THE EFFECT OF CITRIC ACID SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS WEIGHT, TIBIA BONE BREAKING STRENGTH, AND ASH CONTENT OF MALE ROSS 308 BROILER CHICKENS

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DECLARATION

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

Thokwane J (Ms) Date: 03 April 2023

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DEDICATIONS

This work is dedicated to my late grandmother Marth Mokgoatjane who always believed in me and provided guidance. May her soul continue resting in peace. I am highly motivated because of her.

ABSTRACT

Two experiments were conducted to determine the effect of citric acid inclusion level in the diet on growth performance, carcass weight, tibia bone breaking strength and ash content of male Ross 308 broiler chickens aged one to 35 days. The first experiment determined the effect of citric acid inclusion level in the diet on growth performance traits of male Ross 308 broiler chickens aged one to 21 days. The experiment commenced with 200 male day-old Ross 308 broiler chickens with an initial average live weight of 40±1.6g per chick. The chicks were assigned to five treatment groups in a completely randomized design, each replicated five times, and each replicate having ten chicks. The citric acid inclusion levels were at 0, 12.5, 25 or 50g per kg DM of feed. The second experiment determined the effect of citric acid inclusion level in the diet on growth performance, carcass weight, tibia bone breaking strength and ash content traits of male Ross 308 broiler chickens aged 22 to 35 days. The experiment commenced with 180 male Ross 308 broiler chickens aged 22 days. The chickens were assigned to four treatment groups, each having three replicate pens of eight chickens per replicate in a completely randomized design. Data was analysed using the General Linear model (GLM) procedures of the Statistical Analysis of System, version 9.3.1 software program. Where there were significant differences ($P<0.05$) between the treatment means, Tukey Multiple Comparison Test was used for mean separation.

Citric acid inclusion in the starter diets improved ($P<0.05$) live weight and growth rate of male Ross 308 broiler chickens aged one to 21 days. Citric acid inclusion in the starter diets did not affect ($P>0.05$) daily feed intake, body weight gain and feed conversion ratio of male Ross 308 broiler chickens aged one to 21 days.

The inclusion of citric acid did affect ($P<0.05$) live weight, body weight gain, feed conversion ratio and growth rate of chickens aged 22 to 35 days. Citric acid inclusion levels used in the present study influenced ($P<0.05$) DM and CP digestibility, ME intake and N-retention of male broiler chickens aged 22 to 35 days. The results of the current study showed that citric acid inclusion in a diet improved ($P<0.05$) chicken bone morphology. Thus, positive relationships were observed between citric acid inclusion level and right tibia bone weight, diameter, calcium, phosphorous and Magnesium contents of chicken bones aged 35 days. There were positive relationships between citric acid inclusion level and breast weight of male Ross 308 broiler chickens aged 35 days. Further studies are recommended to ascertain these findings.

Keywords: Citric acid, broiler chickens, growth performance traits, carcass weight, bones.

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LIST OF ABBREVIATIONS

CA – Citric Acid

SV – Source of Variation

SS – Sum of Squares

DF- Degrees of Freedom

MS- Mean of Squares

LW – Live Weight

DFI – Daily feed Intake

FCR – Feed Conversion Ratio

BWG – Body weight gain

GR – Growth Rate

DR – Dressing Rate

Ca – Calcium

P – Phosphorous

Mg – Magnesium

ME – Metabolizable Energy

N - Nitrogen

CHAPTER ONE

INTRODUCTION

1.1 Background

Poultry production in South Africa is the most common and quickly developing industry (Department of Agriculture, Forestry, and Fisheries, 2017), as well as the cheapest source of protein in terms of meat and eggs consumption and capital investment requirements (Department of Agriculture, Forestry, and Fisheries, 2017). A vast number of people in South Africa rely on broiler chicken production for economical, nutritional, and cultural reasons (Halima *et al.* 2009).

Broiler chickens are characterized by high growth rates and good feed conversion ratios (Tumova *et al.* 2002). The genetic capability of broiler chickens in the present time can be enhanced through proper feeding and management. Supplementing organic acids in the broiler's diet is found to be protecting them against the food borne diseases (Khan and Igbal, 2015) such as Campylobacterious, and enhances their nutrient utilization, growth, and feed efficiency (Islam, 2012).

Citric acid is mostly employed in the food business due to its pleasant sour taste and high-water solubility. It is also frequently used as an acidifier, flavouring agent, and chelating agent (Islam, 2012). Citric acid provides significant antibacterial activity to protect feed from bacterial deterioration while also lowering harmful bacteria levels (Islam,2012).

1.2 Problem statement

Modern broiler chickens are selected due to rapid growth and high meat increased growth rate performance, on the other hand, has resulted in a higher frequency of metabolic problems such as ascites, sudden death syndrome, skeletal deformities, and increased fat deposition (Urdaneta and Leeson, 2000, Petracci, 2015). Excessive fat deposition decreases carcass quality, resulting in meat rejection by consumers (Macajova et al. 2003; Molepo, 2014).

Leg weakness and shattered bones are major difficulties in the broiler industry worldwide, causing enormous financial losses (Poultry world, 2016). Weak legs and other bone deformities linked with various metabolic illnesses are serious issues in rapidly developing broiler chickens, resulting in output losses (Julian, 2005; Waldenstedt, 2006; Dibner et al. 2007).

According to Williams et al. (2000), recent broiler chicken bones are characterized by a lack of calcium and excessive porosity, which may lead to an increased proclivity for bone injury. Fractures are more likely in chickens who have poor bone mineralization (Blake and Fogelman, 2002). Bone abnormalities can reduce broiler chickens' walking capacity, which can lead to feed intake issues, resulting in poor meat quality, lower body weight, and a poor feed conversion ratio (Orban et al. 1999). Customers do not buy chickens with broken bones or legs, which might result in economic losses for the producers.

1.3 Rationale

Citric acid is a natural acid that is used as a natural ingredient to impart an acidic or sour flavour to diets. It has sufficient antibacterial activity to protect feed from bacterial spoiling while also lowering the population of harmful microorganisms in the gastrointestinal system, which enhances chicken development (Deepa et al. 2011; Islam, 2012).

Citric acid is also used in poultry diets to boost growth by acidifying the digesta, which improves feed digestibility and reduces pathogen burdens (Boling et al. 2000; Islam, 2012), and also increases the solubility of feed ingredients by modifying intestinal pH, hence increasing supplement digestion and retention (Nourmohammadi and Afzali, 2013). Citric acid contains phytochemical compounds with antioxidant, antimicrobial, and anti-inflammatory properties that have been shown to improve the growth performance of birds when mixed in their diets (Sharifuzzaman et al. 2020).

According to Islam (2012), high levels of 3 and 6% citric acid seem to reduce feed palatability whereas low inclusion levels of 0.75 and 2% are likely to increased feed consumption in avian species. Citric acid supplementation increases tibia ash content (Chowdhury et al. 2009; Asgar et al. 2013). However, it has been found that including citric acid in diets reduced broiler chicken tibia Ca, Mg, and P levels (Nourmohammadi et al. 2012; Asgar et al. 2013).

According to Liem et al. (2008), the action of citric acid on bone breaking strength helps enhance mineral utilization, resulting in the bones receiving the nutrients needed for stronger bone strength. Diets low in calcium have a negative impact on performance and tibia characteristics, resulting in weak bones and weak bone breaking strength, and the addition of organic acids such as citric acid improved these indices and helped the birds overcome the problems associated with a low-Ca diet, such as weak bone breaking strength (Houshmand et al. 2011).

Higher quantities of citric acid do not appear to improve bone quality (Nourmohammadi et al. 2012). As a result, determining citric acid supplementation doses for optimal tibia Ca, Mg, and P levels is crucial (Islam, 2012). In general, bone ash increases with mineral deposition due to greater availability, resulting in higher bone breaking strength. Increased bone mineral substance, thickness, and breaking quality may reflect the impact of dietary citric acid on the mineral digestion system (Islam, 2012)

However, to the best of our knowledge there is limited information on the effect of citric acid supplementation on growth performance, carcass weight, tibia ash content, and breaking strength in male Ross 308 broiler chickens. As a result, this study will add to our understanding of the potential of citric acid as a growth promoter on male broiler chicken growth performance, carcass weight, bone breaking strength, and tibia ash content. This data will aid in the development of feeding techniques that will improve broiler chicken productivity.

1.3.1 Aim

The aim of the study was to evaluate the effect of citric acid supplementation level that might be used to improve growth performance, carcass weights, tibia bone breaking strength and tibia ash content of male Ross 308 broiler chickens.

1.3.2 Objectives

The objectives of the study were to determine:

I. The effect of citric acid supplementation on growth performance of male Ross 308 broiler chickens.
II. The effect of citric acid supplementation on carcass weights of male Ross 308 broiler chickens.
III. The effect of citric acid supplementation on tibia breaking strength of male Ross 308 broiler chickens.
IV. The effect of citric acid supplementation on tibia ash content of male Ross 308 broiler chickens.

1.3.3 Hypotheses

The hypotheses of the study were as follows:

I. Citric acid supplementation has no effect on growth performance of male Ross 308 broiler chickens.
II. Citric acid supplementation has no effect on carcass weights of male Ross 308 broiler chickens.
III. Citric acid supplementation has no effect on tibia breaking strength of male Ross 308 broiler chickens.
IV. Citric acid supplementation has no effect on tibia ash content of male Ross 308 broiler chickens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Citric acid is widely utilized as a food ingredient and so has a high production and availability. Recent studies have demonstrated that adding 0.5% citric acid (CA) results in higher diet costs but higher profits due to improved growth and feed efficiency (Islam et al. 2008), and the study by Islam et al. (2011a) supports the findings of increased profits. When compared to a control (no supplementation), broiler production enhanced with CA is more profitable (Tolba, 2010).

Organic acids reduce pathogen colonization and the formation of harmful metabolites while increasing the availability of protein, Calcium (Ca), Phosphorus (P), Magnesium (Mg), and Zn. Citric Acid (CA) enhances protein and fiber digestibility, as well as Phosphorus (P) utilization (Atapattu and Nelligaswatta, 2005). Organic acids (citric acid, lactic acid, propionic acid, and so on) used in animal diets may also inhibit pathogenic growth while improving digestion, absorption, mucosal immunity, and topical actions on the intestinal brush boundary (Mroz, 2005).

This review will add to the understanding of the potential of citric acid as a growth promoter on male broiler chicken growth performance, carcass weight, bone breaking strength, and tibia ash content. This data will aid in the development of feeding techniques that will increase broiler chicken productivity.

2.2 Effect of citric acid on growth performance of broiler chickens

2.2.1 Effect of citric acid as growth promoter on broiler chickens

Citric acid provides enough antibacterial activity to protect feed from bacterial spoiling while also lowering the numbers of unwanted microorganisms in the gastrointestinal tract, which eventually increases chicken growth rates (Deepa et al. 2011). To allow young chicks to acclimate and restrict the acidifier's subsequent therapeutic activity, CA should be used in the growth phase rather than the starting phase (Daskiran et al. 2004; Archana et al. 2019). Islam et al. (2012) found that a higher inclusion level of 7.5% resulted in growth depression rather than toxicity. Furthermore, Ragab et al. (2012) discovered a good effect of citric acid inclusion on live body weight and live body weight gain, which could be attributed to the effect of 2% citric acid on mineral consumption, which influences broiler chick growth and chicken growth performance.

Similarly, Rahman et al. (2018) reported that feeding 0.75 % citric acid to broiler chicks had a positive significant influence on their live weight, feed intake, and feed conversion ratio (FCR). Adding 0.75 % to the diet can improve the performance of broilers even when protein and energy levels are low (Sharifuzzaman et al. 2020).

Dietary supplementation of organic acids such as citric acid has improved the body weight and feed conversion ratio (FCR) in broiler chickens (Haq *et al.* 2017). The improved growth rate and mortality reducing effect, with no significant effect on carcass yield in broiler chickens was reported by Brzoska *et al.* (2013) when supplementing with citric acid at inclusion level of 0.3 – 0.9%. Similar results of improvement in growth performance and better carcass characteristics when using organic acids mixture in broiler diets were reported by Fascina *et al.* (2012).

These beneficial effects of dietary acidifiers like citric acid on growth performance could be attributed to their ability to lower pH levels in the feed and digestive tract, thereby preventing bacterial transfer from the diet or environment (Kil et al. 2011), or to selectively increasing acid-loving lactobacillus and having a direct antimicrobial effect (Ghazala et al. 2011; Haq et al. 2017). The effects of citric acid as a growth promoter on broiler chicken are shown in Table 2.2.1.

Table 2.2 1: The effect of citric acid as growth promoter of broiler chickens

Inclusion level	**Breeds chicken**	**Conclusion**	**References**
0, 0.25, 0.75, 1.25%	Ross PM3	0.25%, 0.75% and 1.25% citric acid exhibited increased growth rates of 2%, 5% and 5%respectively	Islam *et al.* (2012)
0.25%	Ross-308	0.25% of citric acid show a decrease in live weight	Mohammed (2018)
0,3 and 6%	Ross 308	3% citric acid to the diet caused improvement in ileal nutrients digestibility, growth performance and increased minerals retention of broiler chickens	Nourmohammadi (2012)
0.5,0.75 and 1%	COBB 500	0.75% citric acid with diet can improve the performance of broiler even under low protein and low energy concentration.	Sharifuzzaman *et al.* (2020)

		effect on the live weight	
3g/kg per DM	Ross 308	3% of citric acid in the broiler diet led to an increase in body weight gain around 4.16% and positive effects on body weight gain of broilers since it could ameliorate nutrients digestibility because of proper intestinal conditions	Nourmohammadi and Khosravinia (2015)
3%	New Hampshire × Columbian male	3% of citric acid had no significant effect on the broiler's performance	Biggs and Parsons (2008)
0.0, 2.5% and 5%)	Ross 308	Improved the live weight gain	Nezhad *et al*. (2007)

2.2.2 Effect of citric acid on feed intake of broiler chickens

The table below shows the influence of citric acid on broiler chicken feed intake (Table 2.2.2). Citric acid has been shown to be effective as a growth stimulant as well as an anti-pathogen agent in the prevention of diseases when added to drinking water and improves feed intake (Paiva and McElroy, 2014; Dittoe et al. 2018). Citric acid inclusion (levels?) in broiler chicken diets has been proven to increase feed intake due to a nice flavour. However, at 60 g/kg of citric acid, feed intake decreases, and performance suffers (Khosravinia et al. 2015).

Good feed intake stems from high palatability and digestibility (Islam, 2012). The effects of adding 3% CA inclusion level were observed to be contradictory. The addition of 3% CA had no discernible effect on growth performance (Biggs and Parsons, 2008). This is consistent with the findings reported by Kopeck et al. (2012), who discovered that organic acid-added diets had no effect on body weight or feed consumption. Nourmohammadi and Khosravinia (2015), on the other hand, found that adding CA (3%) to broiler feed had a positive influence on Body Weight Gain (BWG), Feed Intake (FI), and Feed Conversion Ratio (FCR) because it enhanced nutrient digestibility due to correct intestinal conditions. These differences in outcomes could be attributed to a loss in palatability, as well as the impacts of location and breed.

Table 2.2 2: The effect of citric acid on feed intake of broiler chickens

Inclusion level	Breeds chicken	Conclusion	References
3g/kg per DM	Ross 308	3% of citric acid in the broiler diet led to a positive effect on feed intake of broilers since it could ameliorate nutrients digestibility because of proper intestinal conditions	Nourmohammadi and Khosravinia (2015)
(2.5 and 3 mg/kg feed)	Ross-308	Caused a decrease in the daily feed intake	Al-Amri and Al-Jashami (2019)
1%	Ross 308	No significant effect of citric acid (1.0%) on feed intake	Mohammadagheri *et al.* (2016)
0.25%	Ross 308	No significant effects of diets with the addition of organic acids on body weight and cause a decrease in feed consumption.	Kopecký *et al. (2012)*

2.2.3 Effect of citric acid on feed conversion efficiency of broiler chickens

Higher level of 60g/kg of citric acid appear to diminish feed palatability in avian species, whilst low inclusion level of 30g/kg appear to promote feed consumption (Islam, 2012; Hak et al. 2019). Khosravinia et al. (2015) reported a decrease in feed intake in broilers when 60 g/kg citric acid was added to the diet; this could be related to decreased palatability. According to Mohammadagheri et al., a 1.0% citric acid supplementation has no effect on feed consumption (2016). These findings could be attributed to lower pH causing an increase in favourable bacteria while higher pH inhibits the development of pathogenic bacteria. On the other hand, Atapattu and Nelligaswatta (2005) and Mohammed (2016) found that supplementing with 1.0, 2%, and 0.25% citric acid had no influence on feed conversion ratio in broiler chickens fed rice by product-based diet. These differences may be linked to the addition of acidic conditions into the gut, which causes the release of pepsin, gastrin, and cholecystokinin, all of which play essential roles in feed conversion and hence boost growth performance (Hayat et al. 2014). Table 2.3 presents the results of the citric acid effect on feed conversion ratio.

Table 2.2 3: The effect of citric acid on Feed conversion ratio of broiler chickens

Inclusion level	**Breeds chicken**	**Conclusion**	**References**
3g/kg per DM	Ross 308	3% of citric acid in the broiler diet led to a positive effect on feed conversion ratio of broilers since it could ameliorate nutrients digestibility as a result of proper intestinal conditions	Nourmohammadi and Khosravinia (2015)
30 g/kg and 60g/kg per DM	Ross 308	Improved average daily gain and feed conversion ratio exhibited in the broilers fed on diets supplemented with 30 g/kg CA; this may be related to lower palatability	Khosravinia *et al.* (2015)
0.75%	Cobb 500	0.75% citric acid in the diet had a positive effect on live weight, feed intake and feed conversion ratio	Rahman *et al.* (2018)
3.2 mg/kg feed	Vencob	Improved the feed conversion ration	Archana *et al*. (2019)

2.3 Effect of citric acid on carcass characteristics of broiler chickens

The structure and functionality of the gut microorganism, as well as the bird's well-being and nutrient digestion influence broiler chicken carcass characteristics such as dressed weight, carcass weight, back weight, and rib cage weight, weights of leg/shank, neck, breast, wing, or dressing percentage (Diaz et al. 2019). Adding different amounts of citric acid to broiler meals led in a significant increase in dressing percentage (without skin). Nezhad et al. (2007) mixed citric acid (0.0, 2.5, and 5.0%) with microbial phytase and found no effect on dressing production.

According to Archana et al. (2019), the use of citric acid greatly improved the highest dressing% and carcass weight. Furthermore, dietary acidification increased relative dressing percentage while having no influence on relative breast meat, thigh meat, or giblet weight (Haq et al. 2014). Hassan et al. (2015) discovered no significant effect on carcass metrics (dressing percentage, liver weight, and spleen weight) in broilers. Birds fed a diet containing 0.75% to 1.25% CA had a higher grade and a better European Broiler Index (EBI) (Islam, 2012). These findings support those of Abdel-Fattah et al. (2008) and Ebrahimnezhad et al (2009) who also found that CA levels in broiler diets influenced carcass weight. Despite this, Nourmohammadi et al. (2010) discovered no statistically significant gains in carcass output in broilers fed 6% CA and improvements at 3% CA. This disparity in results could be related to the increased live body weight of these birds. There has been no more research on the carcass quality of broiler carcasses treated with CA, although data from other organic acids supports the use of organic acids to improve carcass quality.

2.4 Effect of citric acid on tibia bone breaking strength of broiler chickens

Several studies have found a strong positive relationship between bone breaking strength and mineral density (Frost and Rowland, 1991; Orban et al., 1993; Rath et al., 1999; 2000; Massé et al., 2003). Islam (2011) discovered comparable effects, with mineral density increasing by 11, 15, and 11% at 0.25, 0.75, and 1.25% dietary CA levels, respectively. The highest maximum average force (Newton (N)) required to break the tibia was required by birds fed a meal containing 0.25% CA, however the 0.75 and 1.25% CA groups were numerically stronger than the control, indicating a dose-dependent response (Islam et al. 2011a; Islam, 2012). Because of the strong mineral deposition and utilization from the citric acid, the low citric acid inclusion level of 0.25% had the highest bone-breaking strength than the larger inclusion levels of 0.75% and 0.25%.

Mineral deposition with bone ash increases due to improved availability, resulting in an increase in bone breaking strength (Rowland et al. 1967; Meyer and Sunde, 1974; Anderson et al. 1979; Islam et al. 2011a; Islam, 2012). Dietary CA's effect on mineral metabolism could be reflected in increased bone mineral content, density, and breaking strength. Figure 1 depicts the average mineral density and bone-breaking strength of tibiae.

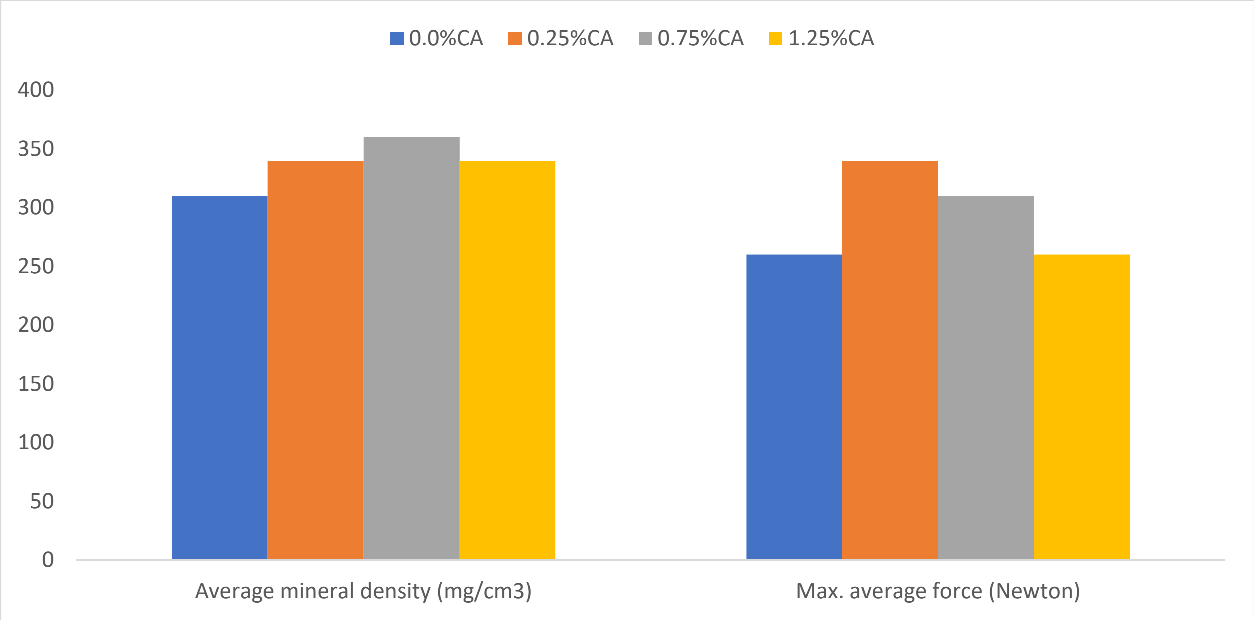

Figure 2.4.1: Mineral density and breaking strength of tibia of birds fed citric acid (CA) supplemented diet (Source: Islam *et al.* 2011a*)*

2.5 Effect of citric acid on ash content of broiler chickens

It was reported, by Islam (2012), that at lower supplementation levels of 0.25 and 0.75% of CA in a standard diet resulted in a numerical increase of dry matter content of bone and found significant differences between the values for ash, Ca, P and Mg content in fresh bone but were lower at the 1.25% CA level compared to 0.25 and 0.75% of CA. Brenes et al. (2003) found that a 2% CA supplementation level raised tibia ash, tibia Ca, and P, although this was not statistically significant. However, Rafacz-Livingston et al. (2005) discovered that increasing the dietary CA from 0% to 4.0% increases the amount of tibia ash linearly. These findings are consistent with those of Atapattu and Nelligaswatta (2005), who discovered that adding 1% and 2% CA levels to broiler diets enhanced the amount of tibia ash. The huge variance in reaction could be related to a lack of minerals in the meal. Citric acid supplementation increases tibia ash rate compared to non-supplemented (Chowdhury et al. 2009). Nourmohammadi et al. (2012), on the other hand, found that adding citric acid to diets decreased broiler chicken tibia Ca, Mg, and P levels, which may be linked to the amount of minerals in the diets.

2.6 Conclusion

Citric acid is used in broiler diets as an alternative to antibiotics because of its ability to improve gut health, its preservative effects on feed, feed conversion efficiency, growth, carcass quality, macro-mineral utilization, bone mineral content and density, and consequently its ability to improve economic profits from commercial production.

In broiler diets, 6% Citric Acid (CA) is the safe level to add without affecting performance. To improve performance, it is indicated that the ideal Citric Acid (CA) amount to utilize in the mash is 0.5% and 0.75% in commercial pelleted diets. Citric Acid (CA) may boost non-specific immunity and boost specific immunity against Newcastle disease, particularly after broiler vaccination. Citric Acid (CA) can compensate for performance losses in broilers fed low protein and energy diets, as well as boost mineral availability to birds. Citric Acid (CA) can be recommended for use in broiler rations and drinking water, but more research is needed to understand its mechanism of action and appropriate utilization in production of Ross 308 broiler chickens, since more information is available for Cobb 500 and Vencob breeds.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the Animal Unit, University of Limpopo. The study area lies at a 23.880870 and longitude 29.739465. The ambient temperatures around the study area ranges from 20°C to 36°C during summer and 10°C to 25°C during winter. The mean annual rainfall ranges from 446.8 – 468.4 mm (Kutu and Asiwe, (2010); Mabelebele, (2014)).

3.2 Ethical consideration

The chickens were managed and raised under the standard conditions that comply with the animal welfare requirement of South African National Standards (SANS) guidelines enforced by the University of Limpopo animal research ethic committee. The ethical clearance was obtained from the University of Limpopo Animal Research Ethics Committee (AREC). AREC registration number: AREC-290914-017 and project number: AREC/05/2020: PG

3.3 Housing and animal management

The house was thoroughly cleansed with water and Virokill disinfectant. After cleaning, the house was left empty for 7 days to disrupt the life cycle of any microorganisms that were not eliminated by the disinfectant. After drying, the house was divided into 20 floor pens of 2m^2 each, with a stocking density of 10 birds per pen. A 7cm layer of new sawdust was used to cover the floor. All the equipment used in the experiment was carefully cleaned and disinfected.

Every day, the footbath was cleaned, and more Jeyes liquid was applied to disinfect the boots before they entered the home. The residence was heated using infrared lights rated at 250 watts. For ventilation, shade rails were installed. The birds were monitored daily for any irregularities on their health checks were performed on the chickens' legs/feet, comb, eyes, feathers, beaks, and activity. . Sick birds were separated from the healthy chicks for each treatment and at the end of the trial euthanasia was utilized.

3.4 Acquisition of experimental animals and materials

A total of 200-day-old male Ross 308 broiler chicks were purchased from Angel Feeds company in Polokwane, South Africa. The chicks were transported with semi well ventilated pickup truck in the morning (time) (to avoid heat stress, which causes mortality during the day) from Angel feeds to University of Limpopo animal unit which is distance of about 30.9 km. A disinfectant (Virokill), 250 watts infrared lights, feed, feeders, and drinkers were also acquired from Angel Feeds, Polokwane, South Africa. Citric acid that was used for supplementation was purchased from Prestige Laboratory Supplies, South Africa.

3.5 Animal diets and experimental design

The chickens were fed in two phases: starter and finisher phases. The four diets at each phase were iso-energetic and iso-nitrogenous but with different citric acid supplementation levels of 0 g/kg DM of feed (T1), 12.5 g/kg DM of feed (T2), 25 g/kg DM feed (T3) and 50 g/kg DM feed (T4). The chickens were fed starter diet from Day 1 to 21 and finisher diet from Day 22 to 35.

3.5.1 Experiment 1

Ross 308 broiler chicks were sexed at hatching, and only males were used in the study because males grow faster, heavier and reach market weight faster than females (Da Costa, 2017). The objective of the first experiment of this study was to determine the effect of citric acid inclusion level in the diet on feed intake, digestibility, feed conversion ratio, growth rate, and live weight of male Ross 308 broiler chickens aged one to 21 days. A total of 200 male Ross 308 broiler chicks with initial mean body weight of 40$\pm$1.6g/bird were assigned to four treatment groups in a completely randomised design, each replicated five times, and each replicate having 10 chicks. The Citric acid inclusion levels in the diets were at 0 (MCA_0), 12.5 ($MCA_{12.5}$), 25 (MCA_{25}), or 50 (MCA_{50}) g per kg DM (Table 3.1). The chicks were fed formulated starter diet (Table 3.2) which was isocaloric and isonitrogenous, which met nutrient requirements of broiler chickens as recommended by the National Research Council (NRC, 1994). The chicks were offered feed and water *ad libitum* and provided light 24 hours per day. The partial one-way analysis of variance (ANOVA) (Table 3.3 for the completely randomised design is presented below.

Table 3. 1: Dietary treatments for Experiment 1

Diet code	**Diet description**
MCA_0	Male Ross 308 broiler chickens fed a 23% CP starter mash without citric acid inclusion.
$MCA_{12.5}$	Male Ross 308 broiler chickens fed a 23% CP starter mash having 12.5g of citric acid per kg DM.
MCA_{25}	Male Ross 308 broiler chickens fed a 23% CP starter mash having 25g of citric acid per kg DM.
MCA_{50}	Male Ross 308 broiler chickens fed a 23% CP starter mash having 50g of citric acid per kg DM.

Table 3. 2: Ingredients and nutrient composition of the starter diets for Experiment 1

	Diet			
	MCA_0	$MCA_{12.5}$	MCA_{25}	MCA_{50}
Feed Ingredients				
Soya oil cake 47%	29,00	29,50	28,63	28,63
Extr soya oil cake 40%	4,00	4,00	3,50	3,50
Sunflower 38%	4,00	3,80	3,80	3,80
Yellow maize	54,92	52,85	52,50	50,00
Soya oil	4,00	4,50	5,50	5,50
Salt	0,50	0,50	0,35	0,35
MCP	0,88	0,90	0,94	0,94
Limestone	1,50	1,50	1,16	1,16
Valine	0,20	0,20	0,18	0,18
Lysine HCL	0,35	0,35	0,30	0,30
Methionine	0,22	0,22	0,22	0,22
Threonine	0,03	0,03	0,02	0,02

Vit premix	0,30	0,30	0,30	0,30
Coccidiostat	0,05	0,05	0,05	0,05
Phytase enzy blend	0,05	0,05	0,05	0,05
Calculated analysis				
Moisture (%)	9,97	9,77	9,50	9,34
Protein (%)	23,03	23,00	23,00	23
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)	3.01	3.01	3.01	3.01
Lysine (%)	1,40	1,40	1,43	1,44
Meth (%)	0,65	0,65	0,64	0,63
CA (%)	0,81	0,90	0,79	0,63
P (%)	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

3.5.2 Experiment 2

The second experiment's goal was to determine the effect of citric acid supplementation on feed intake, digestibility, feed conversion ratio, growth rate, live weight, carcass weight and yields, bone breaking strength, and tibia ash content in male Ross 308 broiler chickens aged 22 to 35 days. The experiment began with 180 male Ross 308 broiler chickens that had been reared up to 21 days on formulated broiler mash (23% CP and 3.01 MJ energy/kg DM). In a perfectly randomized design, the chickens were divided into five treatment groups, each with five replicate pens of nine chickens.

The Citric acid inclusion levels in the diets were at 0 (MCA_0), 12.5 ($MCA_{12.5}$), 25 (MCA_{25}), or 50 (MCA_{50}) g per kg DM (Table 3.3). The diets were isocaloric and isonitrogenous (Table 3.4) and met the nutrient requirements of broiler chickens as recommended by the National Research Council (NRC, 1994). The chickens were offered feed and water *ad libitum* and the light was provided 24 hours per day. The partial one-way analysis of variance (ANOVA) (Table 3.5) for the completely randomised design is depicted below.

Table 3. 3: Dietary treatments for Experiment 2

Diet code	**Diet description**
MCA_0	Male Ross 308 broiler chickens fed a 20% CP finisher mash without citric acid inclusion.
$MCA_{12.5}$	Male Ross 308 broiler chickens fed a 20% CP finisher mash having 12.5g of citric acid per kg DM.
MCA_{25}	Male Ross 308 broiler chickens fed a 20% CP finisher mash having 25g of citric acid per kg DM.
MCA_{50}	Male Ross 308 broiler chickens fed a 20% CP finisher mash having 50g of citric acid per kg DM.

Table 3. 4: Ingredients and nutrient composition of the starter diets for Experiment 2

	Diet				
	MCA_0	$MCA_{12.5}$	MCA_{25}	MCA_{50}	MOM_{25}
Feed Ingredients					
Soya oil cake 47%	24,00	24,00	25,00	25,00	24,00
Extr soya oil cake 40%	3,00	3,00	3,00	3,00	3,00
Sunflower 38%	3,80	3,80	3,80	3,80	3,80
Yellow maize	59,39	58,02	55,33	52,83	59,39
Soya oil	6,50	6,60	7,00	7,00	6,50
Salt	0,35	0,35	0,35	0,35	0,35
MCP	0,79	0,81	0,85	0,85	0,79
Limestone	1,30	1,30	1,30	1,30	1,30
Valine	0,10	0,10	0,10	0,10	0,10

Lysine HCL	0,25	0,25	0,25	0,25	0,25
Methionine	0,15	0,15	0,15	0,15	0,15
Threonine	0,02	0,02	0,02	0,02	0,02
Vit premix	0,30	0,30	0,30	0,30	0,30
Coccidiostat	0,05	0,05	0,05	0,05	0.05
Phytase enzy blend	0,05	0,05	0,05	0,05	0.05
Calculated analysis					
Moisture (%)	10,04	9,83	9,35	9,30	10,04
Protein (%)	20,21	20,07	20,00	20,49	20,21
Fat (%)	8,80	9,24	9,00	9,58	8,80
Fibre (%)	2,62	2,60	2,60	2,59	2,62
Ash (%)	1,28	1,30	1,31	1,32	1,28
AMEN (MJ/kg)	3.22	3.21	3.21	3.11	3.22
Lysine (%)	1,22	1,22	1,24	1,26	1,22
Meth (%)	0,56	0,56	0,56	0,56	0,56
CA (%)	0,81	0,81	0,70	0,70	
P (%)	0,65	0,65	0,65	0,65	
NA (%)	0,19	0,19	0,13	0,13	
CL (%)	0,28	0,28	0,20	0,20	

Table 3. 5: Partial ANOVA

Source of variation (SV)	Sum of squares (SS)	Degrees of Freedom (DF)	Mean of squares (MS)	Variation Ratio (F)
Citric acid Error Total		(t-1) = (4-1) =3 (n-t) = (200-4) = 196 (n-1) = (200-1) = 199		

3.6 Data collection

Growth performance measurements

The initial live weights of the chicks were measured at the commencement of the experiment, from there on, individual live weights were measured at weekly intervals using Adams compact adjust scale of 620g x 0.001g. These live weights were utilized to calculate the growth rate of the chickens.

Daily feed intake per bird was measured by subtracting the weight of feed refusal from that offered per day, and the difference was divided by the entire number of chickens within the pen. Average feed conversion ratio per pen was calculated as add up to feed consumed divided by the weight gain of the birds in that pen (McDonald *et al.* 2010).

Digestibility trials were conducted in specially designed metabolic cages having separated watering and feeding troughs. Two birds were randomly selected from each replicate and used for digestibility determination. The chickens were given 3 days for adaptations in the metabolic cages. The faeces were collected from the fourth day until the seventh day at 09:00 hours every day. Faeces were weighed, dried, and kept for nutrient analysis (McDonald *et al.,* 2010).

Carcass characteristics measurements

After 35 days of the experimental period, following the same sample size and slaughtering method as Khan (2018), 40 male birds, ten per treatment were randomly selected and slaughtered by cervical dislocation. After slaughtering, scalding, plucking, and washing, the feet, head, and neck were removed to get carcass. The carcass was weighed using Adams compact adjust scale of 620g x 0.001g balancing scale to get carcass weight. Dressing percentage (carcass weight/Bodyweight) was calculated and expressed as a percentage.

Carcass yield measurements

Visceral organs (liver, heart, stomach fat, spleen, and digestive tract) were removed after slaughter, plucking and washing, then after they were weighed separately (per bird) using Adams compact adjust scale of 620g x 0.001g. Using a knife, the carcasses were cut separated into primal cuts: drumstick, wing, thigh, and breast. The carcasses were cut into drumsticks (legs), wings and thighs, respectively using a sharp knife; and through the shoulder area to remove the spine from the breast; at that point cuts were weight utilizing the Adams compact adjust scale of 620g x 0.001g. The carcass was chilled overnight at 4^0C in a refrigerator for advance analysis and weighed once more the taking after day to obtain cold carcass weight.

Bone preparation and Bone breaking strength measurements

By separating the thigh and drumstick with a knife, the tibia bones of each bird were removed while the drumsticks and flesh remained unharmed. The drumsticks were labelled with a permanent marker before being placed in a waterproof PVC plastic bag and soaked in boiling water (100 ^{0}C) for 10 minutes to release the tissue from the bone. Tissue was physically removed from the tibia once it had cooled to room temperature.

Measurements of bone biomechanical properties were taken by means of the three-point bending test. Bone-breaking strength was determined using an Instron Materials tester (model 3344, Instron Corp., Universal Testing apparatus machine) at constant speed of crosshead – 10 mm/min and distance between supports – 50 mm. The weights, diameters, and lengths of right tibiae were measured using Adams compact adjust scale of 620g x 0.001g and Empisal measuring tape Emt -001, respectively. Tibia diameters were measured at the narrowest points. Tracing of force was recorded at a constant rate.

Ash content measurements

Ash weight was calculated as a percentage. Tibia ash content was determined by ashing the bone in a crucible? in a muffle furnace for 48 h at 600 °C. The ash percentage was determined relative to the dry weight of ground tibia using the following formula:

Ash (%) = $\frac{\mathrm{w3-w1}}{\mathrm{w2-w1}} \times 100$

Where (w1) is the combined mass of the crucibles and lid weight, W2 is the combined mass of crucible, lid, and approximately 1 g of the ground dry sample, and W3 is the samples after ashing in a muffle furnace.

Bone mineralisation

The left tibia contents of Ca and P were measured using dry-ashed bone samples. Ca in the bones was determined according to the EDTA titration method by Han *et* *al*. (2013). Total P and Mg in the tibia were determined by photometric methods after reaction with ammonium molybdate and ammonium metavanadate.

3.7 Chemical analysis

Dry matter of meat, faecal and feed samples were determined by oven-drying at 105^0C for 24 hours. The ash content of bones and feed samples were analysed by ashing the samples at 600℃ in a muffle furnace overnight. Gross energy values for feeds and meat were determined using a bomb calorimeter according to the method described by Association of Analytical Chemists (AOAC) (2000) at the University of Limpopo Animal Nutrition Laboratory. Citric acid was determined by iron-exchange chromatography at the University of Limpopo Animal Nutrition Laboratory.

3.8 Data analysis

The effect of citric acid on growth performance, carcass weight, bone-breaking strength, and tibia ash content of male Ross 308 broiler chickens were analysed statistically with Statistically Package for Social Sciences (SPSS) 26.0 (2019) using one-way analysis of variance (ANOVA). Data was analysed using the statistical model depicted below:

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

Where: Y_{ij} = response variable (growth performance, carcass weight, bone-breaking strength, and tibia ash content), μ = the overall mean, α_i = the effect of citric acid treatment (T1, T2, T3, and T4) and e_{ij} = random error. The treatment means were compared using the Tukey test with a significance level of $P < 0.05$.

The responses in optimal productivity (growth performance, carcass weight, bone-breaking strength, and tibia ash content) to the level of citric acid supplementation were modelled using the following quadratic equation:

$$Y = a + b_1x_1 + b_2{x_2}^2 + e$$

Where Y = response variable (growth performance, carcass weight, bone-breaking strength, and tibia ash content); 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

4.1 Nutrient composition of the diets

Results of the nutrient composition of the experimental finisher diets are presented in Table 4.1. The starter and finisher diets had similar protein and energy contents of 23% and 3.01MJ/kg and finisher diet also had similar protein and energy contents of 20% and 16.8 MJ/kg DM, respectively. However, diets had different citric acid inclusion levels of 0, 12.50, 25, or 50g per kg DM

Table 4. 1: *Nutrient composition of the diets*

	Citric Acid Inclusion level (g/kg DM of feed)			
Nutrient	**0**	**12.5**	**25**	**50**
		STARTER DIET		
Moisture	9,97	9,77	9,50	9,34
Protein	23,03	23,00	23,00	23
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 MJ/kg	3.01	3.01	3.01	3.01
Lysine	1,40	1,40	1,43	1,44
Meth	0,65	0,65	0,64	0,63
Ca	0,81	0,90	0,79	0,63
P	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
		FINISHER DIET		
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 MJ/kg	3.22	3.21	3.21	3.11
Lysine	1,22	1,22	1,24	1,26
Meth	0,56	0,56	0,56	0,56
Ca	0,64	0,65	0,65	0,66
P	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. The effect of citric acid inclusion level on growth performance of male Ross 308 broiler chickens aged 21 days

Results of the effects of citric acid inclusion in a diet on live weight, DM feed intake, feed conversion ratio (FCR), body weight gain and growth rate of male Ross 308 broiler chickens aged one to 21 days are presented in Table 4.2. Citric acid inclusion in diets affected (P<0.05) live weight, and growth rate of male Ross 308 broiler chickens aged one to 21 days and had no effect (P>0.05) on daily feed intake (DFI), feed conversion ratio (FCR), and body weight gain (BWG).

The chickens fed with 12.50 g/kg CA had heavier (P<0.05) live weights than those fed diets having 0, 25 or 50g of citric acid per kg DM. Similarly, chickens fed a diet having 0, 25 or 50g of citric acid per kg DM had similar (P<0.05) live weights. Whilst chickens fed with 50g of citric acid had the lowest live weight. However, those fed at 0 and 25g of citric acid per gram DM had similar (P>0.05) live weights. A 2.226g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal DM live weight of male Ross 308 broiler chickens aged one to 21 days (Figure 4.2 and Table 4.3).

Male Ross 308 on diets containing 12.50g of citric acid per kg of DM had the highest daily feed intake than those fed at 0, 25, or 50g of citric acid per kg of DM. However, the male Ross 308 broiler chickens fed at 0g had the lowest daily feed intake.

Male Ross 308 broiler chickens fed diet with 12.50g of citric acid per kg of DM had the heaviest body weight gain than those fed at 0, 25 or 50g. While those fed diet with 25g of citric acid per kg of DM had the lightest body weight gain than the those in the diets 0g and 50g of citric acid per kg of DM. Results of the diets containing fed 12.50g of citric acid per kg of DM showed a higher feed conversion ratio, while those male Ross 308 broiler chickens fed at 0 (control) had the lowest feed conversion ratio of 3.37 than those fed at 25 and 50g of citric acid per kg of DM.

Male Ross 308 broiler chickens fed diets having 12.50g of citric acid per kg DM had faster (P<0.05) growth rate than those fed diets with 0, 25, or 50g of citric acid per kg DM. However, male chickens fed diets having 0 or 25g of citric acid per kg DM had similar (P>0.05) growth rate. Moreover, chickens fed diets having 50g of citric acid per kg DM had the slowest (P>0.05) growth rate. A 2.228g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal growth rate of male Ross 308 broiler chickens aged 21 days (Figure 4.3 and Table 4.2)

Table 4. 2: The effect of citric acid inclusion levels on live weights (LW), daily feed intake (DFI), body weight gain (BWG), feed conversion ratio (FCR), and growth rate (GR) of male Ross 308 broiler chickens Aged 21 days

	Diets*			
Variables#	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}
LW (g/bird/day)	$788,75^{ab} \pm 45,98$	$856,40^{a} \pm 49,02$	$810,76^{ab} \pm 37,55$	$742,20^{b} \pm 74,27$
DFI (DM g/bird/day)	1,79 ± 0,35	2,00 ± 0,03	1,98 ± 0,03	1,97 ± 0,05
BWG (g/bird/day)	530,60 ± 43,38	555,06 ± 37,45	484,90 ± 145,72	487,82 ± 51,90
FCR (g DM feed/g live weight gain)	3,37 ± 0,60	3,61 ± 0,22	4,62 ± 2,24	4,05 ± 0,31
GR (g/bird/day)	$37,56^{ab} \pm 2,19$	$40,78^{a} \pm 2,34$	$38,61^{ab} \pm 1,79$	$35,34^{b} \pm 3,54$

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

CA : Citric acid supplemented

** : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA*

 # : Values presented as mean ± standard deviation

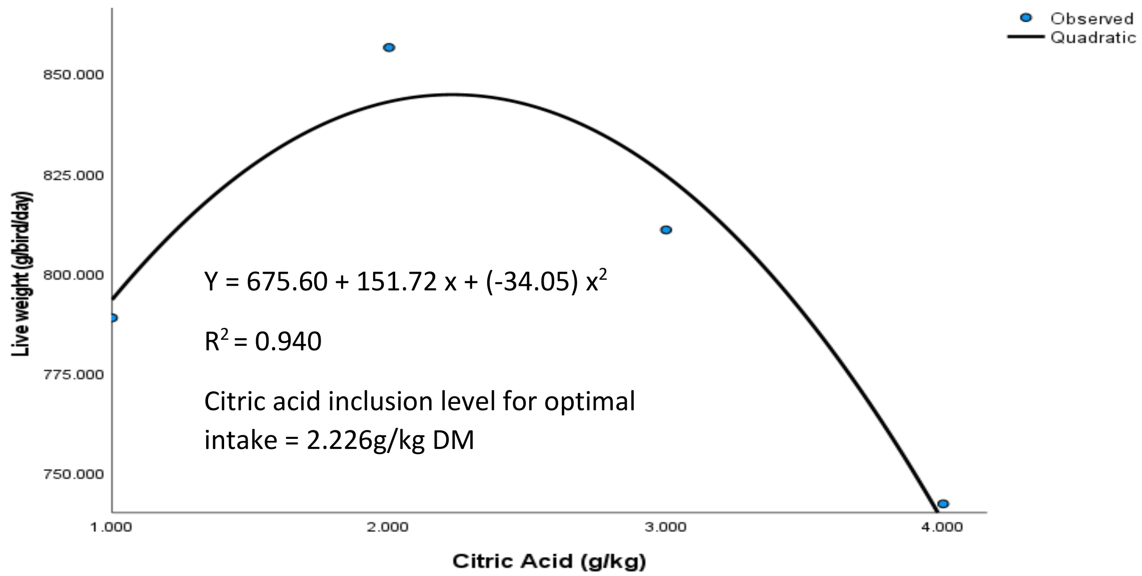

Figure 4. 1: The effect of citric acid inclusion level on live weight of male Ross 308 broiler chickens aged 21 days

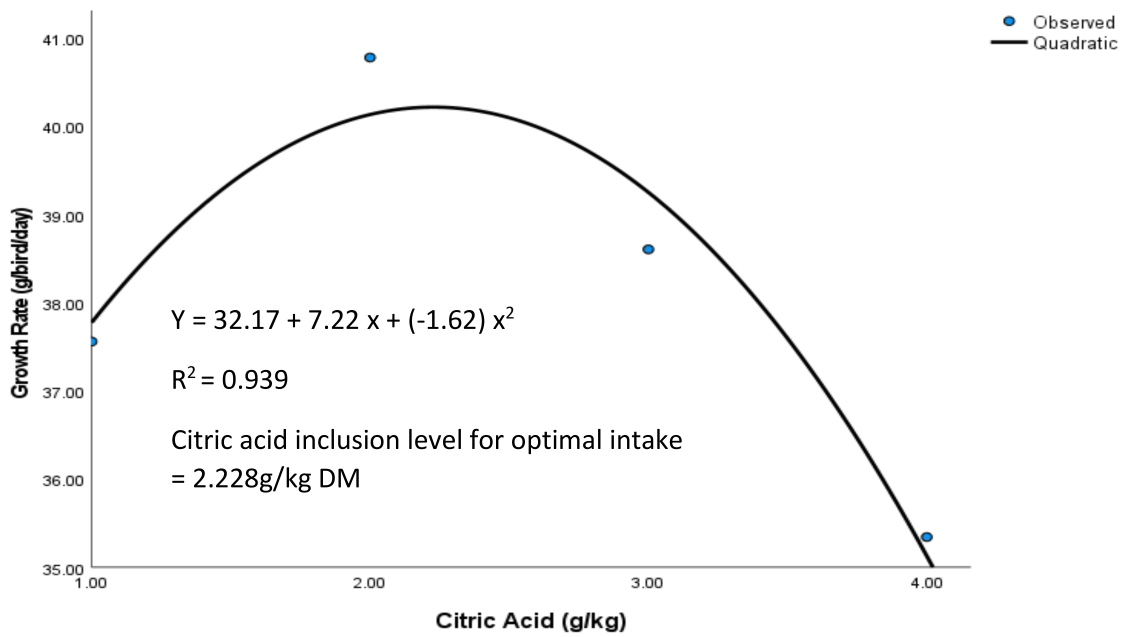

Figure 4. 2: The effect of citric acid inclusion level on growth rate of male Ross 308 broiler chickens aged 21 days

Table 4. 3: Citric acid inclusion levels for optimal live weights (LW), and growth rate (GR) of male Ross 308 broiler chickens Aged 21 days

Factor	Formula	X	Y	R^2	P
LW	$Y = 675.60 + 151.72x + (-34.05)x^2$	2.226	844.06	0.940	0.246
GR	$Y = 32.17 + 7.22x + (-1.62)x^2$	2.228	40.21	0.939	0.246

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

4.3. The effect of citric acid inclusion level on growth performance of male Ross 308 broiler chickens aged 35 days

Results of the effects of citric acid inclusion in a diet on live weight, DM feed intake, feed conversion ratio (FCR), body weight gain and growth rate of male Ross 308 broiler chickens aged one to 35 days are presented in Table 4.4. Citric acid inclusion in diets affected (P<0.05) live weight, feed conversion ratio (FCR), body weight gain (BWG) and growth rate but had no effect (P>0.05) on daily feed intake (DFI) of male Ross 308 broiler chickens aged 35 days.

The chickens fed at 12.50 g/kg CA had heavier (P<0.05) live weights than those fed diets having 0,25 or 50g of citric acid per kg DM. Similarly, chickens fed a diet having 0 or 50g of citric acid per kg DM had similar (P<0.05) live weights. However, male Ross 308 broiler chickens fed diet with 25g of citric acid had the lightest live weight. However, those fed diet with 0 and 50g of citric acid per gram DM had similar (P>0.05) live weights the chickens. A 24.939g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal DM live weight of male Ross 308 broiler chickens aged 35 days (Figure 4.3 and Table 4.4). A positive relationship (r^2 = 0.649) was observed between citric acid inclusion level on live weight of the chickens aged 35 days (Figure 4.3).

Results of the present study indicated that the chickens fed control diet of 0g of citric acid per kg DM had the highest (P<0.05) body weight gain than those fed diets with 12.5, 25, or 50g of citric acid per kg DM. However, male chickens fed diets having 12.5 or 50g of citric acid per kg DM had similar (P>0.05) body weight gain. Moreover, chickens fed diets having 25g of citric acid per kg DM had the lowest (P>0.05) body weight gain. A 2.880g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal body weight gain of male Ross 308 broiler chickens aged 35 days (Figure 4.5 and Table 4.4)

A diet having 25 g/kg citric acid had the highest ($P<0.05$) feed conversion ratio than those fed diets having 0,12.50 or 50g of citric acid per kg DM. Similarly, male broiler chickens fed a diet having 12.50 or 50g of citric acid per kg DM had similar ($P<0.05$) feed conversion ratio. However, male Ross 308 broiler chickens fed at control level 0g of citric acid had the lowest feed conversion ratio. However, chickens fed at 12.50 and 50g of citric acid per gram DM had similar ($P>0.05$) feed conversion ratio male Ross 308 broiler chickens. A 2.893g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal DM feed conversion ratio of male Ross 308 broiler chickens aged 35 days (Figure 4.6 and Table 4.4).

Results of the present study indicated that the chickens fed diets having 12.50g of citric acid per kg DM had faster ($P<0.05$) growth rate than those fed diets with 0, 25, or 50g of citric acid per kg DM. However, male chickens fed diets having 0 or 50g of citric acid per kg DM had similar ($P>0.05$) growth rate. Moreover, chickens fed diets having 25g of citric acid per kg DM had the slowest ($P>0.05$) growth rate. A -25.157g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal growth rate of male Ross 308 broiler chickens aged 35 days (Figure 4.7 and Table 4.4). A positive relationship ($r^2 = 0.649$) was observed between citric acid inclusion level on growth rate of male Ross 308 broiler chickens aged 35 days (Figure 4.3).

Table 4. 4: The effect of citric acid inclusion levels on live weights (LW), daily feed intake (DFI), body weight gain (BWG), feed conversion ratio (FCR), and growth rate (GR) of male Ross 308 broiler chickens Aged 35 days

	Diets*			
Variables[#]	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}
LW (g/bird/day)	$1856,72^{ab} \pm 132,50$	$1886,22^{a} \pm 100,69$	$1699,78^{b} \pm 42,46$	$1725,06^{ab} \pm 92,36$
DFI (DM	2,15 ± 0,15	2,15 ± 0,08	2,14 ± 0,10	2,14 ± 0,10

g/bird/day)				
BWG (g/bird/day)	1278,87[a] ± 128,69	1109,90[ab] ± 75,57	976,90[b] ± 150,81	1123,48[ab] ± 58,05
FCR (g DM feed/g live weight gain)	1,69[b] ± 0,23	1,94[ab] ± 0,10	2,24[a] ± 0,42	1,91[ab] ± 0,02
GR (g/bird/day)	53,05[ab] ± 3,79	53,89[a] ± 2,88	48,57[b] ± 1,21	49,29[ab] ± 2,64

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

CA : Citric acid supplemented

** : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA*

 # : Values presented as mean ± standard deviation

Table 4. 5: Citric acid inclusion levels for optimal live weights (LW), body weight gain (BWG), feed conversion ratio (FCR), and growth rate (GR) of male Ross 308 broiler chickens aged 35 days

Factor	Formula	X	Y	R^2	P
LW	$Y = 1932.03 + 52.87 x + (-1.06) x^2$	24.939	2591.283	0.649	0.592
BWG	$Y = 1666.52 + (-454.35) x + 78.89 x^2$	2.880	1012.337	0.935	0.254
FCR	$Y = 0.99 + 0.81 x + (-0.14) x^2$	2.893	2.162	0.845	0.393
GR	$Y = 55.20 + (-1.51) x + (-0.03) x^2$	-25.167	74.200	0.649	0.592

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

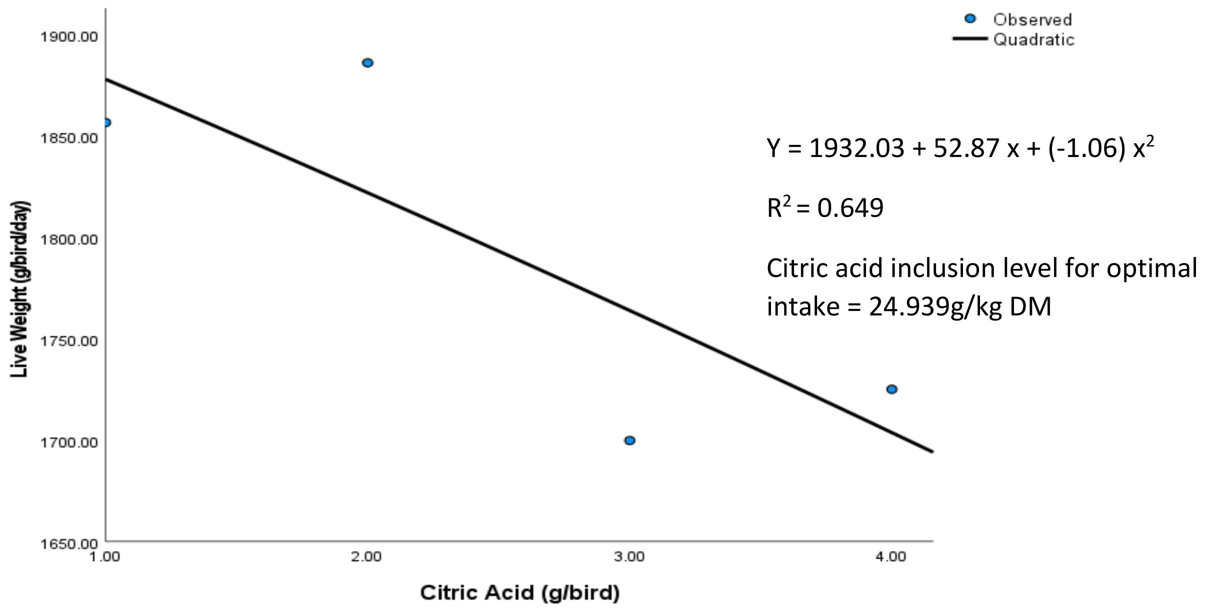

Figure 4. 3: *The effect of citric acid inclusion level on live weight of male Ross 308 broiler chickens aged 35 days*

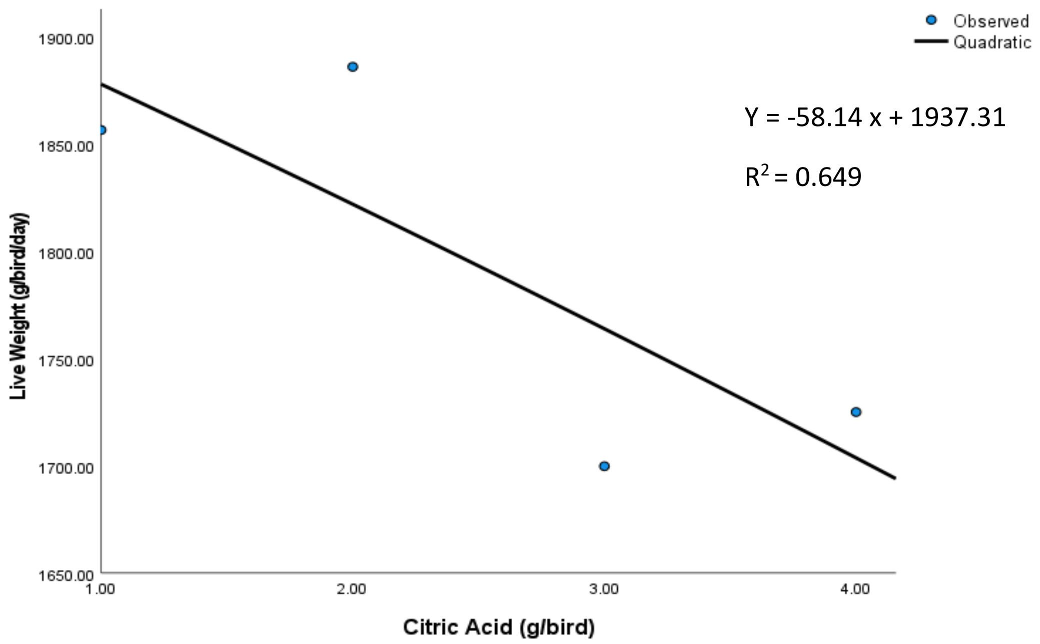

Figure 4. 4: *The relationship between citric acid inclusion level and live weight of male Ross 308 broiler chickens aged 35 days*

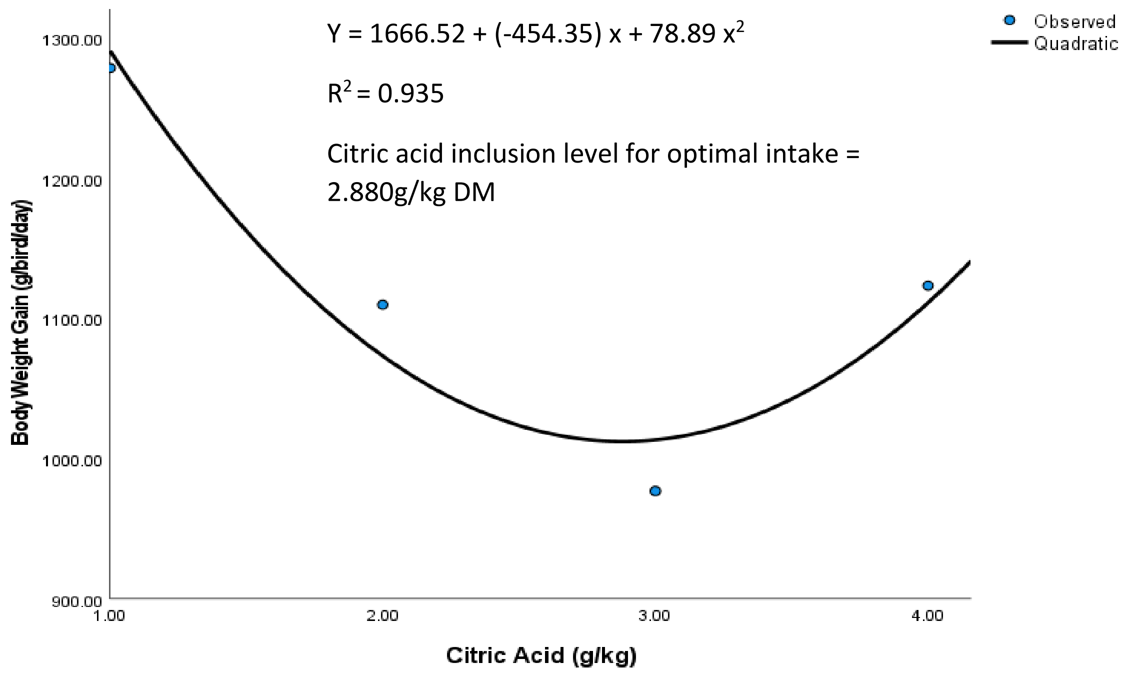

Figure 4. 5: *The effect of citric acid inclusion level on body weight gain of male Ross 308 broiler chickens aged 35 days*

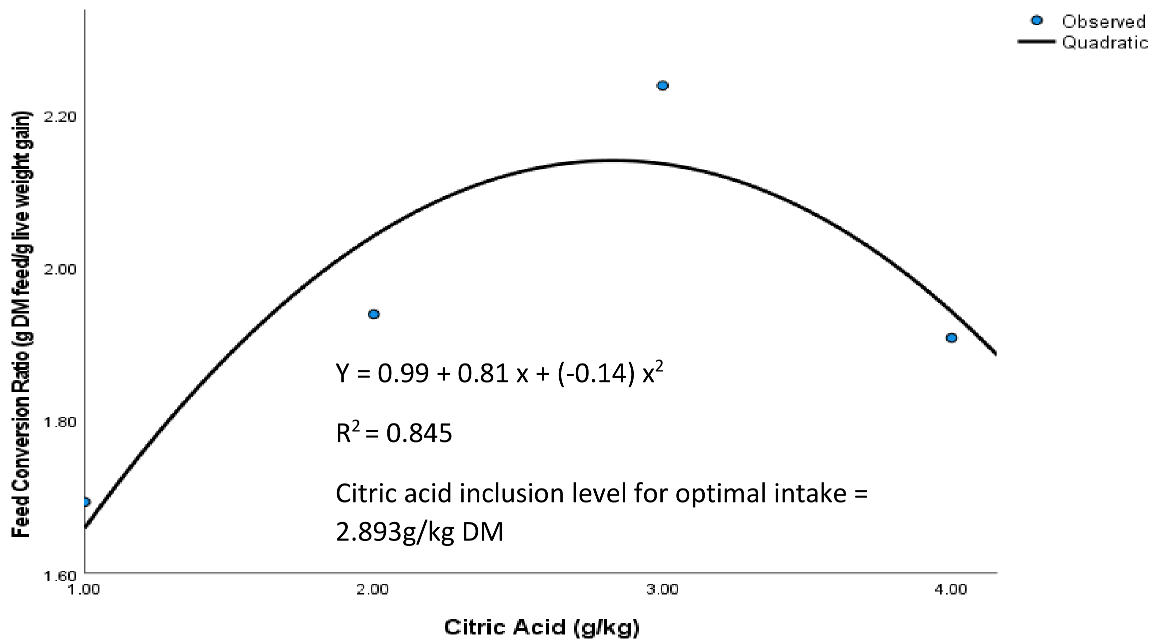

Figure 4. 6: *The effect of citric acid inclusion level on feed conversion ratio of male Ross 308 broiler chickens aged 35 days*

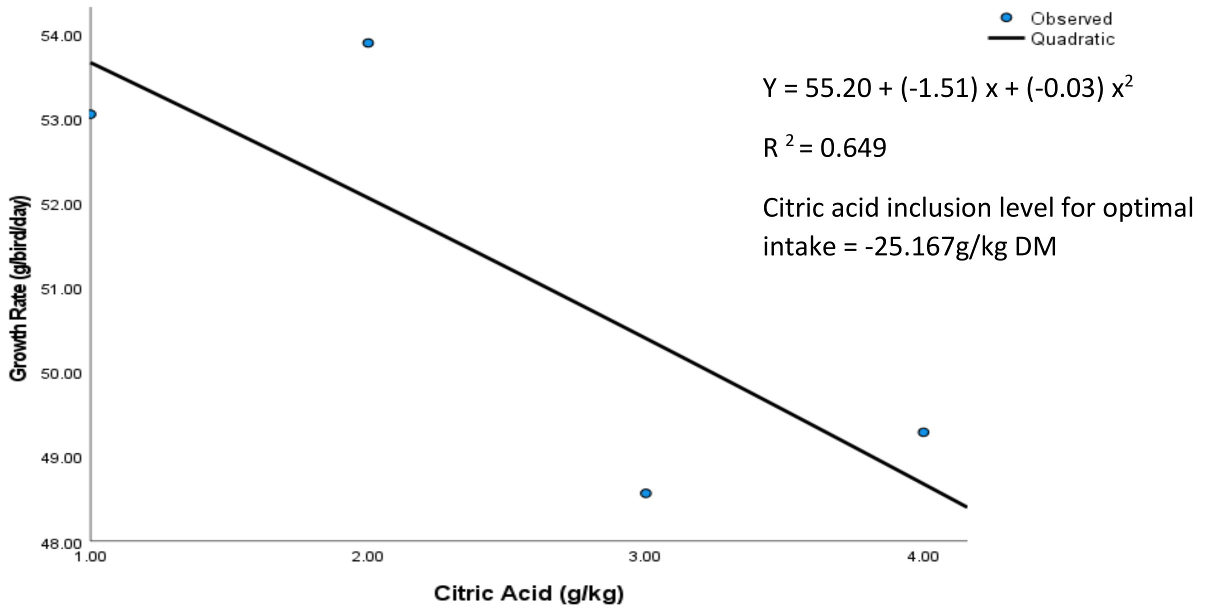

Figure 4. 7: *The effect of citric acid inclusion level on growth rate of male Ross 308 broiler chickens aged 35 days*

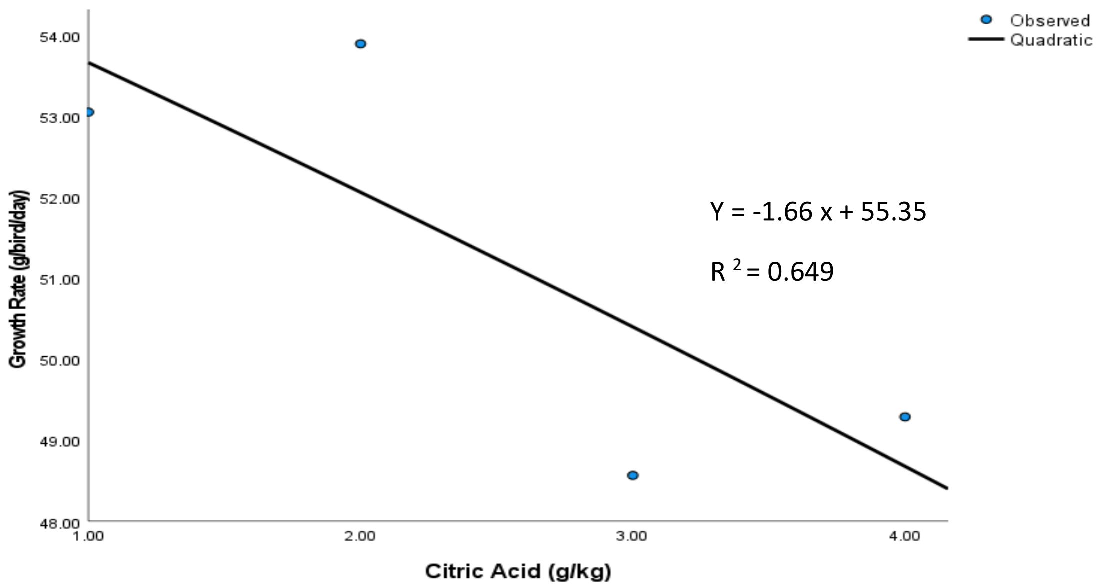

Figure 4. 8: *The relationship between citric acid inclusion level and growth rate of male Ross 308 broiler chickens aged 35 days*

4.4. Effect of citric acid inclusion level in a diet on dry matter, crude protein and ash digestibility, metabolisable energy intake and nitrogen retention of male Ross 308 chickens aged 35

The results of the effects of citric acid inclusion level in a diet on dry matter, crude protein and ash digestibility values, metabolizable energy intake, and nitrogen retention of male Ross 308 broiler chickens aged 35 days are presented in Table 4.6. Citric acid inclusion level in a diet affected ($P<0.05$) dry matter, crude protein and ash digestibility values, metabolisable energy (ME) intake and nitrogen retention values of male Ross 308 broiler chickens aged 35 days. Broiler chickens fed a diet having 25g citric acid per kg DM had higher ($P<0.05$) dry matter digestibility values than those fed a diet having 0, 12.5 or 50g of citric acid per kg DM. However, chickens fed diets containing 12.5g of citric acid per kg DM had the lowest ($P>0.05$) dry matter digestibility values. A 2.59g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal DM digestibility percentage values of male Ross 308 broiler chickens (Figure 4.8 and Table 4.7).

Table 4. 6: Effect of citric acid inclusion level in a diet on dry matter, crude protein and ash digestibility, metabolizable energy intake and nitrogen retention of male Ross 308 chickens aged 35

Factor	Formula	X	Y	R^2	P
DM digestibility	$Y = 47.12 + 7.66x + (-1.48)x^2$	2.59	57.03	0.128	0.934
CP digestibility	$Y = 88.17 + 1.73x + (-0.41)x^2$	2.11	89.99	0.336	0.815
ME Intake	$Y = 3176.53 + 65.87x + (-20.64)x^2$	1.596	3229.08	0.920	0.284
N - Retention	$Y = 8.61 + 0.37x + (-0.08)x^2$	2.3125	9.0378	0.547	0.673

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

Male Ross 308 broiler chickens fed a diet having 25g of citric acid per kg DM had higher (P<0.05) crude protein digestibility values than those fed diets having 0, 12.5 or 50g of citric acid per kg DM. Similarly, broiler chickens fed diets having 0 or 12.5g of citric acid per kg DM had higher (P<0.05) and similar (P>0.05) crude protein digestibility values than those on a diet having 50g of citric acid per kg DM. However, chickens fed diets having 12.5 or 50g of citric acid per kg DM had similar (P>0.05) crude protein digestibility values. However, broiler chickens fed diets having 50g of citric acid per kg DM had lowest (P<0.05) crude protein digestibility values. A 2.11g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal CP digestibility percentage values of male Ross 308 broiler chickens (Figure 4.10 and Table 4.7).

Results of the present study indicate that male Ross 308 broiler chickens fed control diet of 0g of citric acid per kg DM had the highest (P<0.05) metabolizable energy value than those fed diets with 12.5, 25, or 50g of citric acid per kg DM. However, male chickens fed diets having 12.5 or 25g of citric acid per kg DM had similar (P>0.05) metabolizable energy value. Moreover, chickens fed diets having 50g of citric acid per kg DM had the lowest (P>0.05) metabolizable energy value. A 1.596g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal metabolizable energy of male Ross 308 broiler chickens (Figure 4.11 and Table 4.7)

Male Ross 308 broiler chickens fed a diet having 25g of citric acid per kg DM had higher (P<0.05) nitrogen retention values than those fed diets having 0, 12.5 or 50g of citric acid per kg DM. However, male Ross 308 broiler chickens fed diets having 0, 12.5 or 50g of citric acid per kg DM had similar (P>0.05) and lowest nitrogen retention values. A 2.3125g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal nitrogen retention values of male Ross 308 broiler chickens (Figure 4.12 and Table 4.7).

Table 4. 7: Effect of citric acid inclusion level in a diet on dry matter, crude protein and ash digestibility, metabolizable energy intake and nitrogen retention of male Ross 308 chickens aged 35

	Diets*			
Variables#	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}

Digestibility (%)				
Dry Matter	55,06^{b} ± 0,13	51,26^{d} ± 0,23	62,09^{a} ± 0,14	52,38^{c} ± 0,07
CP	89,83^{ab} ± 0,24	89,00^{bc} ± 0,25	90,70^{a} ± 0,63	88,24^{c} ± 0,17
ME Intake (MJ/kg DM)	3227,91^{a} ± 9,85	3207,23^{b} ± 4,34	3206,84^{b} ± 5,93	3103,60^{c} ± 3,21
Nitrogen retention (g/broiler/day)	8,94^{b} ± 0,05	8,96^{b} ± 0,07	9,13^{a} ± 0,07	8,85^{a} ± 0,04

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

CA : Citric acid supplemented

* : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA

: Values presented as mean ± standard deviation

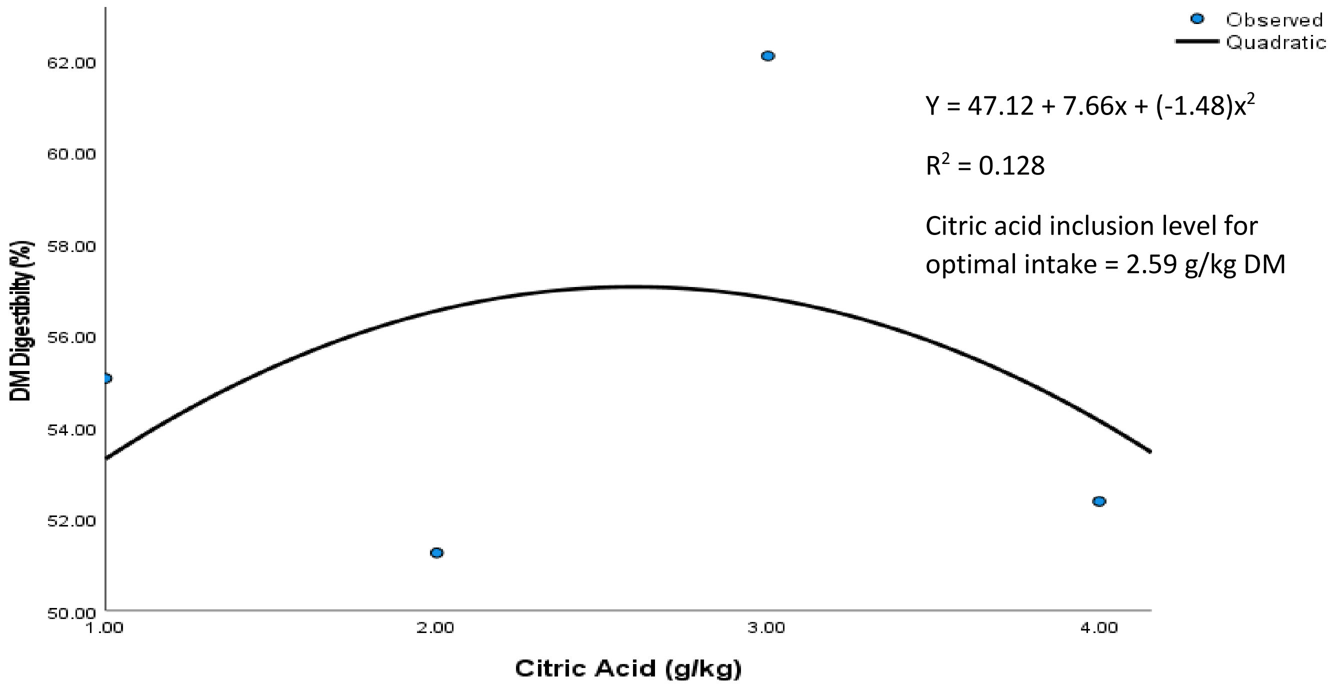

***Figure 4. 9** : The effect of citric acid inclusion levels on DM digestibility of male Ross 308 broiler chickens*

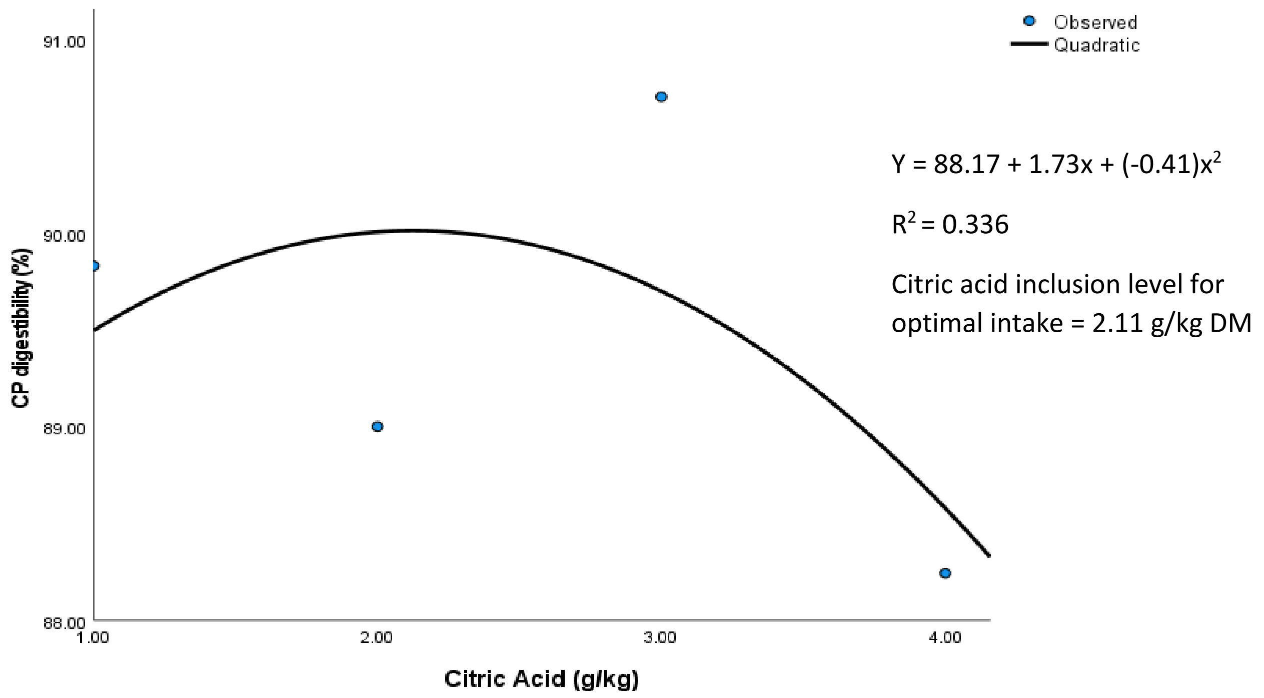

Figure 4. 10*: The effect of citric acid inclusion levels on CP digestibility of male Ross 308 broiler chickens*

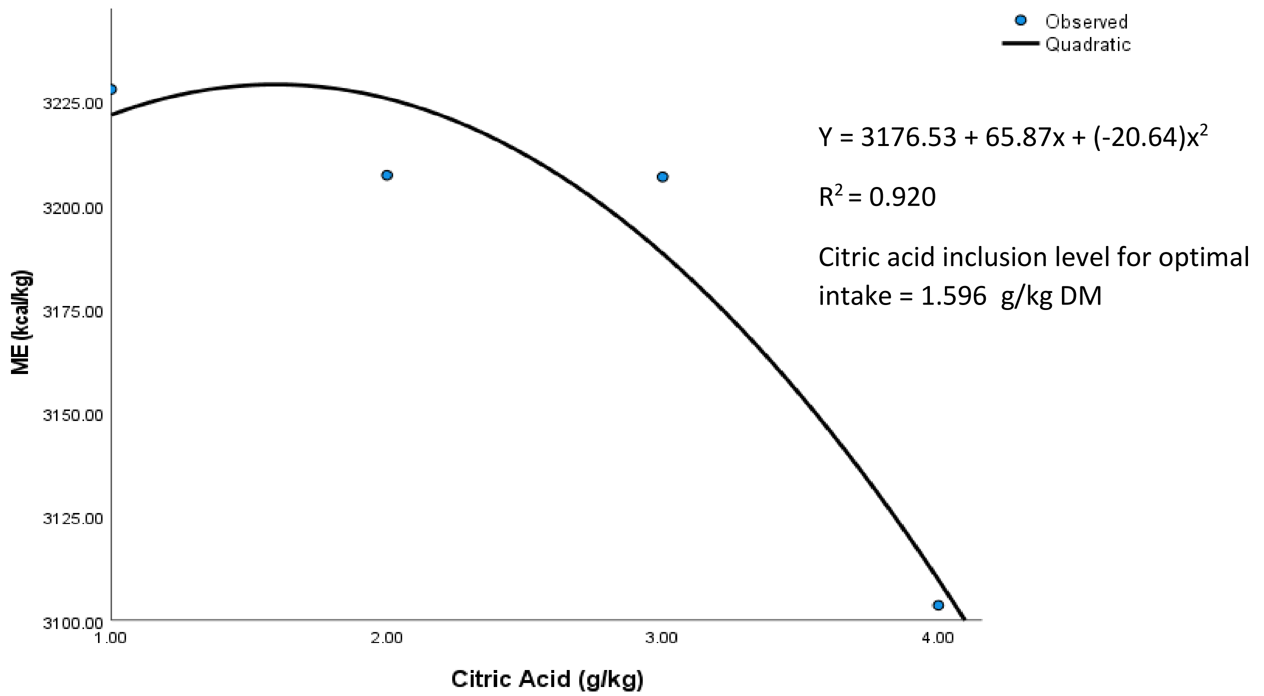

Figure 4. 11*: The effect of citric acid inclusion levels on metabolizable energy of male Ross 308 broiler chickens*

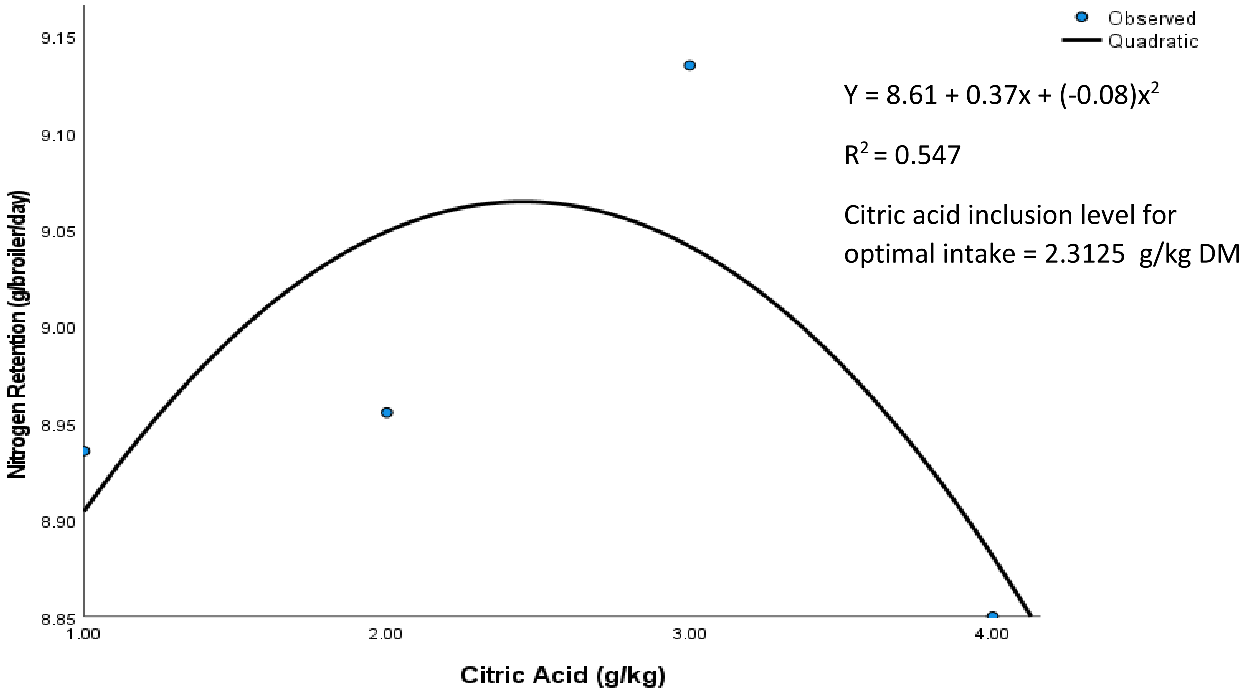

Figure 4. 12*: The effect of citric acid inclusion levels on Nitrogen retention of male Ross 308 broiler chickens*

4.5. The effect of citric acid inclusion level on carcass weight of male Ross 308 broiler chickens

The effects of citric acid inclusion in a diet on carcass weight and dressing percentage of male Ross 308 broiler chickens are presented in Table 4.8. Citric acid inclusion in diets had no effect (P>0.05) on live (LW), carcass weight, and dressing rate (DR) of male Ross 308 broiler chickens.

4.6. The effect of citric acid inclusion level on carcass yield of male Ross 308 broiler chickens

Results of the effects of citric acid inclusion in a diet on thigh, wing, drumstick, breast and internal organs of male Ross 308 broiler chickens are presented in Table 4.9. Citric acid inclusion in diets affected (P<0.05) the breast weight but had no effect (P>0.05) on thigh, wing, drumstick, and internal organs of male Ross 308 broiler chickens. Results of the present study indicate that male Ross 308 broiler chickens fed the control diet having had heaviest (P<0.05) breast weight than those fed diets with 12.50, 25, or 50g of citric acid per kg DM. However, chickens fed diets having 50g of citric acid per kg DM had the lowest (P>0.05) breast weight. A 4.942g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal breast weight of male Ross 308 broiler chickens aged 35 days (Figure 4.13 and Table 4.10).

Table 4. 8: The effect of citric acid inclusion levels on thigh, wing, drumstick, breast and internal organ's weight of male Ross 308 broiler chickens

	Diets*			
Variables#	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}
Thigh	91,21 ± 14,59	95,11 ± 13,07	88,77 ± 5,34	88,71 ± 5,64
Wing	74,82 ± 6,71	77,39 ± 2,33	75,79 ± 4,36	80,74 ± 7,74
Drumstick	89,33 ± 9,12	89,86 ± 9,67	80,46 ± 6,70	84,37 ± 9,74
Breast	$584,59^{a}$ ± 72,48	$538,74^{ab}$ ± 73,21	$478,36^{bc}$ ± 21,72	$466,46^{c}$ ± 21,19
Internal organs	288,43 ± 28,48	277,73 ± 37,22	287,78 ± 27,96	288,76 ± 15,30

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

CA : Citric acid supplemented

** : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA*

 # : Values presented as mean ± standard deviation

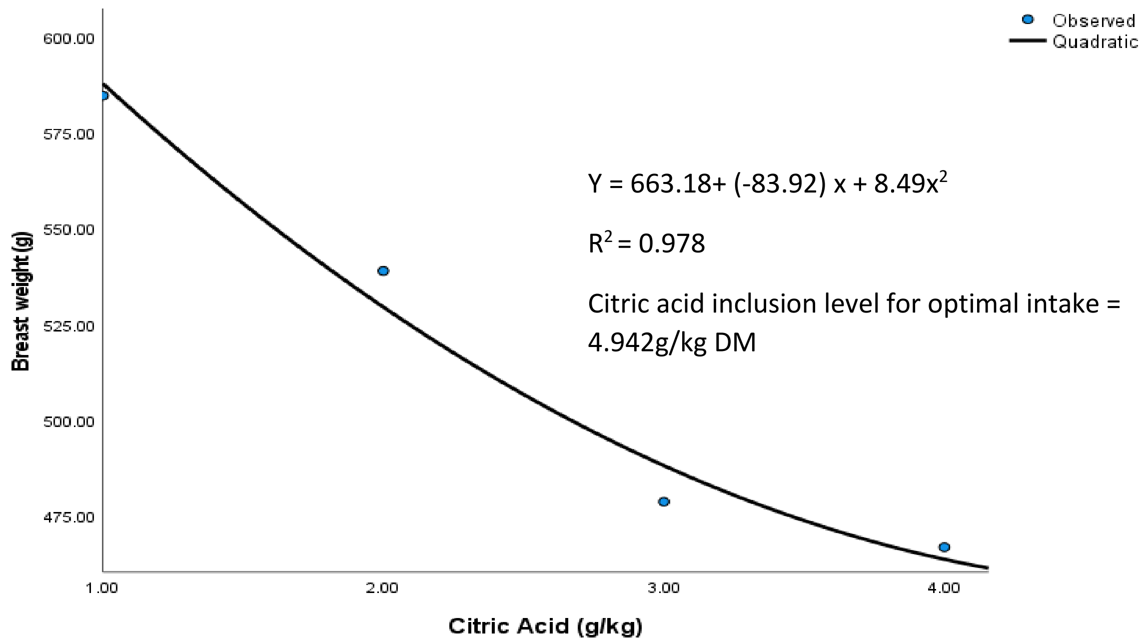

Figure 4. 13*: The effect of citric acid inclusion levels on breast weight of male Ross 308 broiler chickens*

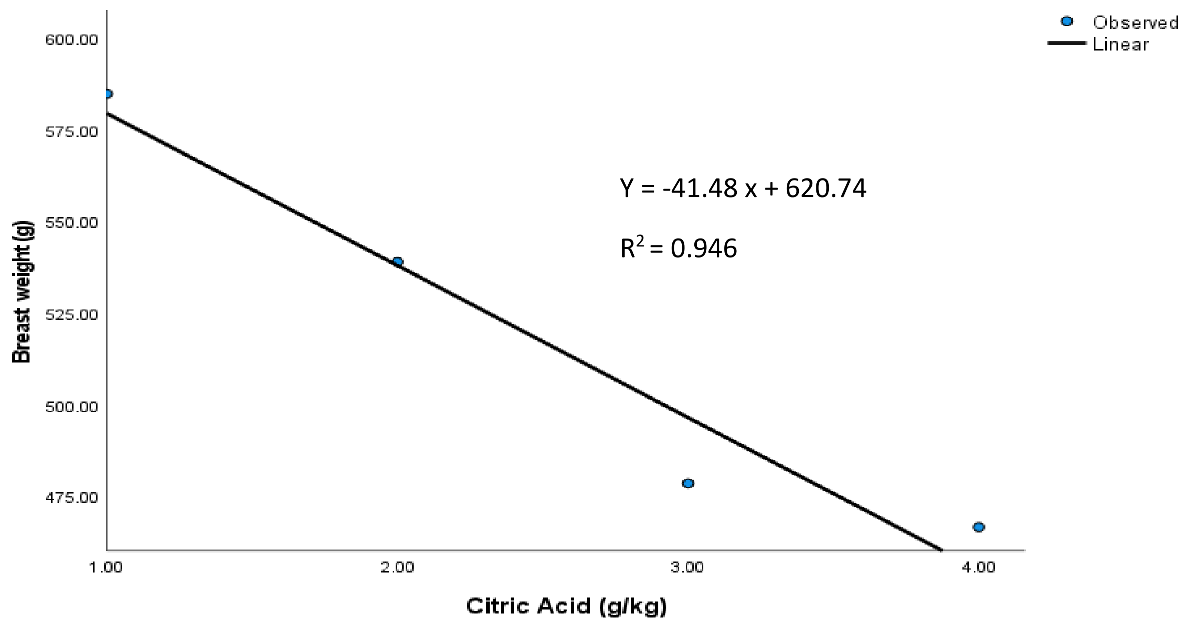

Figure 4. 14: *The relationship between citric acid inclusion levels and breast weight of male Ross 308 broiler chickens*

Table 4. 9: *Citric acid inclusion levels for optimal breast weight of male Ross 308 broiler chickens*

Factor	Formula	X	Y	R^2	P
Breast weight	$Y = 663.18 + (-83.92)x + 8.49x^2$	4.942	455.802	0.978	0.02

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

4.7. The effect of citric acid inclusion level on bone parameters of male Ross 308 broiler chickens

Results of the effects of citric acid inclusion in a diet on bone breaking strength, weight, diameter, length, ash weight percentage, calcium, phosphorous and magnesium of male Ross 308 broiler chickens are presented in Table 4.11. Citric acid inclusion in diets had no effect (P>0.05) on bone breaking strength and weight of air died and oven dried bones and lengths of male Ross 308 broiler chickens. However, it had an effect (P<0.05) on bone weight of air-dried bones and ash weight percentage. A diet having 12.50 g/kg citric acid had the heaviest (P<0.05) bone weight of air-dried bones than those fed diets having 0,25 or 50g of citric acid per kg DM. Similarly, male broiler chickens fed a diet having 0g or 50g of citric acid per kg DM had similar (P<0.05) bone weight of air-dried bones. However, male Ross 308 broiler chickens fed control level 25g of citric acid had the lightest bone weight of air-dried bones. A 2.833g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal DM bone weight of air-dried bones of male Ross 308 broiler chickens (Figure 4.15 and Table 4.12).

Table 4. 10: The effect of citric acid inclusion levels on bone breaking strength Air and Oven dried, Diameter, weight of Air and oven dried bones, and length of male Ross 308 broiler chickens

	Diets*			
Variables[#]	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}
Bone breaking Strength Air dried (N)	80,33 ± 0,15	80,64 ± 0,68	80,60 ± 0,17	80,32 ± 0,15

Bone breaking Strength Oven dried (N)	80,41 ± 0,23	80,84 ± 0,74	80,38 ± 0,20	80,59 ± 0,16
diameter cm	2,62[ab] ± 0,26	2,73[a] ± 0,19	2,42[b] ± 0,30	2,53[ab] ± 0,22
Bone weight Air dried (g)	13,05[a] ± 1,68	11,64[ab] ± 2,05	11,08[b] ± 1,22	11,93[ab] ± 1,09
Bone weight Oven dried (g)	7,30 ± 0,68	7,15 ± 0,88	7,02 ± 0,57	7,09 ± 0,63
Length (cm)	9,79 ± 0,35	9,89 ± 0,44	9,76 ± 0,37	9,51 ± 0,33

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

CA : Citric acid supplemented

* : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA

\# : Values presented as mean ± standard deviation

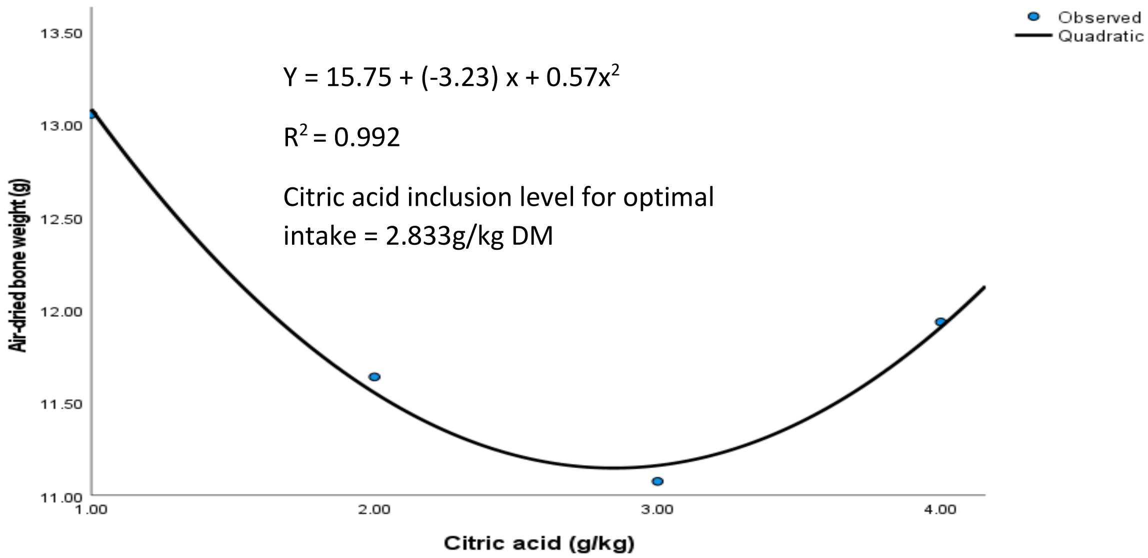

***Figure 4. 15**: The effect of citric acid inclusion levels on air-dried bone weight of male Ross 308 broiler chickens*

Table 4. 11: Citric acid inclusion levels for optimal air-dried bone weight and ash weight percentage of male Ross 308 broiler chickens

Factor	Formula	X	Y	R^2	P
air-dried bone weight	$Y = 15.75 + (-3.23)x + 0.57x^2$	2.833	11.174	0.992	0.088

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

4.8. The effect of citric acid inclusion level on bone mineralisation of male Ross 308 broiler chickens

Results of the effects of citric acid inclusion in a diet on bone mineralisation content of male Ross 308 broiler chickens aged one to 21 days are presented in Table 4.13. Citric acid inclusion in diets affected (P<0.05) bone ash percentage, Calcium (Ca), Phosphorous (P) and Magnesium (Mg) of male Ross 308 broiler chickens.

Results of the present study indicate that male Ross 308 broiler chickens fed diets having 12.50 and 25g of citric acid per kg DM had higher (P<0.05) ash weight percentage than those fed diets with 0, or 50g of citric acid per kg DM. However, chickens fed diets having 50g of citric acid per kg DM had the lowest (P>0.05) ash weight percentage. A 0.922g of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal ash weight percentage of male Ross 308 broiler chickens (Figure 4.16 and Table 4.14).

Tibia bones from chickens fed a diet containing 25g of citric acid per kg DM had higher (P<0.05) calcium contents than the bones from chickens fed diets having 0, 12.5 or 50g of citric acid per kg DM and chickens fed with diets containing 50g of citric acid per kg DM lowest calcium contents. A 2.45 of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal calcium content of male Ross 308 broiler chickens (Figure 4.17 and Table 4.14).

Male broiler chickens fed a diet containing 25g of citric acid per kg DM had higher (P<0.05) phosphorous contents in tibia bones than those fed diets containing 0, 12.5, or 50g of onion meal per kg DM. However, male chickens fed diets containing 12.5 or 25g of citric acid per kg DM had similar (P>0.05) phosphorous contents in the tibia bones. Similarly, Male chickens fed diets containing 0 or 12.5g of citric acid per kg DM had the same (P>0.05) phosphorous contents in the tibia bones. However, diets containing 0 or 50g had similar (P>0.05) phosphorous but diets supplemented with 50g of citric acid inclusion had the lowest phosphorous contents in the tibia bones of male Ross 308 broiler chickens. A 2.40 of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal calcium content of male Ross 308 broiler chickens (Figure 4.18 and Table 4.14).

Tibia bones from chickens fed a diet containing 25g of citric acid per kg DM had higher (P<0.05) magnesium contents than the bones from chickens fed diets having 0, 12.5 or 50g of citric acid per kg DM. However, chickens fed diets containing 0 or 50g of citric acid per kg DM had similar (P>0.05) but lowest magnesium contents. A 2.56 of citric acid inclusion level per kg DM of the diet was calculated using quadratic equations to result in optimal magnesium content of male Ross 308 broiler chickens (Figure 4.19 and Table 4.14).

Table 4. 12: The effect of citric acid inclusion levels on bone mineralisation content of male Ross 308 broiler chickens

	Diets*			
Variables#	CA_0	$CA_{12,50}$	CA_{25}	CA_{50}
Bone Ash percentage (%)	$17,00^{ab} \pm 1,87$	$18,80^{a} \pm 1,79$	$17,80^{a} \pm 0,45$	$15,00^{b} \pm 1,58$
Calcium (mg/L)	$1106,67^{c} \pm 15,28$	$1233,33^{b} \pm 25,17$	$1346,67^{a} \pm 35,12$	$1033,33^{d} \pm 5,77$
Phosphorous (mg/L)	$420,33^{bc} \pm 3,06$	$434,67^{ab} \pm 10,21$	$441,67^{a} \pm 2,89$	$410,00^{c} \pm 3,61$
Magnesium (mg/L)	$37,50^{c} \pm 0,20$	$39,10^{b} \pm 0,36$	$43,23^{a} \pm 0,46$	$36,87^{c} \pm 0,15$

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

CA : Citric acid supplemented

* : Treatments supplemented at different CA level in the diet are 0, 12.5, 25, and 50g/kg CA

: Values presented as mean ± standard deviation

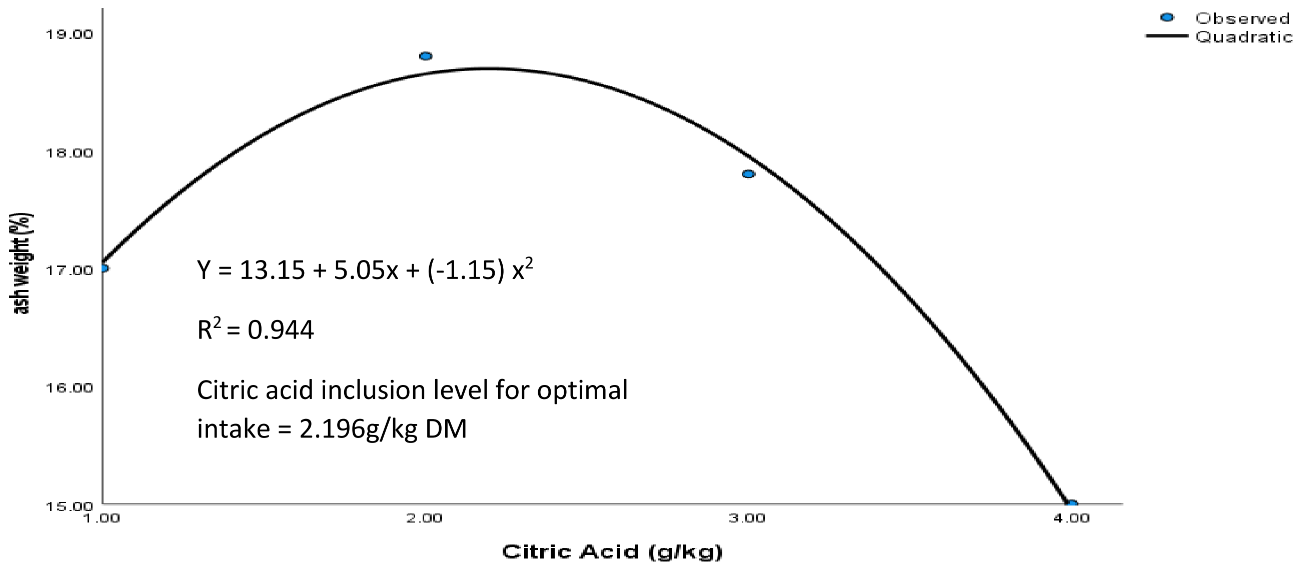

Figure 4. 16*: The effect of citric acid inclusion on ash weight percentage on male Ross 308 broiler chickens*

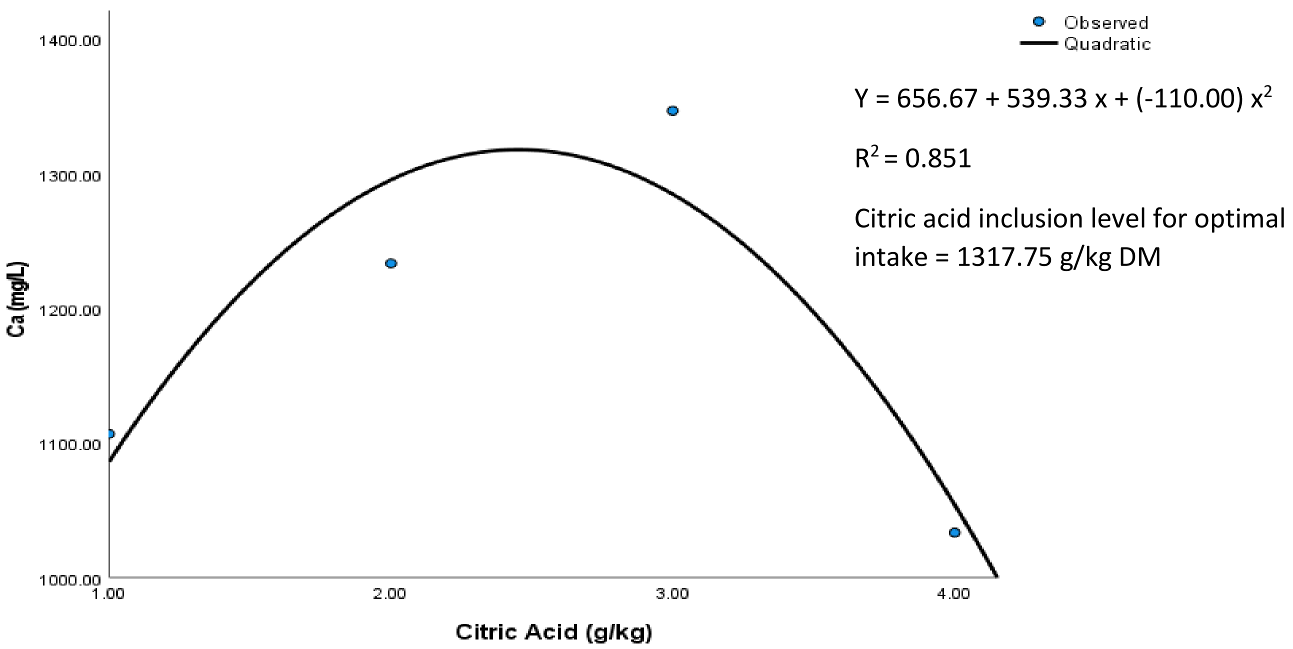

Figure 4. 17: *The effect of citric acid inclusion on ash weight calcium on male Ross 308 broiler chickens*

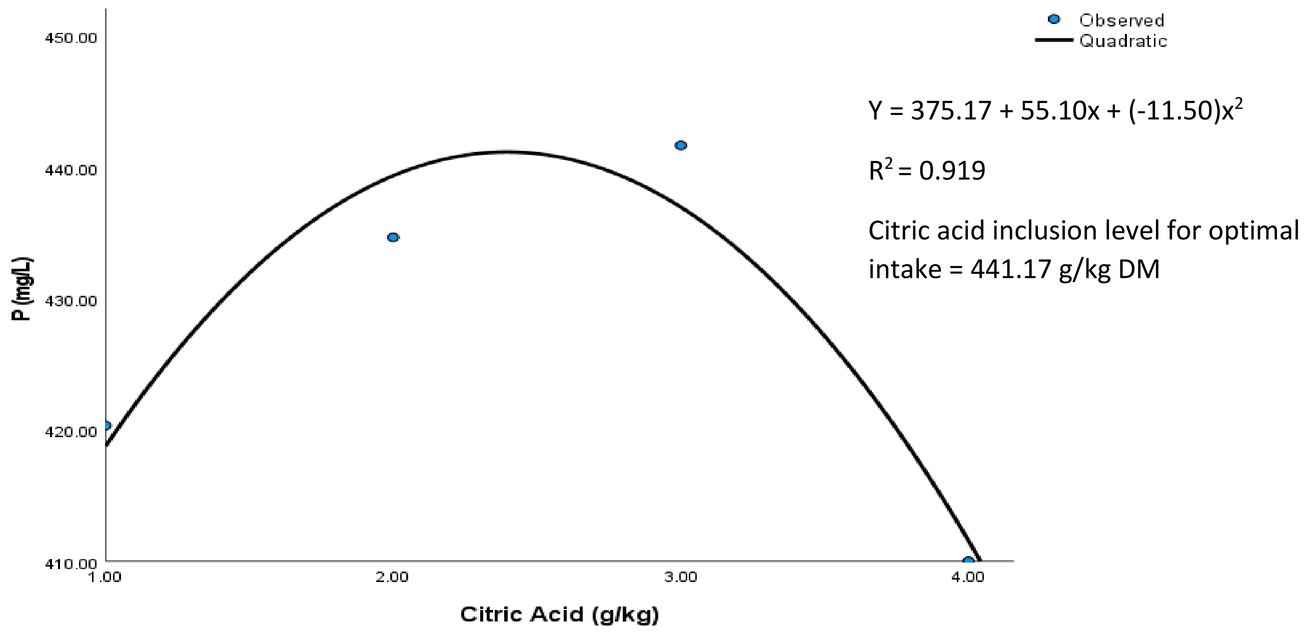

Figure 4. 18*: The effect of citric acid inclusion on phosphorous on male Ross 308 broiler chickens*

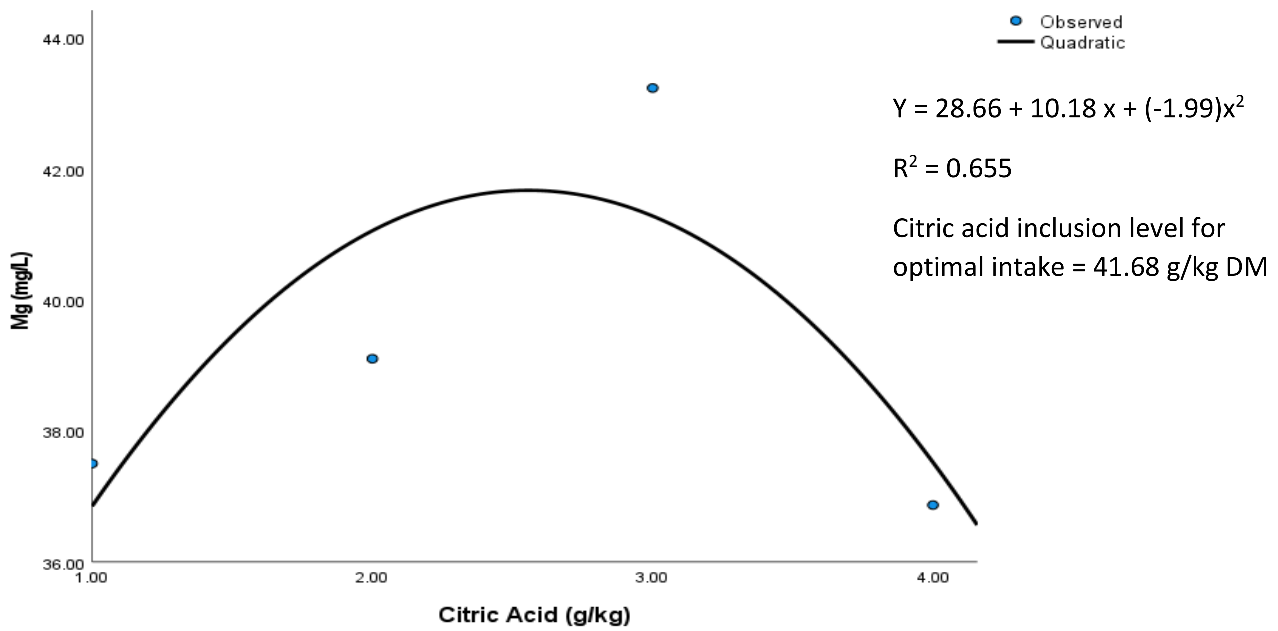

Figure 4. 19*: The effect of citric acid inclusion on ash magnesium on male Ross 308 broiler chickens*

Table 4. 13: Citric acid inclusion levels for optimal ash weight percentage and mineral contents of male Ross 308 broiler chickens

Factor	**Formula**	**X**	**Y**	**R^2**	**P**
Bone ash weight %	$Y = 13.15 + 5.05x + (-1.15) x^2$	2.20	18.70	0.994	0.080
Ca	$Y = 656.67 + 539.33 x + (-110.00) x^2$	2.45	1317.75	0.851	0.385
P	$Y = 375.17 + 55.10x + (-11.50)x^2$	2.40	441.17	0.919	0.285
Mg	$Y = 28.66 + 10.18 x + (-1.99)x^2$	2.56	41.68	0.655	0.587

X: Inclusion level for optimal value

Y: Optimal Y-level

R^2: Coefficient of determination

P: Probability

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The diets used in this study were isocaloric and isonitrogenous and the citric acid inclusion levels were at 0, 12.5, 25, and 50g per kg DM. The diets met the nutrient requirements for broiler chickens as recommended by McDonald *et al.* (2010) and National Research Council (NRC, 1994). Thus, any observed differences in responses by the chickens were attributed to citric acid inclusion in the diets.

The present study indicated that citric acid inclusion in the diets affected live weight and growth rate of male Ross 308 broiler chickens aged one to 21 days. Citric Acid (CA) inclusion in diets improved ($P < 0.05$) live weight and growth rate of male Ross 308 broiler chickens. These observed live weight at inclusion level may be attributed to the citric acid's positive impact of enhancing nutrient digestion (Rehman *et al.* 2016). These results concur with findings by Chowdhury *et al.* (2009); and Islam (2012); Abd-El-Hlim *et al* (2018); Fik *et al.* (2020); Fikry *et al.* *(*2021); who reported that broiler chicks fed CA diets had improved ($P < 0.05$) body weights and growth rate during the production cycle .

However, they are in contrast with the findings by Rafacz-Livingston *et al.* (2005), that live weight and growth rate were not significantly affected by CA in broiler fed diet (Rafacz-Livingston *et al.* 2005). The daily feed intake, body weight gain and feed conversion ratio of broiler chicks fed citric acid (CA) were not significantly influenced by dietary citric acid (CA) and these results concur with the findings of Rafacz-Livingston *et al.* (2005), and this could be because the diet was not highly palatable since CA adds a sour taste to feed hence the low feed intake, weight gain and feed conversion ratio by the chickens.

Chickens fed citric acid affected ($P < 0.05$) live weight, body weight gain, feed conversion ratio and growth rate of broiler chickens aged 35 days. The findings of this study correspond with those of Moghadam et al. (2006); Nezhad et al. (2007); Abdel-Fattah et al. (2008); Shahin et al. (2009); Islam (2012); Asgar et al. (2013) and Mahbuba *et al.* (2014); Allaw (2015); Mohammed (2018); Saad, and Al-Jashami (2019); who discovered a considerable increase in live weight and growth performance rate when citric acids were added to the feed of broiler chickens.

These improvements could be because citric acid supplementation to diets has increased decomposition of protein and amino acids by enhancing the activity of digestive enzymes (Salmata et al. 2008, Salgado-Transito et al. 2011), where citric acid can provide an appropriate medium that assisted in converting pepsinogen into pepsin, thus enhancing protein digestion (Afsharmanesh et al. 2005; Archana et al. 2019).

Supplementation of citric acid reduced feed conversion ratio of the chickens. These findings are similar to those of (Shahin et al. 2009; Islam et al. 2010), who reported a reduction in feed conversion ratio of chickens supplemented with citric acid and this may be due to low nutrient absorption by the chickens. However, Huifang et al. (2005) and Abdel-Fattah et al. (2005), it was found that adding citric acid (CA) at 6% inclusion level to broiler chicken diets improved feed efficiency (2008), because it has enough nutrients to be absorbed and utilized by the body. Lower feed conversion ratios (FCR) and higher levels of productivity are requirements of the modern broiler business, which can be met to some extent, using specialized feed additives (Fascina et al. 2012). Organic acid supplementation enhanced broiler chicken body weight and feed conversion ratio (FCR) (Haque et al. 2010) due to low additives that disturb the chicken's growth.

In this study, daily feed intake of the chickens fed CA diets had no significant difference ($P>0.05$) irrespective of treatment groups. Similarly, (Nezhad, 2007; Chowdhury et al 2009; of Kaya and Tuncer (2009), Aksu et al. (2007), and Brzoska et al. (2013)) discovered that the addition of CA had no effect on feed consumption in broiler chicks.

However, these findings contradicted those of Moghadam et al. (2006), Islam et al. (2008), Koopecky et al. (2012), and Fik et al. (2020), who found that citric acid influenced feed intake. Citric acid increased feed intake, according to Shahin et al. (2009); Denli et al. (2003); Haque et al. (2010); Islam et al. (2010). This increased feed intake appears to be related to the inclusion of lower amounts of citric acid in the diet since they did not make the feed unpleasant for the chickens to consume, which resulted in a rapid growth rate. However, Nourmohammadi and Khosravinia (2015) and Haque et al. (2014), reported that high dietary levels of CA decreased feed palatability while low levels boosted feed consumption in avian species, and this was due to the sourness in diets supplemented with citric acid.

The level of citric acid in the feeds affected the DM and crude protein digestibility values, as well as the metabolizable energy and nitrogen retention values in male Ross 308 broiler chickens. Several researchers have demonstrated that dietary supplementation of citric acids has improved the DM, protein, nitrogen, metabolizable energy and some nutrients (Ao *et al.* 2009, Jongbloed *et al.* 2000; Ghazala *et al.* 2011; and Haq *et al.* 2017) and what could this be attributed to improved nutrient digestibility by the citric acid inclusion. The improved nutrient digestibility resulted could be due to increased enzyme activity, improved digestion of proteins and other nutrients such as nitrogen as well when pH in the stomach is reduced by the citric acid.

According to Haq *et al.* (2017), broiler chickens fed diets containing various inclusion levels of dietary organic acids generally had greater retention of dry matter (DM) and protein than compared to those fed control diets. On the contrary Fik *et al.* (2020) reported that high acidification of dietary CA on the gut content lowered nutrient digestibility and absorption. In order to lower FCR the logical approach is to improve nutrient digestibility Archana *et al.* (2015). The organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with the nutrient transport and energy metabolism causing the bactericidal effect (Hassan *et al.* 2016; Saki *et al.* 2014). Soliman and AL-Youssef (2020) reported that nitrogen and calcium retention of chicks was significantly lower than those fed positive control diet or negative control of CA diets supplemented. Zobac *et al*. (2004) observed an improvement in digestibility of nitrogen by adding lactic acid and phytase into low available phosphorus broiler diets. However, less information on the effect of adding citric acid into broiler diets on metabolizable energy and nitrogen retention of broiler chickens were found.

The current study found that adding citric acid had no effect on the dressing percentage (%) and carcass weight of male Ross 308 broiler chickens. On the contrary, Aksu et al. (2007); Fascina et al. (2012); and Archana et al. (2015) found that supplementing citric acid at 3%, in broiler diets increased dressing percentage, carcass weight, and weight. Citric acid supplementation influenced the breast weight of male Ross 308 broiler chickens. However, Archana et al. (2015) observed that citric acid had an influence on broiler chicken breast weight. The large differences in breast weight are attributable to nutrient absorption. However, in case of breast proportion, a statistical increase ($P<0.05$) was observed in favour of the diet supplemented with citric acid (Fik *et al.* 2020). Several authors (Chowdhury *et al.* 2009; Alciek *et al.* 2004; Hassan *et al.* 2010; Hudha *et al.* 2010; Tollba *et al.* 2010; Fik *et al.* 2020) reported that the addition of citric acid to a broiler diet improved carcass breast weight due to acidification that might increase the cell proliferation and, in this manner, increased the muscle size (Fik *et al.* 2020).

The current study found that include citric acid in the diet had no negative effects on male Ross 308 broiler chicken tibia bone breaking strength, weight, or length. Citric acid addition in broiler diets, on the other hand, had an influence on the diameter, air dried bone weight ash percentage, calcium, phosphorus, and magnesium levels of male Ross 308 broiler chickens. Several studies (Boling et al. 2000; Boling-Frankenbach et al. 2001; Atapattu and Nelligaswatta 2005; Rafacz-Livingston et al. 2005; Martinez-Amezcua et al. 2006) found that CA supplementation increased bone ash %, Ca, P, and Mg in broiler chicken diets. Similar findings were reported by Kalafova et al. (2014), who discovered that citric acid dramatically increased tibia ash, indicating enhanced phosphorus consumption. Liem et al. (2008) discovered that supplementing citric acids enhanced the percentage of tibia ash. A diet containing 60 g of CA has been shown to impair nutrient digestibility and bone mineralization (Nourmohammadi and Khosravinia, 2015). It was indicated by Boling-Frankenbach et al. (2001) and Nourmohammadi and Khosravinia, (2015) that addition of 40 and 60 g CA kg of diet increased tibia ash. Centeno *et al.* (2007) speculated that CA could cohere Ca. Haque *et al.* (2009) found that the percent of tibia ash in CA-fed birds was significantly greater than in the others.

According to Rafacz-Livingston et al. (2005), the amount of tibia ash increases as dietary CA increases. The addition of two different levels of CA (1% and 2%) to broiler chickens enhanced the amount of ash (Atapattu and Nelligaswatta, 2005). Similar outcomes were reported in the study by Islam et al. (2011) until the dietary level of 0.75% CA was achieved. Organic minerals are thus liberated and more easily absorbed. Mineral-deficient diets cause enhanced availability due to the inclusion of CA (Broz et al. 1994; Boling et al. 1999, 2001; Islam et al. 2011). Brenes et al. (2003) found that adding 2.0% CA to broiler chicken feed increased Mg levels. The variances in Ca, P, and Mg in diets may be attributed to the fact that only a particular quantity of Ca, Mg, and P can be absorbed and utilised, and once the optimal level of CA has been reached.

The findings of this study are comparable to those of Soliman and AL-Youssef (2020), who found no significant change in diets supplemented with citric acid. Diets supplemented with citric acid, on the other hand, enhanced tibia weight and length by increasing Ca and P retention (Brenes et al. 2003; and Sacakli et al. 2006). In a study by witkiewic et al. (2010), bone breaking strength of tibias was observed in Bovans Brown hens, who recorded 160 and 166 N averaged across all dietary treatments, and these forces were lower than the values attained by witkiewicz and Koreleski (2005; 2007) with Hy-Line Brown and Lohman Brown hens. This discrepancy may be related to genetic differences across breeds in bone markers. Ascorbic acid supplementation in broiler diet increased bone breaking strength (Orban et al. 1993). Furthermore, in a study conducted by Radcliffe et al., the addition of citric acid to the diet of pigs had no influence on bone breaking strength (1998). When given citric acid, both air dried and oven dried bone breaking strength had no influence in this investigation.) Unfortunately, there is insufficient data on the effect of citric acid inclusion in a diet on male Ross 308 broiler chicken tibia bone weight, diameter, breaking strength, and length. More research is needed to confirm these findings.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion and recommendation

Citric acid's nutritional effects in diets are linked to its ability to provide chemicals that promote growth in broiler chickens. Organic acids, such as citric acid, which are often employed as an acidifier in chicken diets, are emerging as a viable option for enhancing nutrient digestibility and production performance. The current study found that supplementation of citric acid in starting diets boosted the live weight and growth rate of male Ross 308 broiler chickens aged one to 21 days. Chickens grown on diets supplemented with 12.5% citric acid gained more live weight and grew faster. As a result, 12.5g per DM of citric is recommended for optimal production of the chickens, while higher inclusion levels of 50 and 25g per DM for starter and finisher phases, respectively, are not recommended because they produce lower live weights and growth rates of the chickens.

Except for feed intake, citric acid addition had no effect on live weight, body weight gain, FCR, or growth rate in male Ross 308 broiler chickens aged 22 to 35 days. For maximum FCR values of male Ross 308 broiler chickens in our current investigation, 25g per DM citric acid addition is recommended. As a result, these variances may be attributed to the fact that CA addition to the diet alters mineral presence, but only to a point. More research is needed to confirm these findings.

These parameters (DM, crude protein and ash digestibility, and nitrogen retention) were tuned at various degrees of citric acid incorporation. The addition of citric acid to starter and finisher diets had no effect on feed consumption in male Ross 308 broiler chickens aged 22 to 35 days. Thus, improvements in nutrient digestibility and nitrogen retention attributable to citric acid in the diets had no influence on feed intake in Ross 308 broiler chickens aged 22 to 35 days. For high mineral content, 25g of citric acid is the best and most recommended quantity. The addition of CA to the diet alters mineral availability, but only to a point. The four citric acid inclusion levels had no influence on bone breaking strength in this investigation. It is recommended that CA be added to the feed to promote bone strength in order to achieve optimal levels and tougher bone breaking strength. Further research is needed to confirm these findings.

CHAPTER SEVEN

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