EFFECT OF DIETARY CITRIC ACID SUPPLEMENTATION & USE OF NON-LINEAR MODELS ON GROWTH PERFORMANCE IN VENDA CHICKENS

BY

ZULU BLANTINA FANGELE

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SUPERVISOR: DR. B GUNYA

CO-SUPERVISOR: PROF. TL TYASI

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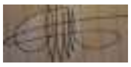
DECLARATION

I declare that this mini dissertation titled “Effect of citric acid supplementation and use of nonlinear models on growth performances in Venda chickens” of Master’s Degree (Agriculture) in Animal Production was carried out by me and submitted to University of Limpopo. This work has not been submitted to this University or other Universities. All the literatures used in this mini dissertation have been listed in the reference section.

Signature:  Date: 02/04/2023

Student name: Zulu BF

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DEDICATION

I dedicate this dissertation to my daughter Constance Nomvula Zulu.

ABSTRACT

The development curve of Venda chicken fed with various amounts of citric acid was evaluated using nonlinear models in an experiment. to ascertain the effects of citric acid supplementation level on feed intake, body weight increase, and linear measurements on Venda chickens. 200 male Venda chickens were used in the experiment which lasted 90 days. The chicks were randomly assigned to four treatments (0, 12.5, 25 and 50g of citric acid inclusion) with 5 replications, resulting in 20 floor pens with 10 chicks per replicate. A completely randomized design was employed as experimental design. Analysis of Variance (ANOVA) was used to determine the effect of citric acid on feed intake, body weight gain, FCR, GR and body linear measurements. Three different non-linear models, namely Gompertz, Weibull, and Richards, were used to define the growth curves of the Venda chickens. The Duncan multiple range test at the 5% level of significance was utilized to detect significant differences between the means. Models were compared using coefficients of determination (R^2) and standard errors (SE). The results indicated that feed intake, body weight, average daily gain and growth rate of Venda chickens were not affected ($p > 0.05$) by citric acid supplementation levels. Similarly, Citric acid supplementation had no effects ($p > 0.05$) on the shank length and wing length of Venda chickens. However, feed conversion ratio (FCR) of Venda chickens was improved ($p < 0.05$) by with an increase in citric acid supplementation. The Venda chicken fed citric acid at grower phase and finisher phase had better growth performances than the starter phase. Citric acid supplementation improved the back length and thigh length of Venda chickens. The Venda chickens fed citric acid at 25g inclusion level significantly higher back and thigh length. The coefficient of determination ranged from 0.00 to 0.98 in all the treatments. The Gompertz Model and Richards Models both exhibited the same coefficient of determination across all treatments. The model with the lowest standard error was found to best describe the growth curve of male Venda across all treatments. The Gompertz model was observed to be suitable for explaining the growth of Venda chickens fed with feed without citric acid (CA_{0g}) and citric acid 125g inclusion ($CA_{12.5g}$). The Richards model was observed to be suitable for explaining the growth of Venda chickens fed citric acid 25g inclusion (CA_{25g}) and citric acid 50g inclusion (CA_{50g}). It is recommended to use a supplement containing lower citric acid to enhance the body linear measurements and growth performance of the chickens. Gompertz and Richards models can be utilized to characterize the growth curve of Venda chickens.

Keywords: chicken, citric acid, growth curve, growth parameters, nonlinear models

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LIST OF ABBREVIATIONS

ADG – average daily gained

ANOVA – Analysis of variance

BD – bulk density

BL – back length

BLM – body linear measurements

BW – body weight

CA – citric acid

FCR – feed conservation ratio

FI – feed intake

GIT -gastrointestinal tract

GR - growth rate

NCSS – number crunchers statistical system software

OA – organic acid

SAS – statistical analysis system

SL – shank length

TL – thigh length

WL – wing length

CHAPTER ONE

INTRODUCTION

1.1 BackgroundIndigenous chickens are the most prevalent livestock species domesticated by resource-constrained rural populations in southern Africa (Mtileni *et al*., 2009). Indigenous chickens (*Gallus domesticus*) are birds that have been raised in large numbers for millennia (Mahendra, 2016). They scrounge for food, have no known description, and are often unimproved (Pedersen, 2002). These chickens serve as a major source of animal protein and perform numerous socio-economic functions in traditional religious and other practices, such as gift payments (McAinsh *et al*., 2004). The produce less meat and fewer eggs in comparison with conventional chickens, they have an important role to play in providing food security and a source of income generation to resource-limited local communities who rely on them at a socio-economic level (Zaman *et al*., 2004).

The poultry business demands birds that can grow quickly and produce a high-quality carcass in a short period of time (Prince, 2002). Growth is defined as an increase in bodily size per unit of time (Porter *et al*., 2010)). The growth and development of chickens can be improved by the use of the additives such as citric acid to their diets (Miles et al., 2006; Pfaller, 2006). It represents an importance economic trait in the poultry industry (Schulze *et al*., 2001). Citric acid has antibacterial activity which protect feed against bacterial spoiling while also lowering the quantities of unwanted bacteria (e.g., *E. coli*.) in the gastrointestinal tract, which can ultimately increase development rate (Falkowski and Aherne,1984; Eidelsburger and Kirchgessner, 1994; Deepa *et al*., 2011). Many linear and nonlinear growth curves have been employed to estimate the growth of living organisms over time (Yakupoglu and Atil, 2001a; b).

1.2 Problem statement

Venda chickens are abundant in South African rural areas (Sil *et al*., 2002). Despite their popularity, Venda chickens are distinguished by their slow growth rate and late maturity (McAinsh *et al*., 2004; Muchadeyi *et al*., 2004). In terms of genetic enhancement, their production has improved only somewhat (Yakupoglu and Atil, 2001; Sengul and Kiraz, 2005). The decreased intake in Venda chickens is caused by the gastrointestinal tract's low pH, which impacts the chicken's feed intake and thus lower growth rate (Mohammed, 2015). The variability in linear body measurements in chicken is caused by genotypic and environmental factors, and the extent of variability varies according on management approaches and environmental conditions (Byarugaba, 2007).

Understanding the relationship between live weight, carcass qualities, and other performance variables in chicken is critical because it allows to anticipate body weight and performance from linear body parts and vice versa (Momoh and Kershima, 2008). The selection of an appropriate model that best depicts an animal's growth pattern is highly debated (Mignon-Grasteau *et al*., 2001). Because these nonlinear models describe growth curve of animal, but each model has different characteristics and limitations (Norris *et al*., 2007). The system that controls and manipulates the feed can boost the growth performance. Many studies (Yami, 1995; Gueye, 1998; Adetayo and Babofunso, 2001; Ijaiya *et al*., 2010) concentrated on determining the effect of citric acid on growth performances but not growth curves as predicted by different nonlinear models, since the nonlinear model can accommodate any form of input, such as varying animal growth over time.

1.3 Rationale

The most popular indigenous breed is Venda chickens (Mtileni *et al*., 2009). They are a dual-purpose breed that produces less egg and meat (Siliga, 2002). Chickens are kept in rural regions as a source of food and revenue. Nonetheless, Venda chickens have a reduced development rate and poorer productivity due to free-roaming and hunting for food, resulting in lesser weight, and they take longer to mature (Magothe *et al*., 2012). Furthermore, lower intake in Venda hens is caused by gastrointestinal system due to low pH, which impacts chicken feed intake and thus lower growth (Mohhammed, 2015).

Because it acidifies the chicken's gastrointestinal tract, citric acid can be given to the diet as a growth promoter (Deepa *et al*., 2011). Citric acid penetrates the bacteria cell wall and disrupts normal pH functioning by making it sensitive to bacteria, causing them to die since they cannot survive an increase in internal and external pH. (Mroz, 2005). However, because it increases nutritional availability to the host animal, it reduces microbial load in the gastrointestinal system and improves weight gain and feed conversion ratio. As a result, higher feed intake (Rahmani *et al*., 2005). Atapattu and Nelligaswatta (2005) found that birds drinking less than 2% citric acid increased their feed consumption.

Citric acid is used all over the world as a growth promoter, thus its productivity and accessibility are higher (Saxena, 2011). It influences feed costs to be lower because it is more popular and has more competition (easily accessible) in markets, while benefiting from higher growth. According to recent research, the addition of 0.5% citric acid raises diet costs while increasing production due to improved growth and feed efficiency (Islam (2012) and Nourmohammadi et al. (2016)). Apart from body weight, various conformation features are thought to be good indications of body growth and market worth of chickens. The association between linear body parameters gives useful information on animal performance and carcass value. The growth curve is useful in characterizing an animal's growth pattern since it can be approximated by using the animal's daily feed intake for growth (Abbas *et al*., 2014). Many growth functions have been developed to describe and fit the nonlinear relationship between animal development and age (Kuhi *et al*., 2003). Nonlinear models such as Gompertz, Weibull, and Richards have been used to suit the growth characteristics of chickens (Norris et al., 2007; Olawoyin, 2007; Magothe et al., 2010; Rizzi et al., 2013; Osei-Amponsah et al., 2014).

Relationships between body weight and body linear measurements are critical in estimating growth performance and can be employed in animal product selection and breeding programs for optimal production. Some studies utilize body linear measurements to predict animal body weight (Attah *et al*., 2004; Sowande and Sobola, 2007; Goe, 2007). However, there is no information on the effect of citric acid supplementation and use of nonlinear models on growth performance in Venda chickens. As a result, the current research will assist farmers in understanding the strategy or approaches that can be employed to adjust feed. The current study will help communal farmers by offering knowledge on growth boosters that will improve feed intake and body weight gain. The current study will also provide data and information on Venda chicken growth performance utilizing various nonlinear models.

1.4 Aim

The aim of the study was to detect the effect of citric acid supplementation level on growth traits of Venda chickens and ascertain the optimal non-linear growth curve model.

1.5 Objectives

The objectives of the study were to:

I. Determine the effect of citric acid supplementation levels on FCE and FCR of Venda chickens.
II. Determine the effect of citric acid supplementation levels on body weight and body linear measurements of Venda chickens.
III. Identify the best nonlinear model that could be used to explain the growth curve of Venda chickens under optimal citric acid supplementation levels.

1.6 Hypotheses

The hypotheses of the study were as follows:

I. Dietary supplementation levels of citric acid have no effect on the FCE & FCR of Venda chickens.
II. Dietary supplementation levels of citric acid have no effect on the body weight, thigh length, back length, wing length and shank length of the Venda chickens.
III. The growth curve of Venda chicken computed from Gompertz, Weibull and Richards non-linear models under varying citric acid supplementation levels have the same effect.

CHAPTER TWO

LITERATURE REVIEW

Part of this chapter was published as a review paper in a peer-reviewed journal (Advances in Animal and Veterinary Sciences).

2.1 Introduction

This literature review discussed the following subtopics: descriptive characteristics of indigenous chickens in south Africa, indigenous chickens' production in Southern Africa, nutritive value of citric acid, use of citric acid in poultry diets, citric acid as a growth promoter in poultry, citric acid as an alternative to antibiotics, effect of citric acid on feed intake, body weight gain, body linear measurement traits of indigenous chickens, financial sustainability of using citric acid in indigenous chickens and common nonlinear growth curve models.

2.2 Descriptive characteristics of indigenous chickens in South Africa

The term "indigenous chicken" refers to chickens that have adapted to their surroundings. Indigenous chickens are domestic creatures that can tolerate harsh cold and heat, as well as wet and dry conditions, whether in cages or free roaming on treetops (Mammo *et al*., 2008). According to Van Marle-Köster *et al*. (2009), the fowl found in rural Southern Africa are mostly named and classified according on their phenotypic and geographical location.

Table 2.1 Descriptive characteristics of indigenous chickens

Breed	Distribution	Phenotypic characteristics	Production Data	References
Naked Neck	Introduced to Africa by traders from Malaysia	Very colourful Naked Neck major gene, plain head and single comb, with medium-size wattles in females and highly-developed wattles in males	Increased weight gain and dressing percentage, adaptation to high environmental temperatures has superior egg production and egg quality and resistance	Ajayi, (2010); Mammo *et al*., 2020;

			against disease	
Venda	Southern Africa	Venda White and black/white & brown plumage, green on feather tips	Quality of egg production, self-sustainment, resistance against diseases, low need for food and broodiness	Van Marle-Köster and Casey (2001); Mammo *et al.,* 2020
Ovambo	Ovambo Northern part of Namibia and Ovamboland	Brown & black plumage, aggressive birds	High egg and meat production	Van Marle-Köster and Casey (2001); Nhleko *et al*., 2003; Grobbelaar *et al*., 2010.
Potchefstroom Koekoek	Southern Africa, Potchefstroom Research Station 1950's	Its black and white striped feathers, with distinct patterns in the roosters and hens	. Lays brown-shelled eggs with an average weight of 55.7 g hatchability rate reaches up to 78%.	Grobbelaar and Fourie, (2006); Tadelle and Fasil, (2016) Mammo *et al.,* 2020
Boschveld	Mantsole ranch in the Limpopo province of South Africa in 1998	Light brown and white feathers	High egg production ability and good meat quality	Ajayi, (2010); McCullough, (2017); Hans, (2020)
Tswana	Botswana	Vary with colours: black, frizzle feathers, typical brown mixed with white colour	Low egg production, egg size range between 38–60 g	Machete, (2017)

2.3 Indigenous chickens' production in Southern Africa

In most rural areas, free-range chicken farming is not commercialized (Manyelo *et al*., 2020). Indigenous chickens are mostly raised as part of mixed farming in extensive systems, and to a lesser extent in semi-intensive systems, as shown in Table 2.2. The free-range poultry production system is characterised by low productivity and low input. The productivity is dependent on the genetics of the stock, the effectiveness of disease control, the quality of supplementary feeds and the availability of pastures (Mapiye *et al*., (2018)). Intensive farming system is characterised by large scale production systems have large number of birds (>10000), have been remained to rely on importation highly producing exotic chickens as the indigenous chickens are fully characterized and improved to the level of commercialization (Nyaga.,2009; Embet *et al*., 2010). The semi-intensive production is medium scale chicken production systems produce from 1,000 to 10,000 chicks and the number of medium scale chicken producing the exotic chickens (Nyaga., 2009; FAO., 2008). Rural residents can use indigenous chickens to convert readily available feed supplies around the house or hamlet into exceptionally nutritious items such as meat and eggs. According to Liswaniso *et al*. (2020) local chicken farming in southern African countries is still in its infancy. In Zambia, for example, only 0.5 % of the total chicken population is sold commercially, with the great majority consumed at home (MFL 2019, Gueye, 2020;). Despite their importance, local chickens have gotten little attention in terms of increasing production rates (Mtileni *et al*., 2012). The rural poultry sector, which is predominantly made up of indigenous chickens, accounts for around 98 percent of the total chicken population (FAO, 2007) and is primarily made up of indigenous chickens (Moreda *et al*., 2013). Several studies have been conducted to improve the productivity of local chickens through efficient feeding (Table 2.3). According to Alabi (Alabi *et al*., 2013) protein, lysine (a base for all other amino acids) and energy requirements of local chickens must be met optimally in order to improve and maximise productivity.

Table 2.2: Indigenous chicken's production system

Production system	Mapiye *et al*., (2018)	Assefa *et al*., (2016)	Tadela *et al*., (2019)
Scavenging/Free range	45(28.1)$^{2+}$	79.6%	100%
Semi-intensive	74(46.2)$^{1+}$	-	-
Cage or confined	45(28.1)$^{2+}$	-	-
Extensive	15(9.4)	-	-

Table 2.3: Recommended protein and energy for improved productivity and performance among local chickens.

Nutrient Level	Improved Production Parameter	Indigenous Chickens Age	Researchers
Protein			
16%	Feed intake per bird increased with increasing dietary protein level	Between 14 and 21 weeks	Kingori *et al*., 2007
17 to 23%	Had similar growth rates and feed intakes	1 and 6 weeks	Ndegwa *et al*., 2001
18–19%	Improved growth and productivity	Between one and six weeks	Mbajiorgu,2010
18%	Improved weight gain	Between one and 13 weeks	Kalinda and Tanganyika (2017)
15.53%	Improved carcass weight and percentage	14 weeks	Charles *et al*., 2017
Energy			
14 MJ ME/kg	Improved growth and productivity	Between one and six weeks	Mbajiorgu,2010
12.34 MJ ME/kg	Improved feed intake,	Between one and	Alabi *al et*., 2013

	growth rate and feed conversion ratios	seven weeks	
12.91 MJ ME/kg	Improved feed intake, growth rate and feed conversion ratios	Between eight and 13 weeks	Alabi *al et*., 2013
2750 kcal/kg ME	Improved growth performance	Between nine and 20 weeks	Mohammad and Sohail (2008)
2842–3200 kcal/kg ME	No significant difference in growth performance parameters	Between six and 9 weeks	Ndegwa *et al*., 2001

2.4 Nutritive value of citric acid

The citric acid-producing organism requires certain trace metals for growth and metabolic reaction. The metals that must be limiting consist of Zn, Mn, Fe, Cu, heavy metals. Fe2+, Mn2+, Zn2+, Cu2+ are identified to be inhibitory to the production of citric acid by Aspergillus niger in submerged fermentation (Yadegary *et al.,* 2013; Sawant *et al*., 2019). Citric acid production by Aspergillus niger from submerged fermentation is very sensitive to trace metals present in starchy and molasses medium. As a result, the concentration of these heavy metals should be reduced in proportion to the concentration of optimal development and maximal citric acid synthesis. Max *et al*. (2010) discovered that low quantities of phosphate result in the highest citric acid synthesis. This effect operates at the level of enzyme activity rather than gene expression. However, although the maximum lead of phosphate to a decrease in carbon dioxide fixation, which might enhance the foundation of specific sugar acids as well as drive development (Soccol *et al*., 2006). Fermentation at phosphorus concentrations ranging from 0.5 to 5.0 g/L is required for optimal citric acid generation.

Potassium dihydrogen phosphate has proven to be a successful source for high-quality citric acid manufacturing. Carbohydrate type and concentration are also crucial elements in determining the synthesis of the desired product. Unlike the influence of other factors, the relative effect of sugar concentration on the main fermentation parameters with filamentous fungi was studied (Ali *et al*., 2016). However, Anwar *et al*. (2009) believe that the high sugar content of fermented medium is favorable for increased citric acid synthesis. According to Chundakkadu *et al*. (2005), the nitrogen source employed in the fermentation directly correlates with the synthesis of citric acid, and ammonium salts such as urea, ammonium chloride, and ammonium sulphate are favoured. Feed makers utilize a variety of pro-nutrients and growth promoters to maximize genetic potential by converting feed to gain more efficiently in a shorter period of time.

2.5 Use of citric acid in poultry diets

Citric acid (CA) is widely utilized in poultry diets to enhance growth by acidifying gastrointestinal contents, increasing nutritional digestibility, and decreasing pathogen burdens (Min *et al*., 2007). It promotes growth by acidifying the gastrointestinal (GI) content and is a key determinant of inadequate nutrient digestion (Boling *et al*., 2000). Furthermore, it affects intestinal pH, improves performance, and increases the solubility of feed materials, as well as the digestion and absorption of nutrients (Nourmohammadi and Afzali, 2013). Improved digestion efficiency cannot be attributed solely to gastrointestinal anatomical changes. Citric acid has been examined for its antibacterial properties in chickens (Patten and Waldroup, 1988). Temperature, oxygen availability, redox potential, and pH all have a significant impact on biogenic amine synthesis (Min *et al*., 2007). Citric acid can limit microbial growth in foods, lowering the BA level. Organic acids, such as citric acid, have mostly been used to sterilize feed in order to avoid salmonella infections in animals (Thompson and Hinton, 1997). Their effect on pathogenic growth in animal diets may also increase digestion, absorption, mucosal immunity, and topical actions on the intestinal brush boundary (Mroz, 2005).2.6 Citric acid as a growth promoter in poultry

Citric acid has enough antibacterial activity to protect feed against bacterial spoiling while also lowering the quantities of unwanted bacteria (for example, E. coli.) in the gastrointestinal tract, which can ultimately enhance development rate (Falkowski and Aherne 1984; Eidelsburger and Kirchgessner 1994; Deepa *et al*., 2011). Cave (1984) observed that high amounts of citric acid could significantly reduce feed palatability, but low levels boosted feed intake in avian species. According to Daskiran *et al*. (2004), early exposure to dietary acidifiers may produce adaptation in birds and diminish the subsequent therapeutic activity of the acidifier. As a result, they advocated using acidifiers in the grower phase rather than the starting phase to avoid economic losses caused by heat stress. However, Nhleko *et al*. (2003) indicated that indigenous hens have a better immune system and are less susceptible to heat stress. Shen Hui Fang *et al*. (2005) demonstrated the best feed conversion ratio in growing hens with the addition of 0.3% CA. A dose titration study of citric acid in diets discovered that up to 6% maintained live weight, although feed intake was lowered by 1.5% and feed conversion efficiency increased up to 6%. (Islam *et al*., 2011c). The greater inclusion level of 7.5% resulted in growth inhibition rather than toxicity. Growth boosters are increasingly recognized as feed additives in the broiler sector for faster growth and more economical meat production (Bhuyan *et al*., 1977). They also increase feed use efficiency (Milligan *et al*., 1955). Citric acid acts by lowering the pathogenic bacteria burden, which lowers the gut pH.

2.7 Citric acid as an alternative to antibiotics

Although organic acids cannot completely replace antibiotics in the development of particular immunity and illness prevention, they can be called growth boosters (Deepa *et al*., 2011). Some experiments have been undertaken to assess the viability of using citric acid instead of antibiotics to improve broiler performance. Haque *et al*. (2010) discovered that 0.5% citric acid feeding promotes weight gain, feed intake, tibia ash deposition, non-specific immunity, feed efficiency, and carcass yield. Several antibiotics are permitted to be used as growth promoters in chicken production (Jones and Ricket, 2003). Sub-therapeutic antibiotic doses in broiler feed have boosted feed efficiency, however the continued use of these antibiotic growth promoters has lingering consequences on their products, such as broiler meat. Because the antibiotic residue increases resistance and cross-resistance to diseases in the animal body and in people, it is now regarded as a public health hazard (Botsoglou and Fletouris, 2001).

There is evidence that antibiotic resistance genes (plasmid pBR322) can be passed from animal to human microbiome (Greko, 2001). Probiotics, prebiotics, organic acids, herbs, and herbal products are some antibiotic replacement approaches in poultry production (Fuller, 1989; Chaveerach *et al*., 2004). Organic acids, among other things, serve in chicken not only as a growth booster (Abdel-Azeem *et al*., 2000; Fushimi 2001; Abdo 2004), but also as a useful tool for reducing all intestinal bacteria, pathogenic and non-pathogenic (Naidu 2000; Wolfenden *et al*., 2007). Citric acid is an organic acid that has been shown to reduce feed intake while increasing daily weight gain and feed conversion efficiency in broilers (Deepa *et al*., 2011).

Citric acid is harmless for humans and can be utilized as a growth booster in broiler production based on these facts. Herbal feed additives are another option to antibiotic growth promoters (AGPs). Because of their antibacterial characteristics, this can be employed in poultry diets (Dorman and Deans, 2000). Many plants and their bioactive components have antibacterial properties (Lewis *et al*., 2003). It can improve digestion by stimulating endogenous enzyme activity and nitrogen absorption (Gill, 2001), as well as inhibiting smell and ammonia control (Varel, 2002).

2.8 Citric acids on indigenous chicken production

2.8.1 Effect of citric acid on feed intake of indigenous chicken breeds

Nutrition and growth are two closely connected and complimentary areas that are studied in applied physiology (Alabi *et al*., 2013). Many researchers are interested in gut issues because they are the main component of the body and are responsible for digestion and absorption. All animals, including birds, have a dynamic digestive system that regulates itself based on physiological requirements and current circumstances. The gastrointestinal tract (GIT) influences chicken feed intake, which is impacted by a variety of factors, including intestinal pH. (Farner, 1942). The rather acidic pH of the avian GIT is also affected by factors such as the chicken's health, the type of nutrients, and, most importantly, the bacteria content of the GIT. Indigenous chickens, like the Indigenous chicken, are scavengers who do not always achieve their nutritional needs. However, there is a mutual relationship between pH and bacteria content, as well as microbiota and nutrition (Sarra *et al*., 1985).

The pH level in specific parts of the GIT determines a specific microbial community and influences the digestibility and absorptive value of most nutrients. The majority of pathogens thrive at pH levels near to or slightly higher than 7. Beneficial bacteria, on the other hand, thrive at an acidic pH (5.8-6.2) and compete with pathogens (Ferd, 1974). Furthermore, decreasing the pH of the GIT using organic acids like citric acid promotes nutrient absorption (Boling *et al*., 2001). The use of antibiotics in chicken rations began 60 years ago (Moore *et al*., 1946), and currently there are various antibiotics that can be used as growth promoters in poultry production (Jones *et al.,* 2003), such as citric acid. Citric acid reduces microbial burden in the GIT and improves weight gain and feed conversion ratio by making more nutrients available to the host, resulting in increased feed intake. Table 2.4 shows the effect of citric acid on feed intake. According to Khooshechin *et al*. (2015), including OA at 3 g kg^{-1} significantly enhanced Average Daily Feed Intake.

The beneficial effect of acidifiers, such as OA, on performance is related to a more efficient use of nutrients and digestibility improvement (Nourmohammadi *et al.,* 2012). On the other, hand Shariffuzzan *et al.* (2020) found that the highest feed intake was observed in birds given 0.75% CA and depressed feed intake was observed on a higher level of CA application (1%CA) as shown in Table 2.4. Similarly, with the findings by Islam *et al*. (2008) found that the feed intake is higher by addition of CA. Islam *et al*. (2008) reported average feed intake was lower in treatment A(control) and higher in treatment D (0.5% CA+ 0.5% Acetic acid).

Table 2.4: Effect of citric acid on feed intake(kg) of chickens

Treatments	Feed intake	References
0%CA	1442±52	Shariffuzzan *et al.* (2020)
0.5%CA	1450±55	
0.75%CA	1470±62	
1.00%CA	1430±27	
BD	1931±42.1	Khooshechin *et al.* (2015)
BD+10Ag Kg^{-1}	1885±42.1	
BD+20 Ag Kg^{-1}	1905±42.1	
BD+30Ag Kg^{-1}	2012±42.1	
Control	2913±142.90	Islam *et al.* (2008)
0.5 Citric acid	3118.6±126.99	
0.5 Acetic acid	3029±223.88	
0.5 Citric acid+0.5 Acetic acid	3101±106.8	

2.8.2 Effect of citric acid on body weight gain of indigenous chicken

Due to a lack of understanding of livestock production systems, advancements in livestock and animal health have not necessarily resulted in long-term advances in farmer welfare or animal productivity. The multiple activities of livestock, as well as the intricate linkages between animal health, nutrition, breeding, and biotechnology, necessitate a systems approach to resource optimization (Kaasschieter *et al*., 1992). As a result, multi-stakeholders focused their efforts on increasing the environmental sustainability of cattle through better measures and methodologies, such as acidification and product fermentation, which provide higher keeping quality (FAO, 2019). Growth and nutrition are closely related and complementary topics in applied physiology. The adoption of various methodologies to investigate this link can improve animal output (Rahmani and Speer 2005; Abdelrazek *et al*., 2016). The pursuit of optimal broiler performance has resulted in the quest for alternative growth promoters, particularly in light of the prohibition on the use of antibiotics as growth promoters, such as citric acid (Fascina *et al*., 2012).

As a result, researchers have created physiological supplements such as organic acids like citric acid to boost immunity and performance. According to Khan (2016), these chemicals help animals develop normal physiological functioning or alleviate deficits. Organic acids are a type of weak acid that improves intestinal function. Correct use of these compounds in conjunction with proper nutrition, management, and biosecurity measures confers several benefits, including improved protein digestion, which leads to improved feed conversion ratio (FCR), growth performance, and immunity, as well as improved mineral absorption from the intestine (Nourmahammadi *et al*., 2012; Wickramasinghe *et al*., 2014). According to Fik *et al*. (2021) and Sharifuzzan *et al*. (2020), citric acid boosted body weight gain in hens, as indicated in Table 2.5. This was supported by Islam *et al*. (2018) and Chowdhi *et al*. (2009), who discovered that feeding birds citric acid-rich diets enhanced their body weight gain. Citric acid's positive effect on gut flora is most likely responsible for the improved body weight increase.

Table 2.5: The effect of citric acid on body weight gain(g) of chickens

Treatments				Reference
T1	T2	T3	T4	
846±38	906±68	926±60	882±41	Sharifuzzan *et al*. (2020)
64.49±5.54	66.83±5.69	66.06±5.73	66.38±5.57	Fik *et al.* (2021)

T0::0%CA, T1:0.5%CA, T2:0.75%, T3:1%CA, T4:1.5%CA

2.8.3 Effect of citric acid on body linear measurement traits of indigenous chickens

Small scavenging enterprises characterize poultry production in most rural areas of South Africa. The majority of the poultry in these farms are indigenous birds with low production. Body linear measurements are commonly used to select or determine the animal's body weight. There are more carcasses from birds fed citric acid-containing diets (Abdel-Fattah *et al*., 2008; Ebrahimnezhad *et al*., 2008). Other studies have showed numerical improvements in carcass, implying that the birds' body linear measures will likewise rise or become larger (Nourmohammadi *et al*., 2010). Organic acids, like as citric acid, are effective in increasing bird body linear measurement.

2.8.4 Financial sustainability of using citric acid in indigenous chickens

Citric acid is widely used as a food additive around the world; therefore, its production and availability are plentiful. As a result, its impact on feed costs would be minimal, but gains from increased growth and lower mortality might be realized. According to Sharifuzzaman *et al*. (2020), the feed cost per bird was highest in the bird with 1.0 percent citric acid added and lowest in the T0 control with 0 percent citric acid added (Table 2.6). The feed cost per bird in the treatment with citric acid was greater because the feed consumption was raised with the supplementation of citric acid. The cost per kg live weight of broiler was highest in the control group, while the cost per kg live weight of broiler was lowest in the citric acid treatment group. According to recent research, adding 0.5%CA to the diet increased diet expenses while increasing production profitability due to increased growth and feed efficiency (Islam *et al*., 2008). Islam *et al*. (2011b) agreed with this conclusion of increased profit in CA fed chickens. Other investigations discovered that adding CA to broiler production increased profitability when compared to an unsupplemented (control) group (Tolba, 2010).

Table 2.6: Financial sustainability of using citric acid in indigenous chickens

Parameters	Dietary treatments			
	T1(0%CA)	T2(0.5%CA)	T3(1%CA)	T4(1.5%CA)
Cost/kg feed	28.02±0.00	29.67±0.00	30.00±0.00	31.12±0.00
Chicken price	45	45	45	45
Feed intake kg/bird	1.60	1.60	1.64	1.57

Cost (Feed/broiler)	44.83±0.80	47.47±1.10	49.2±1.20	49.86±1.20
Cost (feed+chick)/broiler	89.83±0.80	92.47±0.82	94.20±0.85	93.8±0.80
Other cost	25±0.00	25±0.00	25±0.00	25±0.00
Total cost/bird	114.83±0.85	117.47±0.90	199±0.80	118.86±0.81
Cost/kg live weight	115.76±1.07	111.87±1.07	111.40±1.02	115.39±1.08
Sale/bird	124±0.00	131.25±0.00	133.75±0.00	128.75±0.00
Profit/bird	9.17±2.01	13.78±2.10	14.55±2.30	9.86±1.60

Source (Sharifuzzaman1 *et al*., 2020)

2.9 Common nonlinear growth curve models

Non-linear models that are generally used to describe growth curve of animals include Gompertz, Weibull and Richards as used in the study. Non-linear models are more preferred than linear models because the growth of an animal has a sigmoidal shape (Rashad *et al*., 2022). Growth curves have a sigmoidal shape, which allows them to be analysed using non-linear mathematical models (Omotosho *et al*., 2020; Abdelsattar *et al*., 2021). These models provide a chance to abstract data required, understand biological patterns of growth into small set of variables that can be useful when obtaining other important growth characteristics (Teleken *et al*., 2017). The selection criteria used to evaluate the model that best describe the growth curve are mean square error (MSE), coefficient of determination (R^2), percent convergence, Akaike information criterion (AIC), and Bayesian information criterion (BIC) (Omotosho *et al*., 2020; de Sousa *et al*., 2021; Rashad *et al*., 2022).

2.9.1 Gompertz model

The Gompertz model is one of the most frequently used sigmoid models fitted to growth data and other data (Aggrey,2002). Researchers have fitted the Gompertz model to everything from plant growth, bird growth, fish growth, and growth of other animals, to tumour growth and bacterial growth and the literature is enormous (Paine et al., 2012). Some of the re-parametrisations of the Gompertz model found in the literature are more useful than others because they have easy interpretable parameters. One valuable and commonly found re-parameterisation is:

W_t = A* exp(-1*exp(-kt))

Where: W_t = corresponding weight at the time, A = mature or asymptotic weight, B = integration constant, k = maturity rate, t = age of the chickens.

2.9.2 Weibull model

The Weibull model is a flexible and simple function with great potential for application to biological data (Brown,1987). It is often suitable where conditions of strict randomness of the exponential distribution are not satisfied (. The Weibull has been used by several authors for analyzing and describing seed germination (Grsoy et al., 2017). The function's parameters of:

W_t = A- (A - B) exp-(kt)

Where: W_t = corresponding weight at the time, A = mature or asymptotic weight, B = integration constant, k = maturity rate, t = age of the chickens.

2.9.3 Richards model

Richards growth model is the of growth model used to predict the growth process and derive growth parameters (Amir,2013). the Richards growth model fitted is more flexible in describing asymmetrical growth patterns of the dry matter and age data in term coefficients of determination, mean square error, mean absolute percent error for prediction of future or past growth rates (Pommerening and Muszta(2015)).

Wt = A (1 – B * exp(-kt) ^m

Where: W_t = corresponding weight at the time, A = mature or asymptotic weight, B = integration constant, k = maturity rate, t = age of the chickens, m = shape parameter that determines the time and the weight at inflection point.

2.5 CONCLUSIONS

Even when the specific effect of citric acid on indigenous chicken feed intake, body weight gains, and body linear measurements using nonlinear models. It has been well documented that organic acids such as citric acid can acidify the gastrointestinal system of the chicken, improving feed intake and growth performance, including body weight gain and body linear measurements. Common nonlinear model (Gompertz, Weibull and Richards) can be used to identify the best growth curve. Citric acid can improve the gastrointestinal system of chickens, resulting in increased feed intake and the chicken's ability to meet their requirements. As a result, an increase in feed intake will result in an increase in body weight gain and body linear measurements, which are occasionally used to anticipate the animal's body weight.

CHAPTER THREE

METHODOLOGY AND ANALYTICAL PROCEDURES

3.1 Study site

The experiment was conducted at the University of Limpopo Aquaculture Unit, Limpopo Province of South Africa. The University of Limpopo Aquaculture Unit lies at latitude 27.55°S and longitude 24.77°E. The ambient temperatures of the study area range between 20°C and 36°C during summer and between -5°C and 28°C during winter. The mean annual rainfall of the study area is less than 400 mm (Shiringani, 2007).

3.2 Acquisition of materials and chickens

Angel feed in Polokwane, South Africa, supplied the day-old Venda chicken and feeds. Virokill disinfectant, vaccines, medication, injections, 250 watts infrared lights, feeders and drinkers were purchased at NTK, in Polokwane, South Africa. Citric acid from a reputable laboratory supply company was used. The chicks were transferred in the morning by a well-ventilated transport (Van) from Angel feeds to the University of Limpopo, Aquaculture unit, a distance of approximately 30.9 kilometers.

3.3 Preparation of the house

The experimental house was thoroughly cleaned using water and virokill disinfectant. Before splitting the house, it was allowed to dry for roughly 7 days to break down the life cycle of any disease-causing organisms that were not killed by the disinfectant. The house was separated into 20 equal-sized floor pens after adequate drying (2m2 each). The floor was covered with 5cm of fresh sawdust. The drapes served as a means of ventilation. The house was heated using infrared lights rated at 250 watts. For biosecurity, a footbath at the chicken house's entrance was used.

3.4 Experimental diet and design and procedures

A total of 200 male Venda chickens were used in this study. The Venda chicks were randomly assigned to four treatment groups (Table 3.1) of citric acid supplementation level of 0g or feed without citric acid given (CA_{0g}), citric acid 12.5g inclusion ($CA_{12.5g}$), citric acid 25g inclusion (CA_{25g}) and citric acid 50g inclusion

(CA_{50g}) in a completely randomized design with five replicates of ten Venda chicks in each (Table 3.3). The treatments of varying levels of citric acid were indicated as CA1 (0g/kg of diet), CA2 (12.5g/kg of diet), CA3 (25g/kg of diet) and CA4 (50g/kg of diet). The experiment was carried out for 90 days. The chickens were fed a starter diet (30 days), grower diet (30 days) and finishers diet (30 days). The diets were iso-energetic and iso-nitrogenous. A control maize-soybean meal-based diet was prepared based on National Research Council (NRC 1994) recommendations. The experimental diet (Table 3.02) was formulated to meet nutritional requirements of Venda chickens and it was isoenergetic and iso-nitrogenous (12.14 MJ ME/kg DM diet and 180g CP/kg DM diet, respectively). Feed and water were available throughout the feeding trial (*ad libitum*).

Before data collection, the Venda chickens initial body weight was measured using an electronic weighing balance scale (AE Adam). Prior to the start of the experiment and throughout, the resident veterinarian at the University of Limpopo assessed the health of the chickens. The fate of all chickens (sick and healthy) following treatment was humane death utilizing the cervical dislocation procedure developed by Khan *et al*. (2018) with the assistance of a veterinarian.

Table 3.1 Dietary treatments

Treatments	Diet description
Control	Unsexed Venda chickens on a control yellow maize - soyabean meal diet without citric acid supplementation.
$CA_{12.5g}$	Unsexed Venda chickens on a control yellow maize – soyabean meal diet supplemented with 12.5 g citric acid per kg DM feed.
CA_{25g}	Unsexed Venda chickens on a control yellow maize – soyabean meal diet supplemented with 25 g citric acid per kg DM feed.
CA_{50g}	Unsexed Venda chickens on a control yellow maize – soyabean meal diet supplemented with 50 g citric acid per kg DM feed.

Table 3.2 Ingredients and nutrient composition of the diet for the experiment

	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 3.3 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		
Total		(n-1) = (200-1) = 199		

3.5 Growth performances measurements taken

Feed intake (FI), body weight (BW) and body linear measurement were measured to determine the growth rate of the Venda chicken. Feed intake was measured every day while body weight was measured weekly. The voluntary feed intake was measured by subtracting the difference between the feed offered to chickens per day and leftovers and then divide by total number of chickens per pen. The feed offers and leftovers was measured using the 0.01g electronic weighing balance scale (AE Adam).

Feed intake(kg)= (intake – leftover) ÷ total no of chicken per pen

= (intake – leftover)/ total no of chicken per pen

Body weight (BW) was calculated by subtracting the difference between final and initial body weight from the weekly readings. Each animal's body linear measurements included shank length (SL), wing length (WL), back length (BL), and thigh length (TL) were measured at day 90. The 0.01g electronic balance scale was used to determine body weight in grams (g) (AE Adam). A tape ruler was used to measure the body linear measurements such as shank length in centimeters (cm).

Body weight (g) = BW final – BW initial

Feed conversion ratio was calculated using the daily average feed intake and weight gain. Average feed intake was divided by average weight gain to find the FCR value (McDonald *et al*., 2010). Feed conversion efficiency was the amount of feed eaten for production.

FCR = average feed intake(g) ÷ average weight gain(g/days)

3.6 Growth rate determination using non-linear models

The selected nonlinear models used to fit the growth data of Venda chickens set by the Number Cruncher Statistical System (NCSS) software (2015) as indicated below.

Table 3.4 Growth curve using nonlinear models

Nonlinear models	Mathematical expression
Gompertz	W_t = A* exp(-1*exp(-kt))
Weibull	W_t = A- (A - B) exp-(kt)
Richards	W_t = A (1 – B * exp(-kt) ^^{m}

Where:

Wt = corresponding weight at the time.

A = mature or asymptotic weight

B = integration constant

k = maturity rate

t = age of the chickens

m = shape parameter that determines the time and the weight at inflection point

3.7 Statistical analysis

The effect of citric acid supplementation level on feed intake, growth rate, FCR and body linear measurements were analyzed using a one-way analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 2003). Where there were significant differences ($P<0.05$), the treatment means was separated using Tukey's (HSD) test at $P<0.05$.

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

Where:

Yij = Response variables (feed intake, feed conversion ratio, body weight and body linear measurements)

μ = Constant

Ti = The effect of citric acid inclusion levels

eij = Random errors

The optimal responses in Venda chicken body weight, growth rate and FCR 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

$-b_1/2b_2$ = x value for optimal response.

The Venda chicken were individually weighed weekly in the morning and body weights were recorded on every week for 90 days. The average body weights of the birds and number were used as the data for the growth curve. Three growth curve models Gompertz, Weibull and Richards were fitted with data using curve fitting procedure of the NCSS(NCSS,2015) for the evaluation of the growth parameters. To select the best model, two principal criteria of adjustment, Coefficients of determination (R^2) and standard errors (SE) were used. For each of these criteria, the R^2 and SE were compared on each model and treatment in order to choose best model using Number Crunchers Statistical System software (NCSS, 2015) at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Nutrient composition of the diets

Results of the nutrient composition of the experimental starter diets are presented in Table 4.1. 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 4.2. 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 4.3. 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 4.1 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 4.2 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 4.3 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 4.4. 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. Male Venda 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, male 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 similar (p>0.05) growth rate. Similarly, male 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 male Venda aged one to 30 days (Figure 4.1 and Table 4.5).

Male 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, male 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, male 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, male Venda chickens supplemented with 0,12.5 or 50g of citric acid per kg DM had the same (p>0.05) live weight. A 2.536g of citric acid supplementation level per kg DM of the diet was calculated using quadratic equation to result in optimal live weight of male Venda aged one to 30 days (Figure 4.2 and Table 4.5).

Table 4.4 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)	$0.027±0.0007^{ab}$	$0.026±0.0043^{ab}$	$0.031±0.0014^{a}$	$0.025±0.0008^{b}$
ADG (kg/bird/day)	$0.0185±0.0003^{ab}$	$0.0232±0.005^{a}$	$0.0232±0.0015^{ab}$	$0.1642±0.0022^{b}$
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)	$0.563±0.0150^{ab}$	$0.555±0.0911^{ab}$	$0.648±0.0299^{a}$	$0.525±0.0173^{b}$

* : 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 supplementation in the diet of 0(MCA_0),12.5($MCA_{12.5}$),25 ($MCA_{25.0}$) or 50g/kg DM of feed.

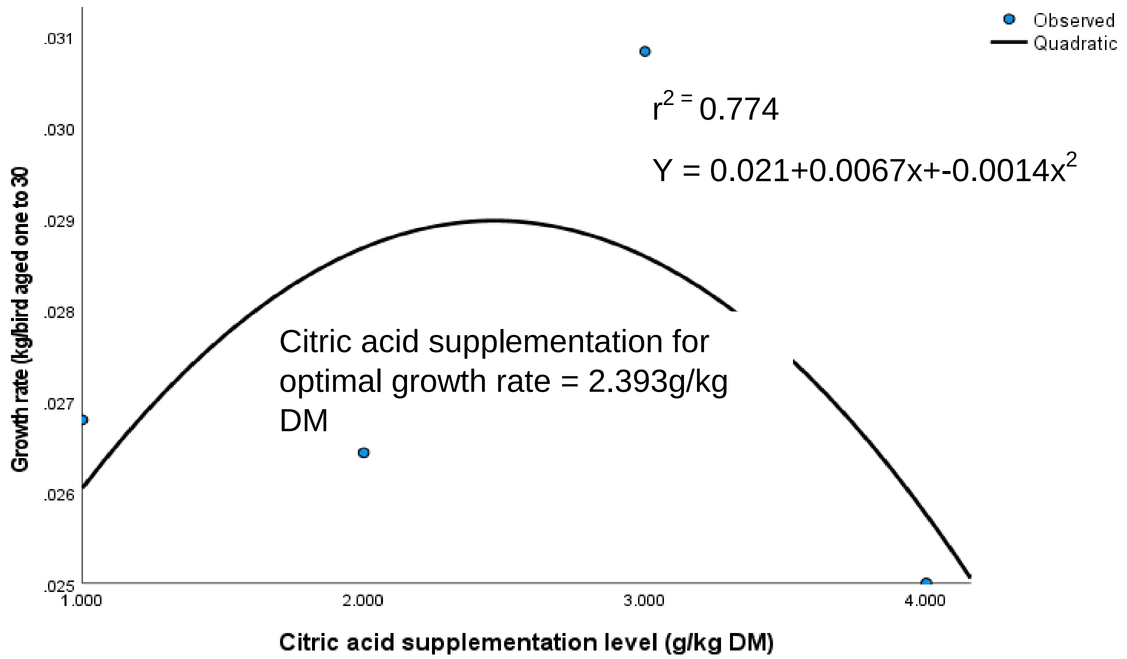


Figure 4.1 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged one to 30 days

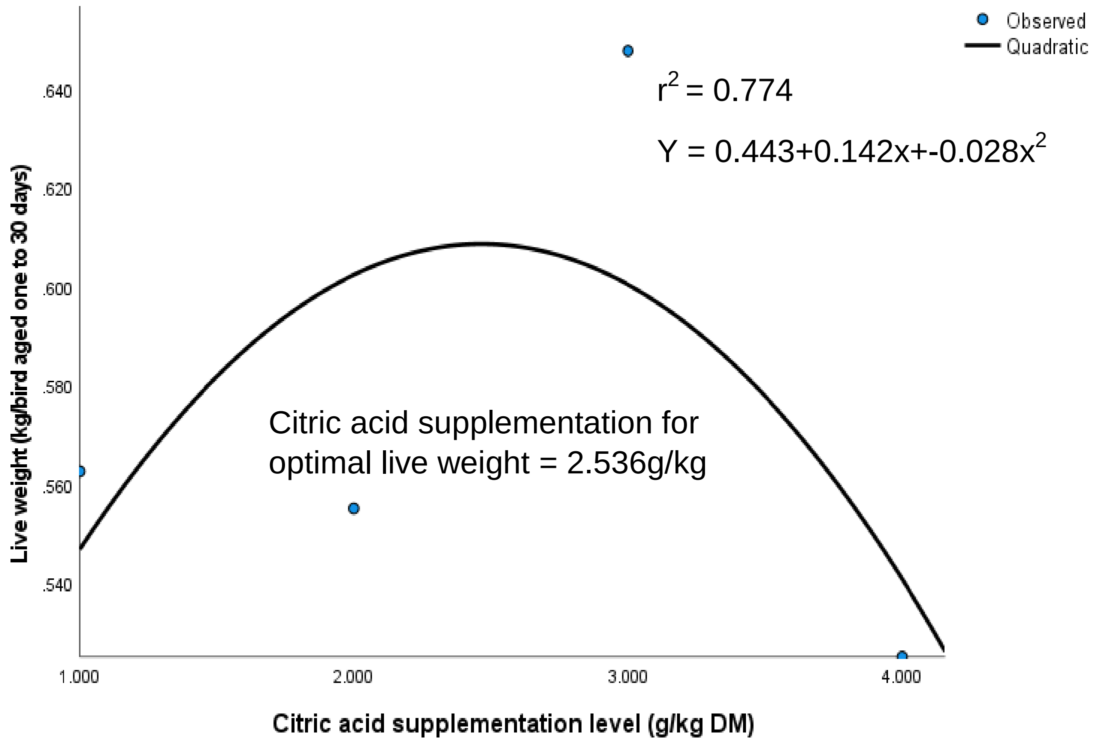


Figure 4.2 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged one to 30 days

Table 4.5 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

r^2 : Coefficient of determination

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

4.3 Results of the effect of citric acid supplementation on feed intake, growth rate, feed conversion ratio and live weight at 60 days of male Venda chickens are presented in Table 4.6. Citric acid supplementation affected (p<0.05) DM feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens aged 31 to 60 days. Male 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, male 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, male Venda chickens supplemented with 0, 25 or 50g of citric acid per kg DM had similar (p>0.05) DM feed intake. Similarly, male 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 male 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, male 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 male Venda aged 31 to 60 days (Figure 4.4 and Table 4.7).

Male 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, male 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, male Venda chickens supplemented with 0, 25 or 50g of citric acid per kg DM had similar ($p>0.05$) feed conversion ratio value. Similarly, male 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 male Venda aged 31 to 60 days (Figure 4.9 and Table 4.7).

Results of the present study indicate that male 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, male 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 male Venda aged 31 to 60 days (Figure 4.10 and Table 4.7).

Table 4.6 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 ± 0.0283^b	1.033 ± 0.0301^b	1.120 ± 0.0280^a	1.047 ± 0.0575^{ab}
Growth rate (kg/bird/day)	$0.025^b \pm 0.0030^b$	0.031 ± 0.0010^a	0.030 ± 0.0015^a	0.020 ± 0.0017^c

FCR (kg DM feed/kg live weight gain)	2.485±1.0244[ab]	1.322±0.1695[b]	1.549±0.1864[ab]	2.949±0.9248[a]
Live weight (kg/bird aged 31 to 60 day	1.033±0.1255[b]	1.288±0.0403[a]	1.243±0.0634[a]	0.843±0.0699[c]

* : 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 1(The treatments were supplementation of varying citric acid levels at 0, 12.5g/kg DM of feed, 25g/kg DM of feed or 50g/kg DM of feed.

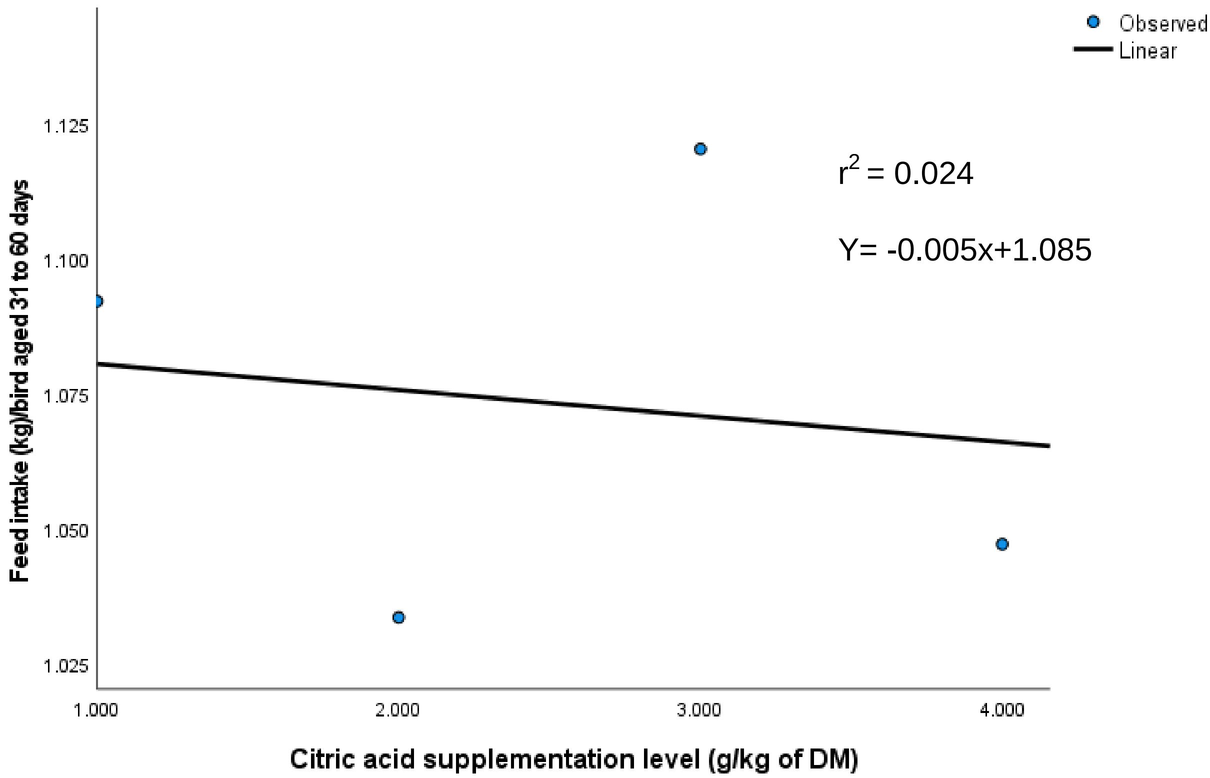


Figure 4.3 Relationship between citric acid supplementation in a diet and feed intake of male Venda chickens aged 31 to 60 days

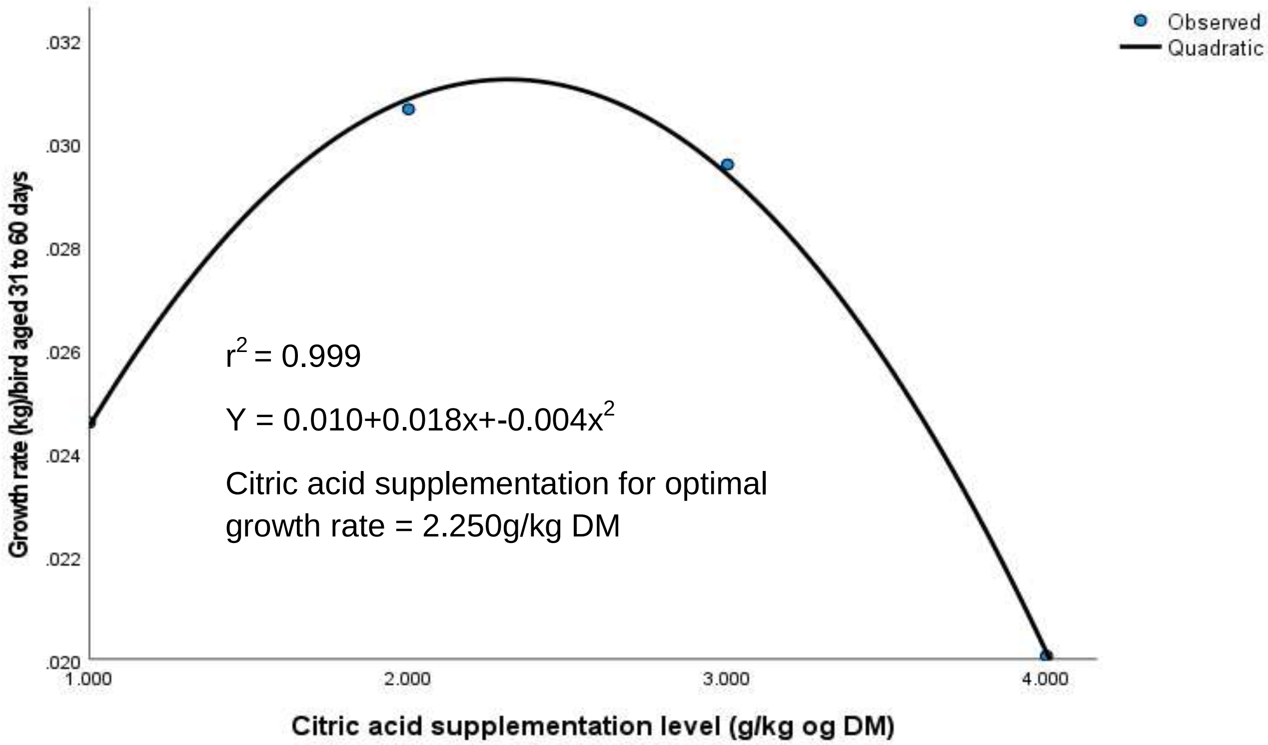


Figure 4.4 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged 31 to 60 days

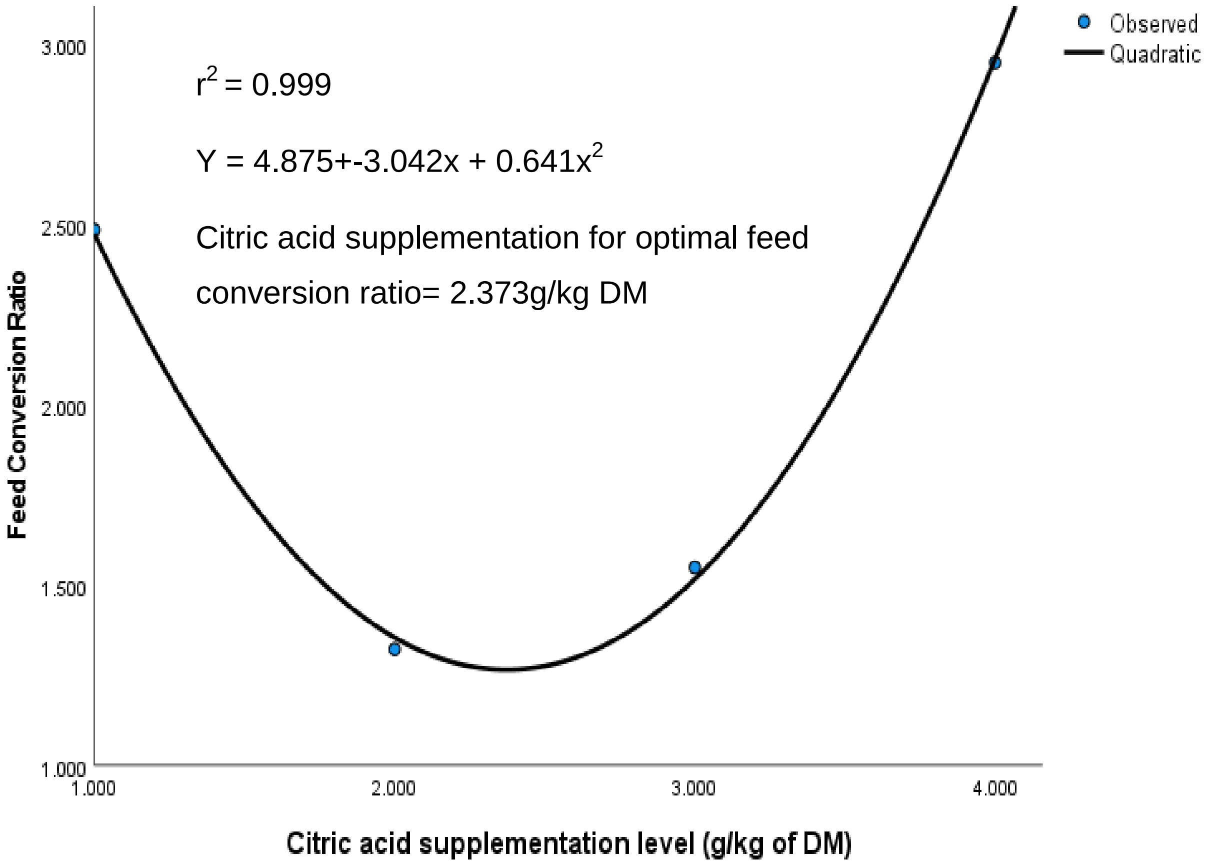


Figure 4.5 Effect of citric acid supplementation in a diet on feed conversion ratio of male Venda chickens aged 31-60 days

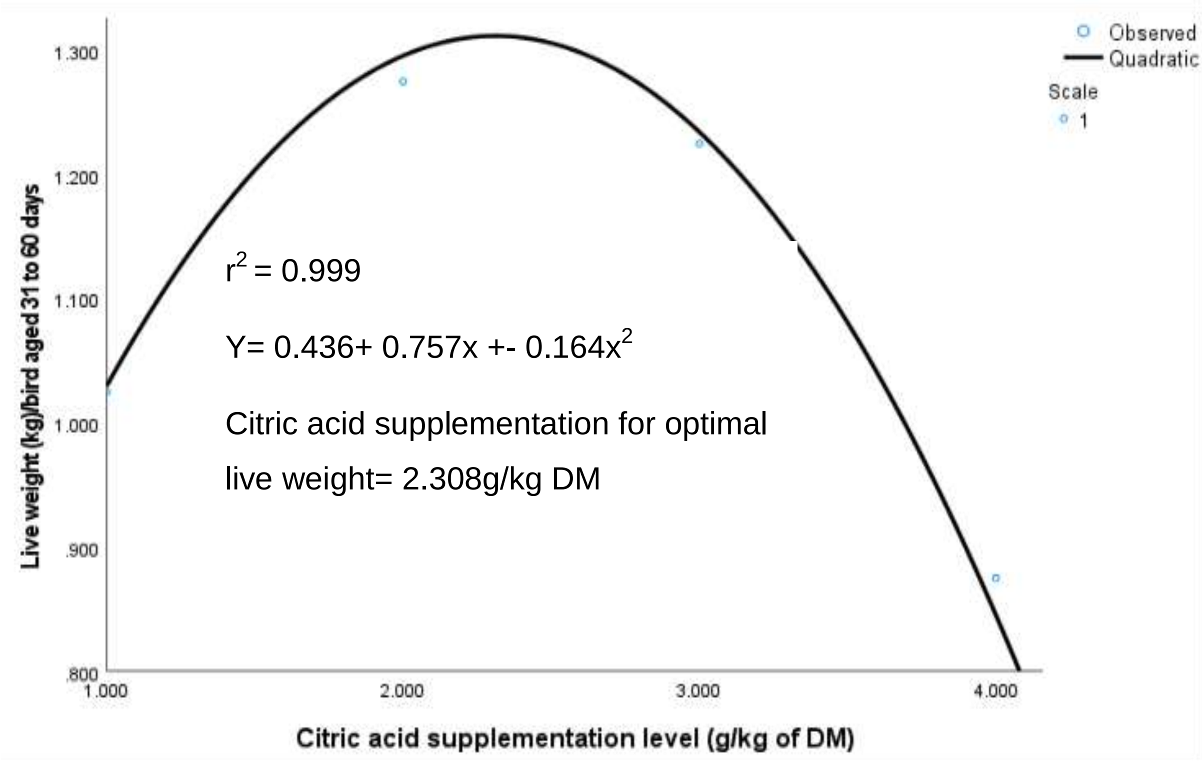


Figure 4.6 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged 31 to 60 days

Table 4.7 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

r^2 : 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 male Venda chickens aged 61 to 90 days are presented in Table 4.8. Citric acid supplementation affected (p<0.05) DM feed intake, growth rate, feed conversion ratio and live weight of male Venda chickens aged 61 to 90 days. Male 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, male 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, male 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, male Venda chickens supplemented with 0 or 12.5g of citric acid per kg DM had the same (p>0.05) DM feed intake. Similarly, male 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 male Venda aged 31 to 60 days (Figure 4.7 and Table 4.9).

Results of the current study indicate that male 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, male 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, male 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 male Venda aged 61 to 90 days (Figure 4.8 and Table 4.9).

Male 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, male 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, male Venda chickens supplemented with 0 or 50g of citric acid per kg DM had the same (p>0.05) feed conversion ratio value. Similarly, male 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 male Venda aged 61 to 90 days (Figure 4.9 and Table 4.9).

Results of the current study indicate that male 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, male 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, male 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 male Venda aged 61 to 90 days (Figure 4.10 and Table 4.9).

Table 4.8 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 ± 0.0658^{ab}	1.524 ± 0.0271^{a}	1.418 ± 0.0181^{b}	1.307 ± 0.0445^{c}
Growth rate (kg/bird/day)	0.026 ± 0.0016^{b}	0.031 ± 0.0006^{a}	0.029 ± 0.0009^{a}	0.022 ± 0.0014^{c}
FCR (kg DM feed/kg live weight gain)	1.476 ± 0.1428^{ab}	1.192 ± 0.0859^{b}	1.275 ± 0.0688^{b}	1.622 ± 0.2249^{a}
Live weight (kg/bird aged 31 to 60 day)	1.578 ± 0.0946^{b}	1.840 ± 0.0356^{a}	1.763 ± 0.0532^{a}	1.340 ± 0.0841^{c}

* : 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 1(The treatments were

supplementation of varying citric acid levels at 0, 12.5g/kg DM of feed, 25g/kg DM of feed or 50g/kg DM of feed.

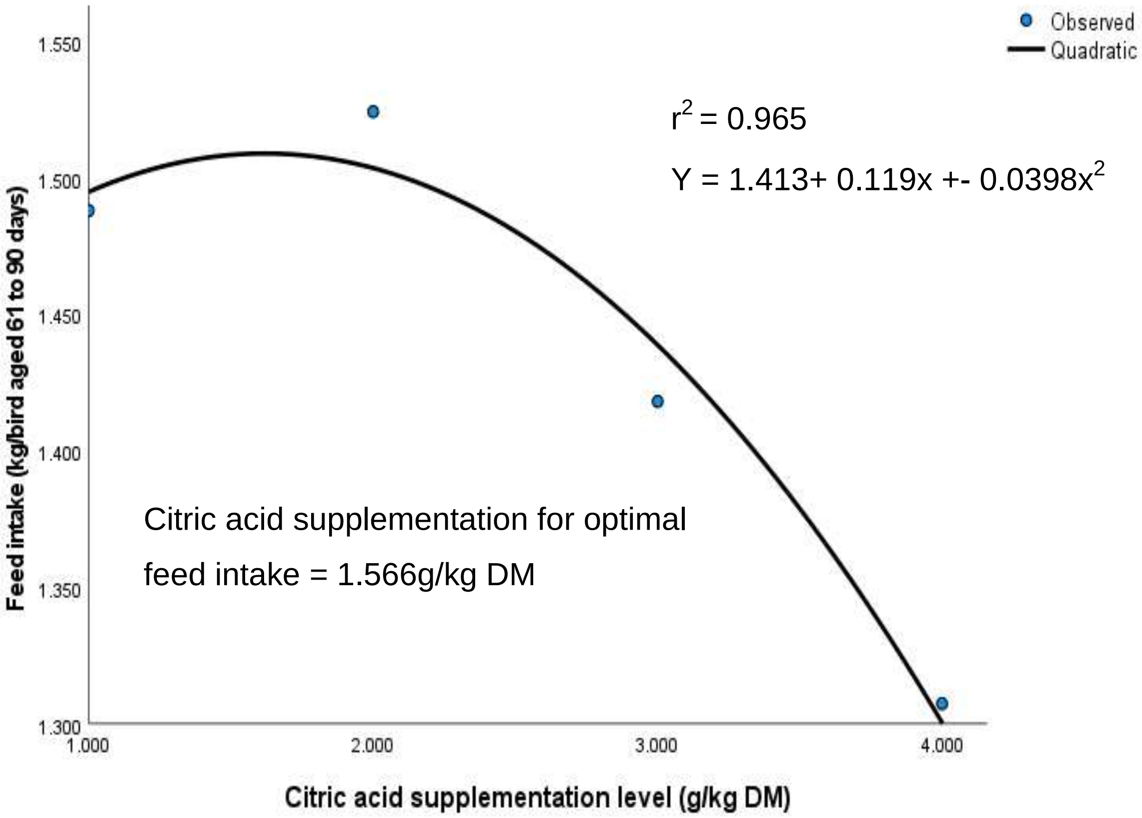


Figure 4.7 Effect of citric acid supplementation in a diet on feed intake of male Venda chickens aged 61 to 90 days

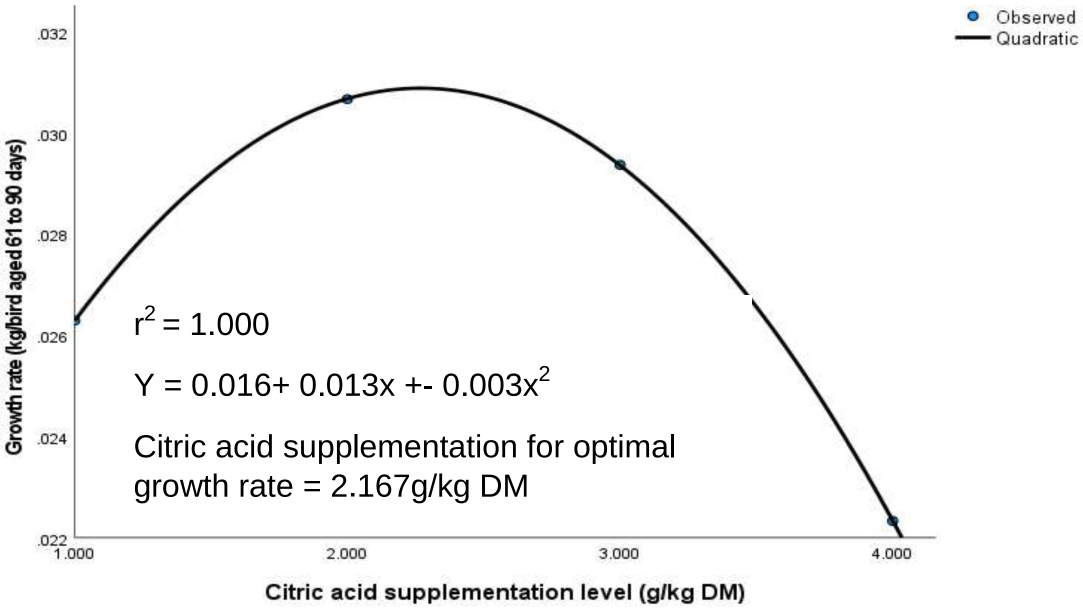


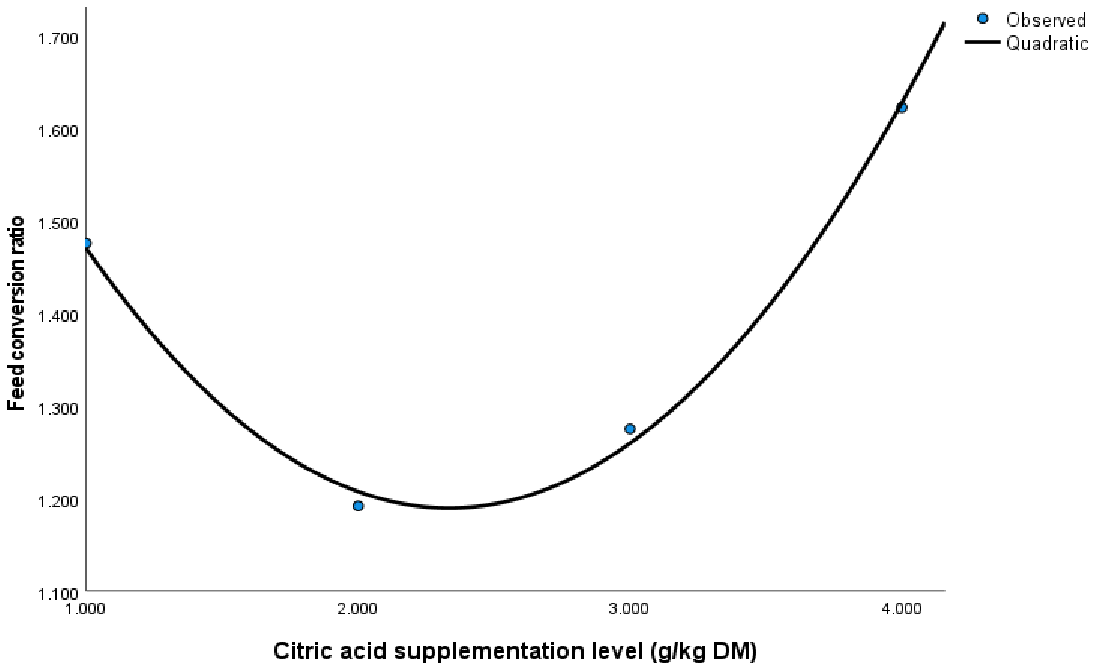
Figure 4.8 Effect of citric acid supplementation in a diet on growth rate of male Venda chickens aged 61 to 90 days

$r^2 = 0.995$

$Y = 2.050 + 0.737x + - 0.158x^2$

Citric acid supplementation for optimal feed conversion ratio = 2.333g/kg DM

Figure 4.9 Effect of citric acid supplementation level in a diet on feed conversion ratio of male Venda chickens aged 61 to 90 days



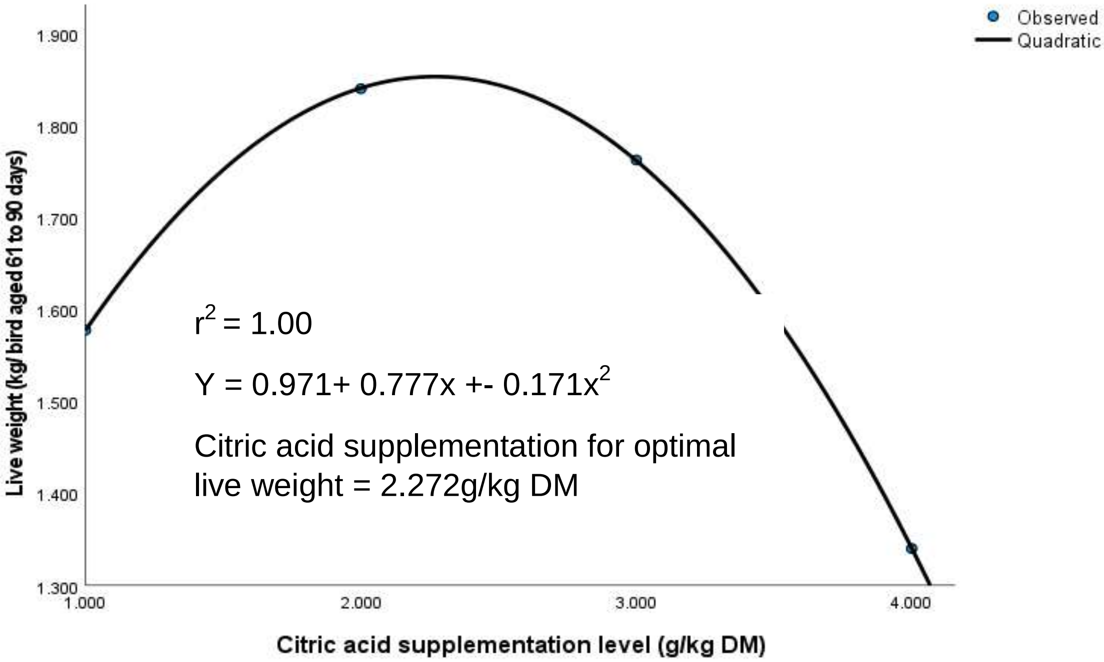


Figure 4.10 Effect of citric acid supplementation in a diet on live weight of male Venda chickens aged 61 to 90 days

Table 4.9 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

r^2 : Coefficient of determination

4.5 Effects of citric acids supplementation on body linear measurements of Venda chickens

Results on the effect of citric acid supplementation levels on body linear measurements of Venda chickens are represented on Table 4.10. Citric acid inclusion had no significant effect ($p > 0.05$) on the shank and wing length. However, citric acids inclusion had effects ($p < 0.05$) on the back and thigh length among the treatment means. Venda chickens fed control, citric acid inclusion 12.5g and 25g had higher back length than those fed citric acid inclusion 50g. Venda chickens fed citric acids inclusion 25g had higher thigh length than those fed control. However, Venda chickens fed citric acids inclusion 25g,12.5g and 50g had similar ($p > 0.05$) thigh length.

Table 4.10 The effect of citric acid supplementation levels on body linear measurements of Venda chickens

	Treatments			
Traits	CA_{0g}	$CA_{12.5g}$	CA_{25g}	CA_{50g}
Shank length(cm)	10.62 ± 0.51	10.80 ± 0.56	10.95 ± 0.53	10.02 ± 0.49
Back length(cm)	17.75 ± 0.78^{a}	16.31 ± 0.79^{a}	17.57 ± 0.84^{a}	14.00 ± 0.77^{b}
Thigh length(cm)	6.13 ± 0.25^{b}	6.53 ± 0.32^{ab}	7.33 ± 0.36^{a}	6.37 ± 0.38^{ab}
Wing length(cm)	11.13 ± 0.49	9.80 ± 0.47	10.68 ± 0.56	10.60 ± 0.54

SEM-standard error of mean. ^{a,b} a together with b means in the same row with different superscripts are significantly different($p < 0.05$). CA_{0g} control; $CA_{12.5g}$ citric acid inclusion 12.5g; CA_{25g} citric acid inclusion 25 g and CA_{50g} citric acid inclusion 50g.

4.6 Effects of citric acid supplementation on growth curve models of Venda chickens

Results on the growth curve models of Venda chickens fed different citric acid supplementation levels are represented on Table 4.6. In control group, Gompertz model (R^2=0.96; SE=650.02) explained the growth curve of Venda chickens better than the Weibull and Richard models. In citric acid inclusion 12.5g group, Gompertz model (R^2=0.97; SE=6.21) explained the growth curve of Venda chickens better than the Weibull and Richards models. In citric acid inclusion 25g group, the Richards model (R^2=0.98; SE=4.81) explained the growth curve of Venda chickens better than Gompertz and Weibull models. In citric acid inclusion 50g, Richards model (R^2 =0.93; SE=1.46) explained the growth of Venda chickens better than the Gompertz and Weibull model. Therefore, Gompertz model is the best model to describe the growth curve of Venda chickens fed the control and citric acid inclusion 12.5g group. Richards model is the best model to describe the growth curve of Venda chickens fed citric acids inclusion 25g and 50g group.

Table 4.11 Effects of citric acids supplementation on growth curve models of Venda chickens

Treatment	Model	R^2	A	SE
CA_{0g}	Gompertz	0.96	290.49	650.02
	Weibull	0.00	51.42	0.00
	Richards	0.96	3262.19	118494.01
$CA_{12.5g}$	Gompertz	0.97	24.19	6.21
	Weibull	0.00	10.56	0.00
	Richards	0.97	29.38	42.38
CA_{25g}	Gompertz	0.98	30.34	7.40
	Weibull	0.00	12.48	0.00
	Richards	0.98	16.23	4.81
CA_{50g}	Gompertz	0.93	20.10	6.79
	Weibull	0.00	10.36	0.00
	Richards	0.93	10.08	1.46

R^2-coefficient of determination. A- asymptotic weight, SE- standard error, CA_{0g} control; $CA_{12.5g}$ citric acid inclusion 12.5g; CA_{25g} citric acid inclusion 25 g and CA_{50g} citric acid inclusion 50g.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The study objectives were to identify the influence of citric acid supplementation on growth performances. 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 had effect on the growth rate and live weights of male Venda chickens aged one to 30 days. Citric acid supplementation doses of 2.393 and 2.819g per kg DM of the feed were calculated to result in optimal growth rate and live weight of male Venda chickens aged one to 30 days. This study, however, corresponds with the findings of Nourmohammadi *et al*. (2010), who found no significant effects on feed consumption in chickens fed a diet enhanced with citric acid. In contrast, Moghadam *et al*. (2006) found that the effects of citric acid on feed consumption of chicks were significant and similar results were found by Atapattu and Nelligaswatta (2005). However, this observation agrees with the findings of Nezhad *et al*. (2007) who reported that there was no significant effect on feed intake in chicks fed a diet supplemented with citric acid.However, 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.in contrast, 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. In contrast, Snow *et al*. (2004), who discovered that adding dietary citric acid boosted body weight increase and similar results were found by Afsharmanesh and Pourreza (2005). Moghadam *et al*. (2006) discovered a similar result. This suggests that the effects of citric acid on weight gain are mediated through its effects on feed intake.

Citric acid supplementation had 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 Panda et al. (2009). 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). Highest weight gain on 0.5% citric acid agreed with previous findings of Shen-HuiFang et al. (2005); Denil et al. (2003) and Stipkovits et al. (1992) where improved live weight, weight gain, feed intake and FCR was observed with administration of citric acid in diets at 0.3, 0.5 and 0.7%, respectively as compared to control birds. The results contradict with the findings of previous researchers Pinchasov et al. (2000) where depressed weight gain was observed with application of acetic acids in diets. The improved growth performance could be attributed to citric acid's beneficial effect on gut morphometry and size. According to (Abdel-Fattah et al. 2008, Adil et al. 2011a,). however, Celik et al. (2003) discovered improved feed conversion ratio in broilers fed an acidifier diet, while Hassan et al. (2010) found the same results. However, 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). However, other studies reported that supplementation of citric acid to feed has no effect on performance of chickens (Kaplan, *et al*. (2003); Nourmahammad *et al.* (2012)).

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 chicken fed 2 or 3% CA-supplemented diets had significantly greater live weight, weight gain and feed conversion ratio compared to the control. Similar results were found by Rafacz-livingston *et al*. (2005) and 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) reported a linear increase in weight gain and feed intake in chicks fed diets supplemented with up to 3% of citric acid. Isabel and Santos (2009) discovered that organic acid has a substantial effect on feed conversion ratio (FCR). The increase in feed conversion ratio 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 lower feed consumption because of citric acid. Lower feed conversation ratio 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). 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 above findings suggest that the effects of citric acid on weight gain are mediated through its effects on feed intake.

However, the citric acid had effect on back length and thigh length on body linear measurements. This is a typical natural phenomenon and genotype on growth performance, although citric acid inclusion can improve some features. The findings supported the existence of traits disparities in body linear measurements, as previously reported by (Deep and Lamont (2002), Ajayi and Ejiofor (2009)). Similar, results were found by Olawumi (2015), who showed that traits did not differ in body linear measurements. Khetani *et al*. (2009) found no significant effect of diet restriction on body linear measurements in hens. The average thigh lengths and back lengths obtained in this study were lower than the average thigh length of indigenous guinea fowl in Lafiya, Nasarawa state (Ogah ,2012). In contrast, Fajemilehin ,2010 reported that guinea cocks were higher in average thigh length whereas guinea hens were lower over the reported the pearl, ash and black indigenous guinea fowls. Venkatesan *et al.* (2015), who observed no significant difference in mean shank length of guinea hen and guinea cocks. The mean shank length was higher than the value (6.60cm) reported by Momoh and Kershima (2008) for female local chickens. However, the observed result of shank length across all hybrids (Arbor Acre, Marshall, and Ross 308 broiler) was not statistically different, and it is in line with the findings of (Udeh and Ogbu,2011) who observed insignificant differences in shank length among all the above listed hybrids of broiler chicken. Wing length reported by Fayeye *et al*., (2006), who observed no significant difference in mean for naked neck, frizzle and normal feathered Nigerian local chicken. In contrast, with Abdul-Rahman et al. (2015), who observed higher shank length of guinea cocks and guinea hens. These findings suggest that some of the traits can be associated with other traits such as beak, neck, head and chest circumference.

This study also focused on finding the best model to describe the growth curve of Venda chickens. The Gompertz model was the best to explain the growth curve of Venda chickens fed with feed without citric acid and citric acid 12.5g inclusion. However, the Richards model was the best to explain the growth curve of Venda chickens fed citric acid inclusion of 25g and 50g. A parameter values, which is the asymptotic limit of the weight when age (t) approaches infinity does not imply that A is the heaviest weight attained by the individual, but it indicates the average weight of the mature animal, independent of short-term fluctuations in weight due to temporary environmental effects (Lopez de Torre *et al*., 1992; Berry *et al*., 2005). The models (Gompertz and Richards) have higher R^2 values than the Weibull model among citric acid supplementations.

The Weibull model does not seem appropriate for description of the growth data in this study. The Gompertz and Richards (Tholon and Queiroz, 2007) and Weibull (Khamis *et al*., 2005) growth models were used to explain the weight-age relationship in broiler chickens. Sengul and Kiraz (2005) found high R^2 values for Gompertz and Richards in a study of growth curves in turkeys. Yakupoglu and Atil (2001) also found high R^2 values in a study of growth in broilers using Gompertz model. Similar results obtained by Kuhi *et al*. (2003) who found the same growth functions in France. The comparison based on R^2 values, R^2 showed that it is difficult to identify the model which is significantly better than the others to fit growth curve based on this criterion. Tompić *et al*. (2011) found that the R^2 values of the Gompertz, Richards and Weibull function were ranged from 0.988 to 0.995 in Ross 308 broiler chickens. The results of the present study disagreed with the previous results reported by several researchers (Gous *et al*., 1999; Santos *et al*., 2005) who confirmed that the Gompertz function is the best function for describing growth curve in broiler chickens.Some studies found that the Richards function gave the best fit for growth curve in broiler (Kuhi *et al*., 2003; Tompić *et al*., 2011; Moharrery and Mirzaei, 2014). The difference of these finding may be due to the difference of breeds, environment conditions and other errors, obtaining a good comparison among different research results was difficult (Wang *et al*., 2004; Sun *et al*., 2006). These findings suggest that more studies are needed using different breeds or same breed and feeds.

5.2 CONCLUSIONS

In conclusion, citric acid had influence on growth performances during grower and finisher diet on Venda chicken development. The citric acid had influence on back length, and thigh length on Venda chickens. During starter citric acid had no effect on Venda chickens, it might be a period where the chicken adapted to the feed. Citric acid can be added in small amounts to chicken feed to increase growth performance because it was observed that Venda chickens fed citric acid 50g had lower growth performances. Because certain animals are accustomed to wild behavior and are picky about what they eat. The Gompertz and Richards model can be used as the best growth curve model to describe the growth of Venda chickens. The use of mathematical models to describe growth patterns allows for precise comparison. Mathematical models of growth can be used to visualize growth trends throughout time. The equation can be used to forecast the weight of a group of animals at a given with same effect.

5.3 RECOMMENDATIONS

Based on the findings

It is recommended that village farmers can use citric acid as a supplement to improve the performance of animals. The must uses 12.5g or 25g of citric acid per kg.

The farmers especially communal farmers should have knowledge on body weight and body linear measurement and to predict the growth curve of the animals.

More studies are needed on the effect of citric acid supplementation using large sample size more than 200 chickens and more nonlinear models can be used to determine the best growth curve for Venda chickens.

Farmers should be educated on how to measure the body linear measurements to determine the performances of the animal.

Farmers should be educated about the mathematic models that are used to determine the growth curve of Venda chickens or any animals using the software not manually so that they can be able to use the nonlinear models to describe the growth curve.

CHAPTER SIX

REFERENCES

6.1 REFERENCES

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