on effect of citric acid supplementation in diet on DM feed intake,growth rate, FCR, and live weight of male Venda chickens aged 61 to 90 days is represented by Table 4.05. The supplementation of $CA_{12.5}$ in diet of male Venda chickens aged 61 to 90 days significantly improved ($P$ < 0.05) feed intake much better than $CA_{25}$ and $CA_{50}$ supplementation but there was no significant difference ($P$ > 0.05) in feed intake on chickens supplemented with $CA_{12.5}$ and $CA_{0}$ diet. Chickens fed diet supplemented with $CA_{0}$ and $CA_{25}$ had no significant difference ($P$ > 0.05) on feed intake. The supplementation of $CA_{50}$ in diet reduced ($P$ < 0.05) feed intake on male Venda chicken aged 61 to 90 days.

Chickens supplemented with $CA_{12.5}$ and $CA_{25}$ significantly increased ($P$ < 0.05) growth rate better compared to $CA_{0}$ and $CA_{50}$ supplementation. However, $CA_{12.5}$ and $CA_{25}$ had no significant difference ($P$ > 0.05) in growth rate of male Venda chickens.$CA_{0}$ improved growth rate ($P$ < 0.05) compared to $CA_{50}$ supplementation in diet of male Venda chickens. The supplementation of $CA_{50}$ in diet reduced ($P$ < 0.05) growth rate of male Venda chicken.

Chickens fed diet supplemented with $CA_{12.5}$ and $CA_{25}$ had better ($P$ < 0.05) FCR compared to those fed diet supplemented with $CA_{0}$ and $CA_{50}$. However, chickens fed diet supplemented with $CA_{0}$, $CA_{12.5}$ and $CA_{25}$ resulted in FCR that was not signicantly different ($P$ > 0.05). Chickens fed diet supplemented with $CA_{50}$ had poor ($P$ < 0.05) FCR compared to those fed diet supplemented with $CA_{12.5}$ and $CA_{25}$. But, $CA_{0}$ and $CA_{50}$ supplementation resulted in similar ($P$ > 0.05) FCR in male Venda chickens aged 61 to 90 days.

Chickens fed diet supplemented with $CA_{12.5}$ and $CA_{25}$ had better ($P$ < 0.05) live weight compared to chickens fed diet supplemented with $CA_{0}$ and $CA_{50}$. However, chickens fed diet supplemented with $CA_{12.5}$ and $CA_{25}$ had similar ($P$ > 0.05) live weight. Chickens fed diet supplemented with $CA_{0}$ had better ($P$ < 0.05) live weight compared to those fed diet supplemented with $CA_{50}$. Live weight in chicken fed diet supplemented with $CA_{50}$ decreased ($P$ < 0.05).

A 1.566g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal feed intake of male Venda chickens aged 61 to 90 days (Table 4.06 and Figure 4.06) and a 2.161g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal growth rate of male Venda chickens aged 61 to 90 days (Table 4.06 and Figure 4.07). A 2.332g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal FCR of male Venda chickens aged 61 to 90 days (Table 4.06 and Figure 4.08) and a 2.272g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal live weight of male Venda chickens aged 61 to 90 days (Table 4.06 and Figure 4.09).

**Table 4.05** Effect of citric acid supplementation in diet on feed intake, growth rate, feed conversion ratio (FCR), and live weight of male Venda chickens aged 61 to 90 days old

| 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 61 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

a, b, c : Means in the same row not sharing a common superscript are significantly different ($p < 0.05$)

# :The treatments were citric acid supplmentation level in the diet ($MCA_0$ ),( $MCA_{12.5}$ ) ($MCA_{25}$), and ($MCA_{50}$) in 1kg of DM of feed.

**Table 4.06** 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 old

| Variable | Formula and answers | X | Y | $r^2$ | Probability |
|---|---|---|---|---|---|
| Feed intake (kg/bird/day) | $Y=1.413+0.119x+-0.038x^2$ (1.51;1.69) | 1.57 | 1.51 | 0.97 | 0.186 |
| Growth rate (kg/bird/day) | $Y=0.016+0.013x+-0.003x^2$ (0.03; 0.06) | 2.17 | 0.03 | 1.00 | 0.002 |
| FCR (kg DM feed/kg live weight gain) | $Y=2.050+-0.737x+0.158x^2$ ( 1.19;4.58) | 2.33 | 1.19 | 0.99 | 0.068 |
| Live weight (kg/bird aged 61 to 90 days) | $Y=0.971+0.777x+-0.171x^2$ (1.85;3.62) | 2.27 | 1.85 | 1.00 | 0.003 |

$r^2$= Coefficient of determination

X= Optimal citric acid level

Y= Optimal variable

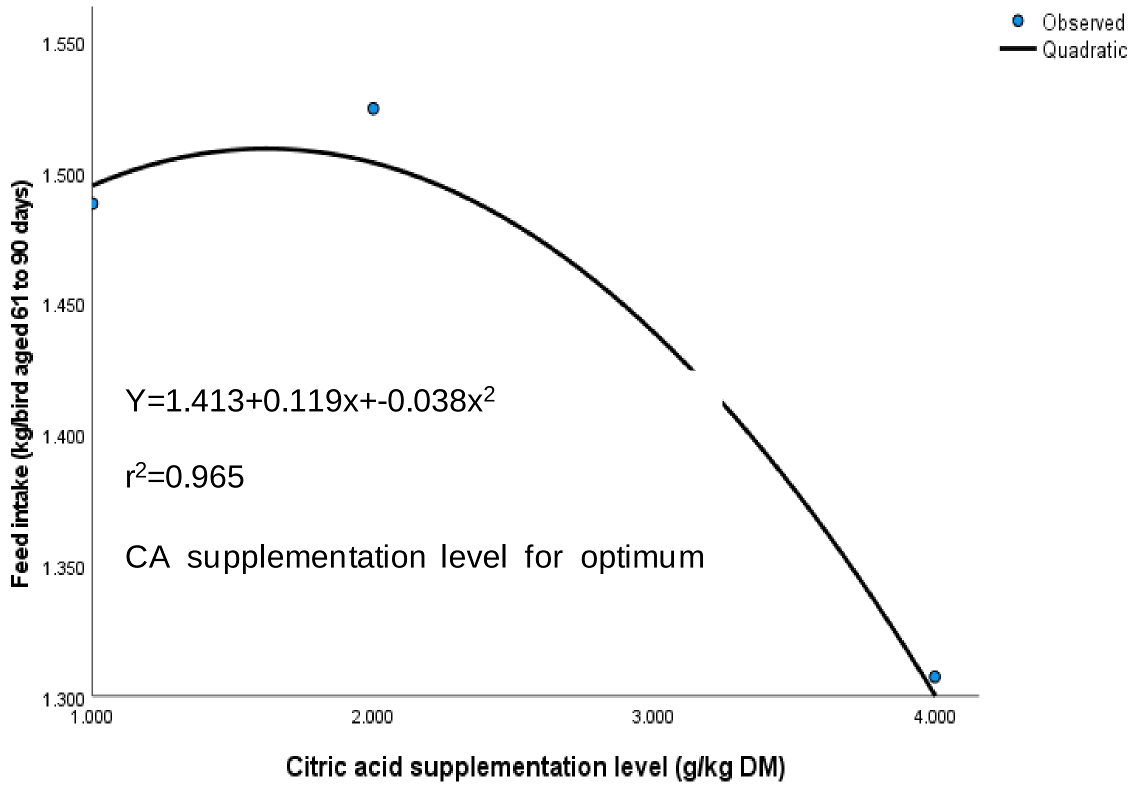


**Figure 4.06** Effect of CA supplementation on feed intake of male Venda chicken aged 61 to 90 days old

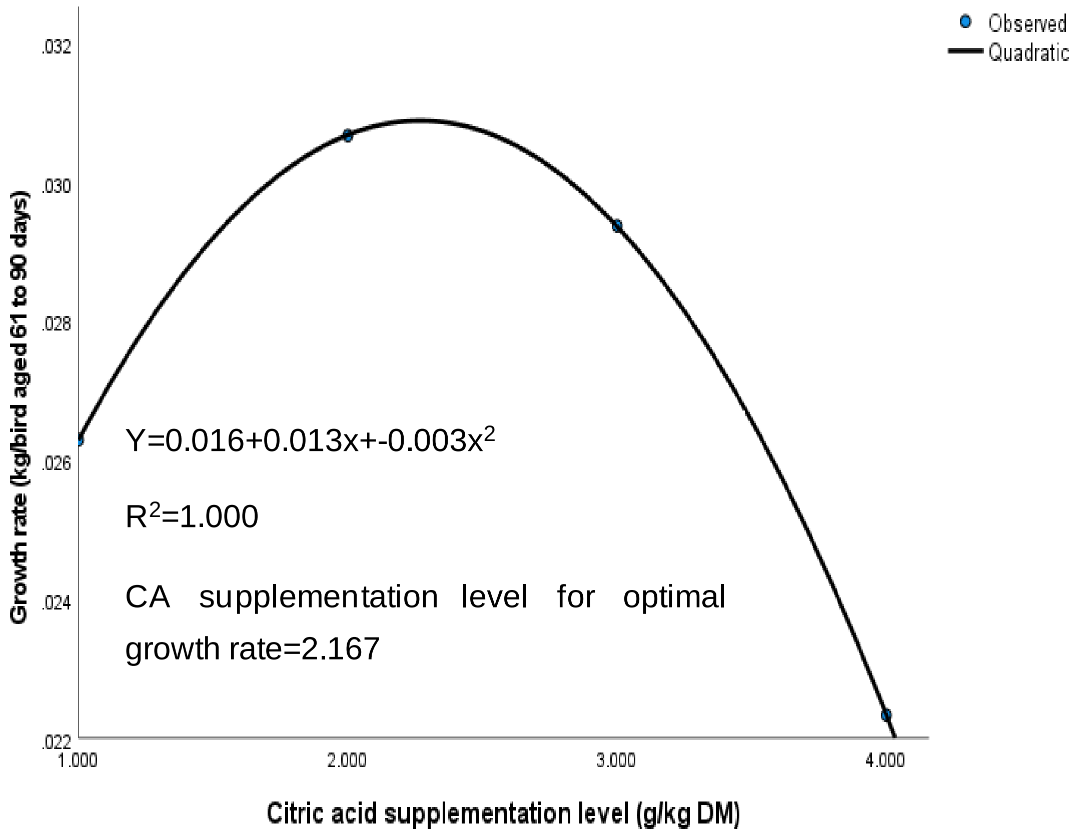


**Figure 4.07** The effect of CA supplementation in diet on growth rate of male Venda chicken aged 61 to 90 days old

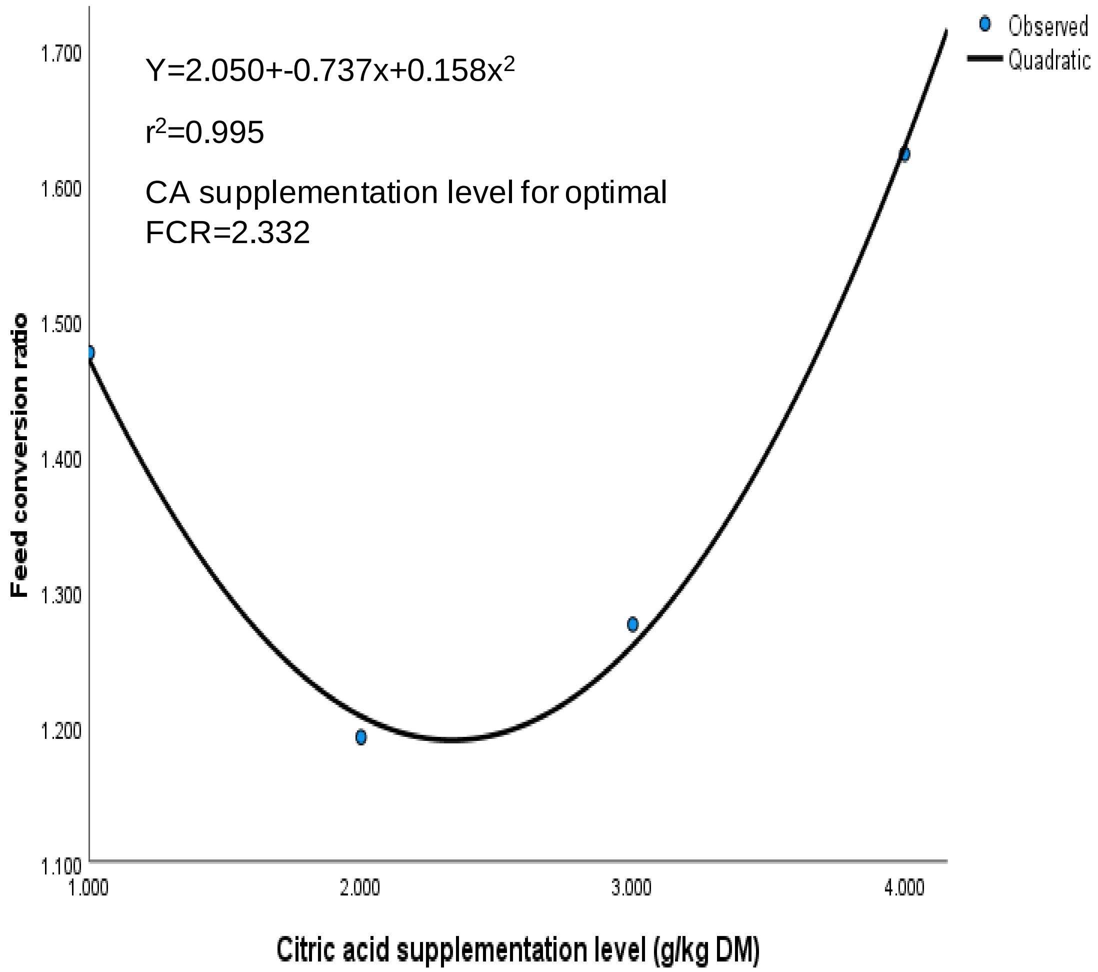


**Figure 4.08** The effect of CA supplementation in diet on FCR of male Venda chicken aged 61 to 90 days old

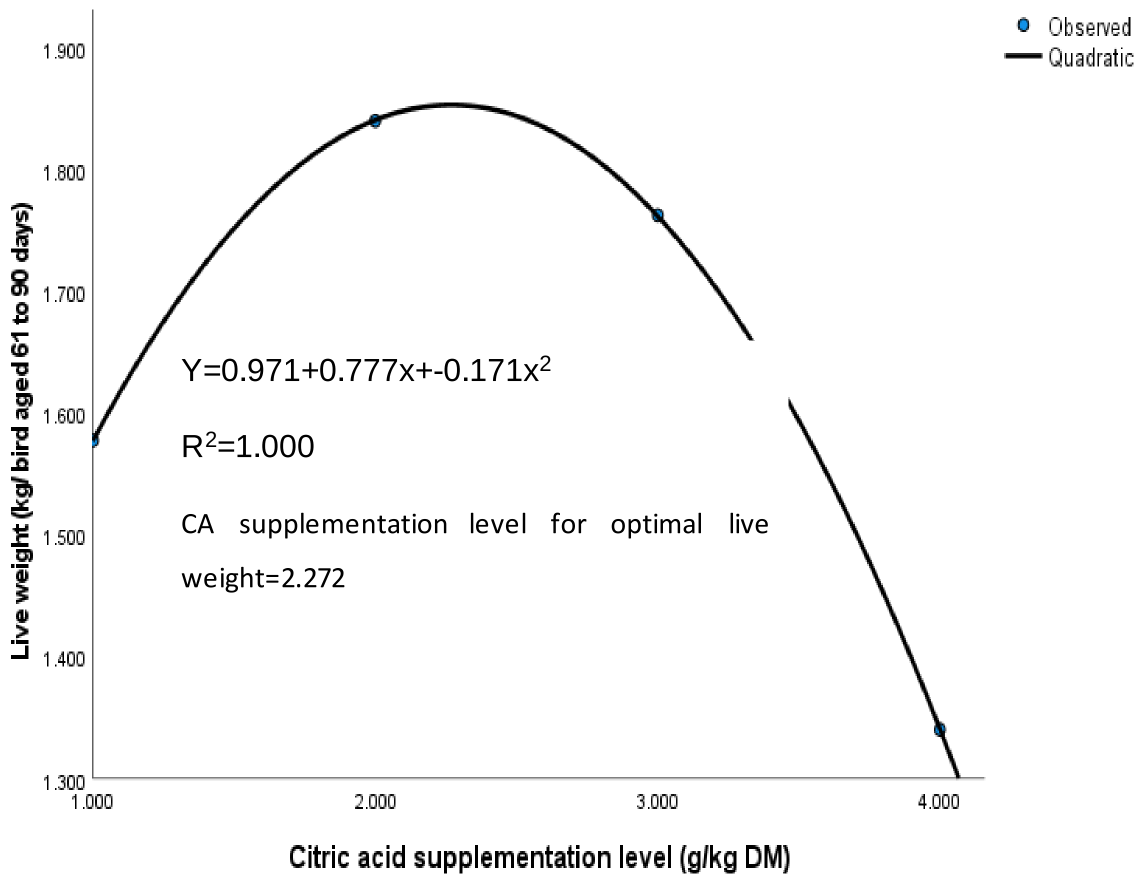


**Figure 4.09** The effect of CA supplementation in diet on Live weight of male Venda chicken aged 61 to 90 days old

**4.4 Effect of citric acid supplementation in diet on gut digesta weights crop, gizzard, pro-gizzard, duodenum, jejunum, ileum, and ceca), gut digesta pH (crop and gizzard pH) and intestines length (cm) (small intestine and large intestine) of male Venda chickens reared from one to 90 days**

The effect of dietary citric acid supplementation on crop, gizzard, pro-gizzard, duodenum, jejunum, ileum and ceca weight(g), crop and gizzard pH and small and large intestine length of male Venda chickens reared from one to 90 days is represented by Table 4.07. Supplementation of citric acid in diet at all levels do not affect ($P > 0.05$) weight of crop; pro-gizzard; duodenum; jejenum; ileum; GIT and the length of small and large intestines of male Venda chickens reared from one to 90 days. However, CA supplemetation significantly influenced ($P < 0.05$) gizzard weight, large intestines, crop pH and gizzard pH of male Venda chickens.

$CA_{25}$ in diet greatly ($P < 0.05$) improved weight of large intestine compared to 12.5g of CA /kg and 50g of CA/kg DM supplementation. However, chickens fed $CA_0$, $CA_{12.5}$ and $CA_{50}$ diets had no significant difference ($P > 0.05$) on weight of large intestine.

Citric acid supplementation in diet at all levels significantly reduced ($P < 0.05$) pH of crop and gizzard, with the lowest pH obtained in chicken supplemented with $CA_{50}$ in both crop and gizzard and the highest pH was seen in chickens fed control diet $CA_0$. These results shows that the more you increase the CA level the more the pH of the crop and gizzard declines in male Venda chicken reared from one to 90 days.

A 1.66g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal large intestine weight of male Venda chickens reared from one to 90 days (Table 4.08 and Figure 4.10) and a 3.84g of citric acid supplementation level per kg DM of the diet was calculated using a quadratic equation to result in optimal gizzard pH of male Venda chickens reared from one to 90 days (Table 4.08 and Figure 4.11).

**Table 4.07** Effect of citric acid supplementation in diet on gut digesta weights (crop, gizzard, pro-gizzard, duodenum, jejunum, ileum, and ceca), gut digesta pH (crop and gizzard pH) and intestines length (small intestine and large intestine) of male Venda chickens aged 90 days old

| | DIET# | | | |
|---|---|---|---|---|
| **Variables*** | $CA_0$ /1kg | $CA_{12.5}$ /1kg | $CA_{25}$ /1kg | $CA_{50}$ /1kg |
| **Gut digesta weights (g)** | | | | |
| Crop | 4.00 ± 1.70 | 5.60 ± 1.67 | 4.10 ± 1.56 | 4.70 ± 1.57 |
| Gizzard | $60.60^{ab}$ ± 9.51 | $52.10^{b}$ ± 5.78 | $64.84^{a}$ ± 5.25 | $54.90^{ab}$ ± 5.91 |
| Pro-gizzard | 6.90 ± 1.52 | 6.70 ± 0.67 | 6.70 ± 0.67 | 5.50 ± 0.50 |
| Duodenum | 10.40 ± 2.22 | 11.30 ± 0.91 | 11.10 ± 1.02 | 10.80 ± 1.04 |
| Jejunum | 13.40 ± 2.86 | 11.80 ± 1.44 | 12.90 ± 1.71 | 11.40 ± 0.65 |
| Ileum | 12.50 ± 2.55 | 13.10 ± 3.70 | 12.00 ± 2.09 | 10.50 ± 0.94 |
| Ceca | 10.00 ± 3.14 | 8.70 ± 1.30 | 8.60 ± 1.14 | 9.60 ±1.52 |
| Large intestine | $20.60^{ab}$ ± 7.45 | $15.30^{b}$ ± 7.09 | $28.50^{a}$ ± 3.39 | $17.00^{b}$ ± 4.30 |
| GIT | 168.80 ± 30.44 | 154.90 ± 15.73 | 179.04 ± 4.08 | 153.90 ± 7.82 |
| **Gut digesta Ph** | | | | |
| Crop | 6.16 ± $0.14^{a}$ | $4.60^{b}$ ± 0.23 | $4.20^{c}$ ± 0.15 | $3.08^{d}$ ± 0.30 |
| Gizzard | $5.43^{a}$ ± 0.27 | $3.12^{b}$ ± 0.18 | $2.55^{c}$ ± 0.26 | $1.90^{d}$ ± 0.15 |
| **Intestine length (cm)** | | | | |
| Small intestine | 114.62± 11.41 | 117.44 ± 10.77 | 113.18 ± 6.07 | 116.04 ± 8.32 |
| Large intestine | 8.80 ± 1.25 | 9.56 ± 1.12 | 9.52 ± 1.28 | 9.56 ± 0.93 |

* : Values presented as a mean ± standard deviation

a, b, c : Means in the same row not sharing a common superscript are significantly different ($p < 0.05$)

# :The treatments were citric acid supplmentation level in the diet ($MCA_0$ ),( $MCA_{12.5}$ ) ($MCA_{25}$), and ($MCA_{50}$) in 1kg of DM of feed.

**Table 4. 08** Effect of citric acid supplementation in diet on weight of gizzard and large intestine and pH of crop and gizzard of male Venda chicken aged 90 days old

| Variables | Formula and answers | X | Y | $r^2$ | Probability |
|---|---|---|---|---|---|
| Large intestine weight (g) | $Y=12.0+7.99x+-1.55x^2$ (20.99;29.53) | 1.66 | 20.99 | 0.096 | 0.951 |
| Gizzard pH | $Y=8.12+3.19x+-0.42x^2$ (14.18; 26.56) | 3.84 | 1.981 | 0.977 | 1.53 |

$r^2$= Coefficient of determination

X= Optimal citric acid level

Y= Optimal variable

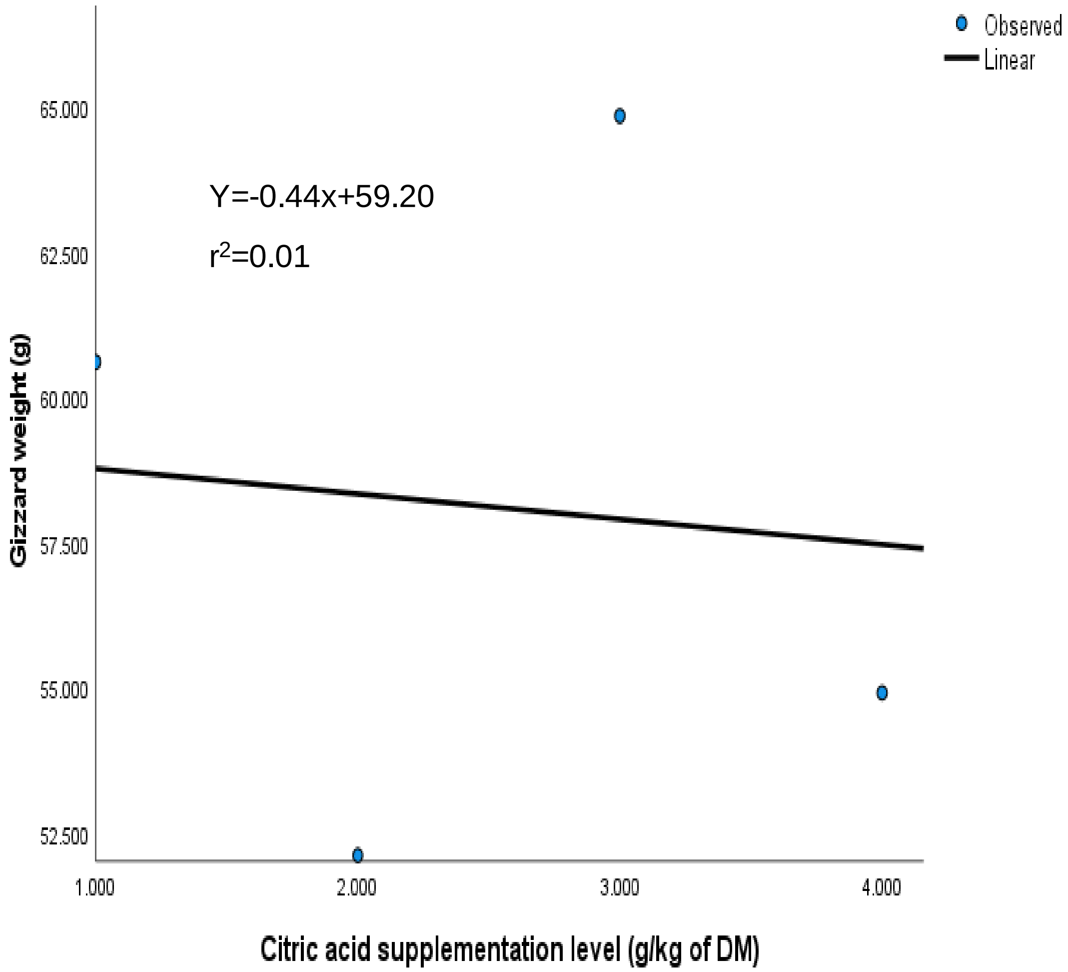


**Figure 4.10** The relationship between CA supplementation in diet on gizzard weight (g) of male Venda chicken aged 90 days

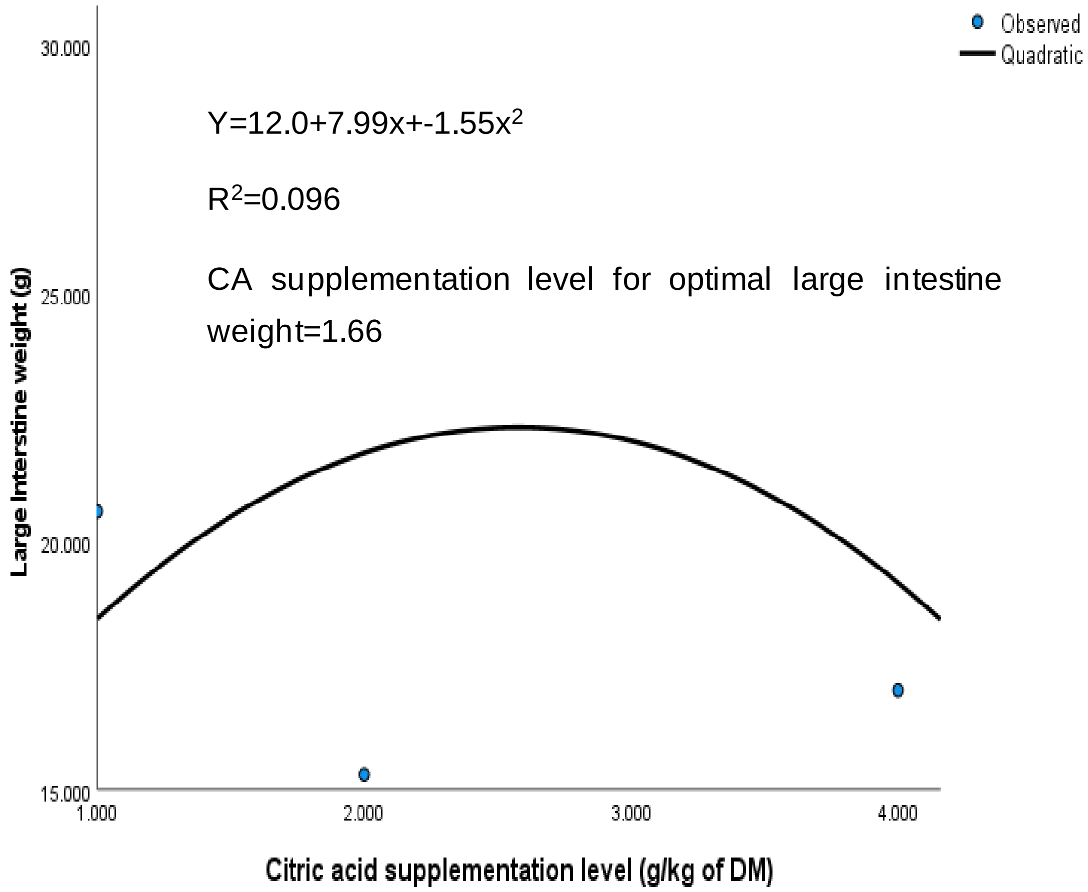


**Figure 4.11** Effect of CA supplementation in diet on large intestine weight (g) of male Venda chicken aged 90 days old

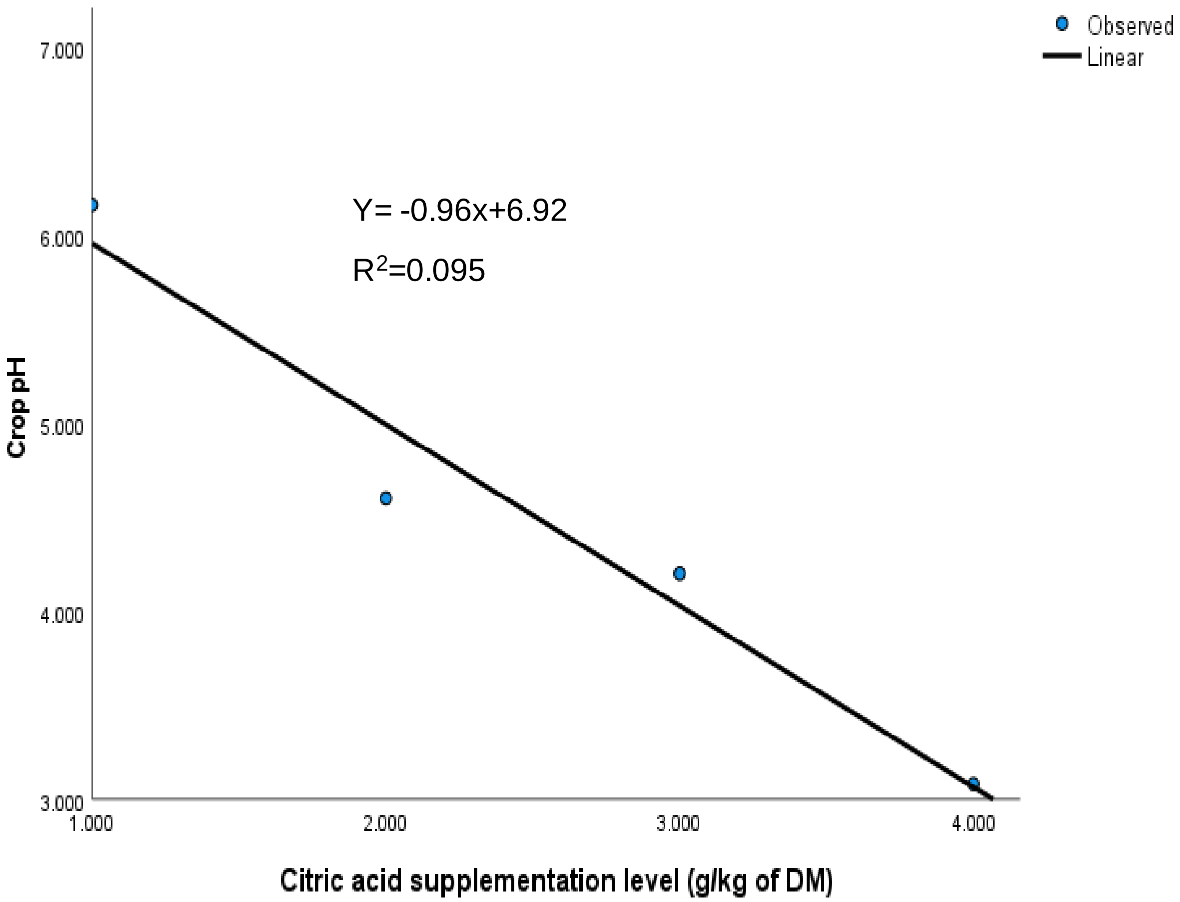


**Figure 4.12** The relationship between CA supplementation in diet on crop pH of male Venda chicken aged 90 days old

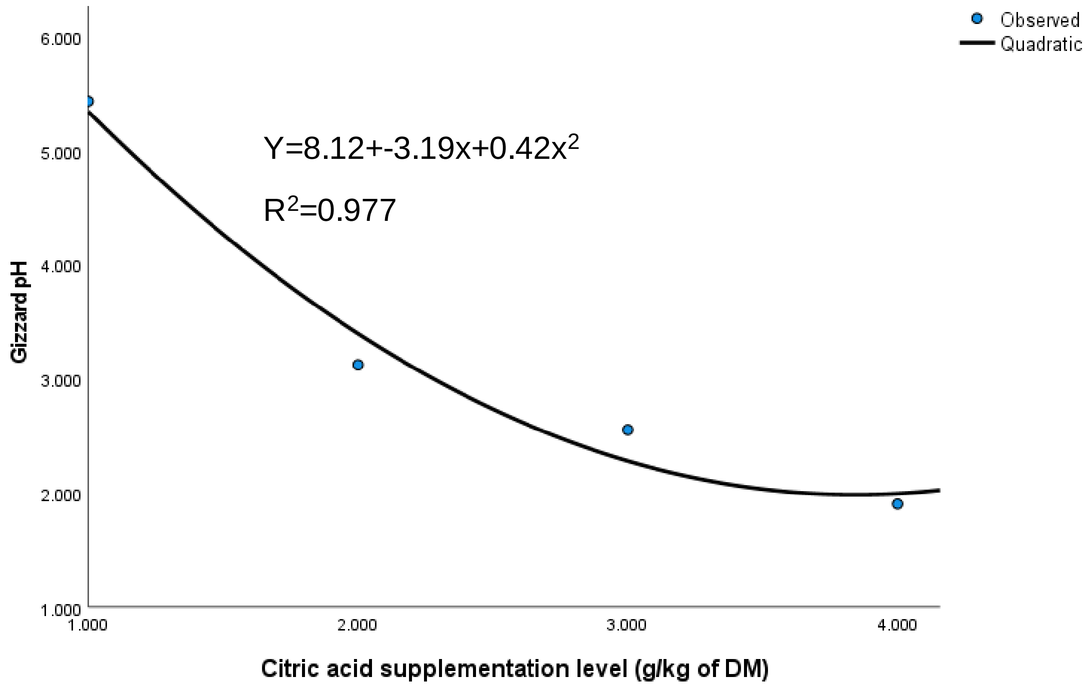


**Figure 4.13** Effect of CA supplementation in diet on gizzard pH of male Venda chicken aged 90 days old.

# CHAPTER FIVE

# DISCUSSION, CONCLUSION AND RECOMMENDATIONS

# DISCUSSION

The current study found that citric acid supplementation at all levels in the diet had no effect on feed consumption of male Venda chickens from one to 30 days (starter phase). These feed intake findings are comparable with those of Adil et al. (2010), who discovered no significant effect of organic acids (butyric acid, butyric acid, fumaric acid, fumaric acid, lactic acid, and lactic acid) on broiler chicken feed consumption. Furthermore, organic acids had no effect on broiler chicken feed intake, according to Hernandez et al (2006). Nguyen and Kim (2020) found that adding citric acid to the diet reduces feed intake in broiler chicks during the beginning phase (1 to 7 days). The disparities in the foregoing findings could be attributed to differences in the nutrient composition of the chickens.

The current study revealed that $CA_0$ supplementation in diet slightly improved ($P$ <0.05) growth rate of male Venda chicken compared to other supplementation levels during the starter phase. This could be because citric acid supplementation in chicken diets alters the nutritional value such as with minerals and vitamins, therefore too much citric acid supplementation level may negatively modify the nutritional value and reduced growth rate. Similarly, Nguyen and Kim, 2020 discovered that increasing citric acid levels reduce growth rate in broiler chicks during the starting phase (1 to 7 days). The lower growth rate with the highest CA inclusion level could be owing to a sour taste caused by higher amount of citric acid inclusion, which limits intake and hence negatively affects growth rate. In contrast, Araujo et al. (2019) discovered that organic acids in broiler chicken have no effect on growth rate. The incongruities among the findings regarding growth rate might be due to different organic acids (lactic, acetic, and butyric acid) used in the diet and may also be influenced by different nutrients composition of the diet.

This study found that adding citric acid to the feed had no effect on the FCR of male Venda chickens aged one to 30 days. This could be because younger chickens had better FCR since they grow faster than older chickens, hence supplementing CA at all levels resulted in no discernible difference in FCR at the younger stage. These findings are consistent with those of Nguyen and Kim (2020), who discovered that organic acid supplementation had no effect on FCR in broiler chicks aged 7 to 21 days. Araujo et al. (2019) and Elmi et al. (2020) found no change in FCR between broilers fed diets supplemented with CA and acetic acid aged 1 to 35 and 1 to 42 days. Furthermore, Omogbenigun et al. (2003) found that organic acids such citric acid had no influence on the FCR of early weaned piglets. In contrast, Salgado et al. (2011) and Islam (2012) found that birds fed a CA supplemented diet had considerably higher FCR than those provided a control diet. Hassan et al. (2016) found that including 1.50% citric acid in a 16% protein diet enhanced FCR in broiler ducks. The discrepancy in FCR findings could be attributed to various CA inclusion levels and different age of chickens.

The current study found that adding citric acid to the meal improves the live weight of male Venda chickens but declines when the CA supplementation amount increases (50g of CA/kg DM) during the beginning phase. This could be because very high CA supplementation levels add a sour flavour to the feeds, resulting in decreased feed intake and, as a result, lower live weight in male Venda chickens. Similarly, Makofane et al. (2022) found that CA has a good effect on the gut, which leads to improved body weight gain, and Salgado et al. (2011) reported that inclusion of 50g of CA/kg DM in the diet inhibits body weight gain. Several researchers have observed an increase in the live weight of chicks fed a CA-enriched diet (Islam et al., 2018; Sharifuzzaman et al., 2020; and Fik et al., 2020). Boling et al. (2000), observed that supplementing the feed with 60g of CA/kg DM raised the live weight of chicks by 22%, which contradicts the current study. The disparities between the findings could be attributed to differences in nutrient composition of the feed and breed type of the chickens.

Citric acid supplementation enhanced feed intake in male Venda chickens aged 31 to 60 days, according to the current findings (grower phase). This could be because CA increases feed flavour and smell, which leads to increased feed intake. Similarly, Langhout (2000) discovered that organic acids in chicken feed increase feed intake. Araujo et al. (2019) found that organic acids have no effect on broiler chicken feed intake. Furthermore, Khosravi et al. (2010) found no effect on feed intake in broiler chicks treated with organic acids aged 1 to 42 days. The differences in feed intake between the aforementioned data could be influenced by the sex, breed, and age of the chickens.

The current study found that FI increased with inclusion levels of 12.5g of CA/kg DM relative to controls and decreased dramatically with increasing CA supplementation levels (25g of CA/kg DM and 50g of CA/kg DM) in male Venda chickens throughout the finishing phase. A variety of factors influence how much feed is consumed, but palatability is the most important. According to Adil et al. (2010), organic acids have a strong sour flavour, which may have reduced the palatability of the feed and resulted in fewer feed intake. Similarly, Cave (1984) found that high dietary CA levels lowered feed tastiness while low levels boosted feed intake in avian species. Organic acids also reduced feed consumption in broiler chicks, according to Leeson et al. (2000). Furthermore, organic acids increase the amount of nutrients available from feed, reducing the amount of feed consumed by quails (Pakhira and Samanta, 2006). Sultan et al. (2015), on the other hand, discovered that varying levels of organic acid throughout the finisher phase in broiler chicks did not significantly change feed consumption. The discrepancies in the above findings could be attributed to differences in experiment duration, seasons in which the studies were conducted, and acidifier supplementation doses.

The current study revealed that $CA_0$, $CA_{12.5}$ and $CA_{25}$ supplementation in diet slightly improved ($P$ <0.05) growth rate of male Venda chicken during the grower phase but decreased with the maximum ($CA_{50}$) supplementation level during grower phase. This may be because too much citric acid in diet makes sour taste of the feed resulting in reduced growth. Similarly, Cave (1984) reported lower growth in avian species fed diet supplemented with higher level of CA. However, Citric acid treatment during finisher phases (day 31 to 90) enhanced the growth rate and FCR of male Venda chickens. This could be because CA improves the nutrient content of the diet, making more nutrients available for absorption at low feed intake, resulting in increased growth rate and FCR in male Venda chickens. Similarly, a food supplemented with citric acid boosted growth rate and feed efficiency in other monogastric animals, such as developing pigs (Upadhaya et al., 2016). Additionally, adding citric acid to drinking water enhanced body weight and feed conversion ratio in broilers during the finisher stage (Sultan et al., 2015). Furthermore, additional research discovered that organic acid supplementation in broiler chicken diets had a significant effect on body weight gain (BWG) and FCR (Owens et al., 2008; Kaczmarek et al., 2016). Reduced unwanted bacteria in the GIT may make more nutrients available to the host animal for absorption, resulting in improved growth performance (Adil et al., 2010). The improvement in growth rate and feed efficiency could be attributed to the expansion of lactobacillus bacteria, which enhances intestinal function, preventing pathogen development and survival due to low pH caused by acid produced by Lactobacillus fermentation (Nguyen and Kim, 2020). Woyengo et al. (2010) and Oryza et al. (2021) discovered, that CA with phytase in the diet had no effect on growth rate or FCR. The differences in the aforementioned data could be related to climatic conditions, such as birds eating more in the winter to produce metabolic heat because it is too cold and eating less in the summer, which could have a major impact on growth rate and FCR.

The current results showed that CA supplementation had an equal effect on live weight during the grower and finisher phases, with higher live weight achieved with 25g of CA/kg DM supplementation, which is significantly different from 50g of CA/kg DM and non-significant to 0g of CA/kg DM and 12.5g of CA/kg DM in male Venda chickens. Similarly, Fascina et al. (2012) observed that broiler chicks fed a diet enriched with cinnamon and CA had enhanced muscle growth. On the other hand, Oryza et al. (2021) found no significant difference in live weight between Thai native broiler chickens fed a CA supplemented diet throughout the experiment. The lower live weight in chicken fed a feed supplemented with 50g of CA/kg DM could indicate acidosis. The disparities in live weight results could be attributed to diverse environmental factors influencing growth performance differently in different breeds of chickens.

Improved feed intake, live weight, growth rate, and FCR of male Venda chickens in the current study as a result of citric acid addition in diet leads to improved growth performance of male Venda chickens. However, gut functions have a huge impact on the growth performance of male Venda chickens (Sugiharto, 2016). Hence, the second objective is to determine the effect of citric acid supplementation in the diet on the intestinal morphology of male Venda chickens.

The current study found that adding CA to the diet of male Venda chickens had no influence on gut digesta weight (crop, pro-gizzard, ceca, and small intestine) or intestine length. Similarly, Fik et al. (2020) found no significant difference in crop, pro-gizzard, or ceca weight in birds fed a CA supplemented diet. In contrast, Hassan et al. (2010) found that CA supplemented diet considerably increased small intestine length while Hudha et al. (2010) found that CA supplemented diet had a detrimental effect on small intestine weights. The disparities in small intestine weight and length could be attributed to differences in breed type and CA inclusion levels.

The current study found that CA supplementation in the food improved the weight of the large intestine but had no effect on the length of the large intestine in male Venda chickens. Similarly, Nourmohammadi and Khosravinia (2015) discovered an increase in bursa of fabricius proportion in birds fed 30 and 60g of CA/kg DM supplemented diets. Fik et al. (2020), on the other hand, found no significant effect on large intestine weights. The unrelated large intestine outcomes could be attributed to the different diets fed to the trial birds.

The current study found that CA supplementation improved gizzard weight and decreased crop and gizzard pH in male Venda chickens aged 90 days. Males naturally grow larger and stronger than females, which could explain the improved gizzard weight. The reason for the lower gizzard pH could be that CA in the meal lowers the diet pH, which could explain the lower gizzard pH in male Venda chickens. The findings of Abdel-Fattah et al. (2008) are consistent with the current study regarding pH; the researcher observed that pH values of GIT segments decrease as CA incorporation in bird feed increases. Citric acid ingestion lowers gastric pH according to Park et al. (2009), ceacal digesta pH according to Jozefiak and Rutkowski, (2005), crop and gizzard according to Andrys et al. (2003), and digesta pH (Dibner and Buttin, 2002). In contrast, Salgado et al. (2011) found that CA inclusion had no effect on the pH of the proventriculus or the gizzard, while Hassan et al. (2016) discovered that 1.50% CA supplementation in a 16% protein diet has no effect on the weight of internal organs (gizzard and liver) in broiler ducks. The disparities in internal organ weight (gizzard weight) and pH values could be attributed to differences in bird breed genetic makeup, buffering ability of the test meal, anticoccidial drugs, microbiota of the GIT, and the environment itself.

## CONCLUSION AND RECOMMENDATIONS

In conclusion, control diet improves growth rate in male Venda chickens aged one to 30 days but the inclusion of 0g, 12.5 g and 25g kg of CA to the diet significantly enhances feed intake, growth rate, and live weight in the grower and finisher phases (31 to 90 days). Citric acid supplementation had no effect on feed intake or FCR in male Venda chickens aged 1 to 30 days (starter phase). However, supplementing 12.5g of CA /kg DM throughout the grower and finisher phases improves FCR the most. Citric acid in the meal has little effect on weight or length of the small intestine, but it significantly lowers the pH of the crop and gizzard in male Venda chickens at 50g of CA /kg DM supplementation. To improve the growth performance of indigenous chickens, 12.5% citric acid in the food is recommended. More research is needed, however, to validate the levels of citric acid application in indigenous Venda chicken.

# CHAPTER SIX

# REFERENCES

# REFERENCES

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