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# Citric acid in the diet of male Venda chickens: Effects on carcass characteristics and physico-chemical attributes

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## Abstract

This experiment was conducted to determine the effects of citric acid (CA) on carcass characteristics and physico-chemical attributes of male Venda chickens. A total of 200 male, one-day-old Venda chicks were distributed in a completely randomized design with four treatments and five replicates of 10 chickens each. The treatments were as follows: four diets with citric acid supplementation of 0, 12.5, 25, and 50g CA/kg. In this study, the live weight, carcass weight, thigh weight, drumstick weight, and carcass yield were higher in $CA_{25}$ than in other treatments. The wing weight was higher in $CA_{12.5}$ than in $CA_0$. The highest value was determined in $CA_0$, followed by $CA_{12.5}$, and $CA_{25}$, respectively, according to $pH_{24\ h}$, $pH_{48\ h}$, $pH_{72\ h}$, cooking loss, as well as shear force. The lowest value was recorded in $CA_{50}$ as expected. The $pH_{24\ h}$, $pH_{48\ h}$, $pH_{72\ h}$, cooking loss, and shear force were found to be similar. The current study indicates that 12.5 and 50 g CA/kg can be used easily in the diet of male Venda chickens without any negative effects.

**Keywords:** indigenous chickens; meat quality; organic acid; poultry

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## Introduction

The Venda chicken breed was first observed in the Venda district of Limpopo Province (Mogesse, 2007a). It is a multicolored chicken with basic colors similar to the white, black, and brown of the region's indigenous cattle and goats (Van Marle-Koster and Nel, 2000). It has a single comb but can also have a rose comb and has five toes. Venda chickens produce few eggs but are broody and have outstanding mothering ability (Mogesse, 2007a; Mngonyama, 2012). They are fairly large in contrast to other indigenous chicken species and lay huge, colorful eggs. These chickens reach sexual maturity at 143 d, weighing an average of 2.1 kg in cocks and 1.4 kg in hens at 20 w of age (Mogesse, 2007a). The average weight of the cockerels and hens can reach up to 2.9–3.6 kg and 2.4–3.0 kg (Manyelo et al., 2020). The productivity of these chickens is generally low while mortality is high, implying that appropriate nutritional and management interventions are needed to realize their optimal production (Okitoi *et al*., 2006; Mbajiorgu *et al*., 2011).

The increase in the cost of nutritional ingredients is one of the most serious challenges for the poultry industry. The decrease in available land for food grains and feed production, climatic changes, and limited water resources influence the cost of animal feed production (Rao, 2015). To overcome this challenge, feed additives are considered essential for optimum performance and productivity in poultry production (Shahid *et al.,* 2015). In the past, antibiotics were used to maximize the utilization of nutrients

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in feed and enhance the growth performance and production of poultry (Huyghebaert *et al.*, 2011). However, consumers are rejecting the use of antibiotics in animal feeds because of their association with human and animal health risks (Gonzalez & Angeles, 2017). Although the inclusion of alternative growth promoters (AGP) in animal feed was banned in the European Union countries in 2006, it is widely practiced in the South African poultry industry, where the effects of AGP are alleviated by feeding an AGP-free 'withdrawal' diet before slaughter. This has encouraged the exploration of alternative means to improve health, including non-therapeutic options such as enzymes, probiotics, prebiotics, herbs, essential oils, immunostimulants, and organic acids. In this regard, organic acids are selected as a promising feed additive in poultry production due to their ability to maintain gut barrier cellular integrity, modulate intestinal microbiota, improve digestion and nutrient absorption rate, and contribute to improved production performance (Dai *et al.,* 2020). Citric acid as a tricarboxylic acid (TCA) has gained considerable attention in poultry production as it is used as an energy source for prime enterocytes (Hosna, 2018) or for the bactericidal efficacy against harmful species (for example, *Escherichia coli*) (Shah *et al.,* 2018). It is volatile and corrosive in its free form; thus, CA is commercially-produced in salt forms (Huyghebaert *et al.,* 2011) to increase palatability and bioavailability in the gut of birds (Khan & Iqbal, 2016).

Citric acid acts as a growth promoter by acidifying gastrointestinal (GI) content and is considered a favourable determinant of effective nutrient digestion (Boling *et al.,* 2000). It also improves the performance and increases the solubility of feed ingredients and the digestion and absorption of nutrients (Nourmohammadi & Afzali, 2013). Various experiments have been conducted to quantify the effects of citric acid on poultry, but varying, and sometimes contradictory, results have been observed. Data indicate that it generally results in bodyweight and feed conversion improvements, whilst decreasing feed intake (Chowdhury *et al.,* 2009; Haque *et al.,* 2010; Nourmohammadi *et al.,* 2010). Several studies have demonstrated the effect of citric acid on the physico-chemical and sensory properties of meat (Hassan *et al.,* 2015; Kim *et al.,* 2019). Moreover, citric acid is generally recognized as a safe antimicrobial agent, and the dilute solutions of organic acids (1%-3%) are generally without effect on the desirable sensory properties of meat (Kotula & Thelappurate, 1994). According to Langhout (2000), using an acidifier may improve the development rate and carcass quality of broiler chickens.

The current study was designed to determine whether increasing levels of citric acid in male Venda chicken diets would improve carcass characteristics and physico-chemical attributes more than those without citric acid.

## Materials and methods

The use of animals and approval for all experimental protocols were granted by the Animal Research Ethics Committee of the University of Limpopo (Project number: AREC/10/2020: PG). The study was conducted at Animal Unit of the University of Limpopo, South Africa. Four experimental maize and soybean meal-based diets were formulated as follows: $CA_0$ = control diet; $CA_{12.5}$ = control diet supplemented with 12.5 g CA/kg feed; $CA_{25}$ = control diet supplemented with 25 g CA/kg feed; $CA_{50}$ = control diet supplemented with 50 g CA/kg feed (Table 1). Birds received different treatment levels of citric acid in the diet: 0, 12.5, 25,and 50 g CA/kg (Table 2). Diets were fed in three phases: formulated maize–soybean meal-based starter from day 1 to day 30, formulated maize–soybean meal-based grower from day 31 to day 60, and formulated maize–soybean meal-based finisher from day 61 to day 90.

Four dietary treatments with various CA levels were randomly assigned to 200 male, day-old Venda chicks. A completely randomized design (CRD) was used with four treatments, five replicates per treatment, and 10 chickens per replicate. The chickens were reared on bedded floor pens with 7 cm of fresh sawdust in an environmentally-controlled house for 90 d. The chickens were offered clean water and food *ad libitum.* The chickens were monitored regularly, sick chickens were isolated and treated accordingly by the veterinarian. Dead chickens were taken immediately from the experimental house to the laboratory for post-mortem. Chickens received 23 h of light from 0 to 3 d of age, 20 h of light from 4 to 7 d of age, and 16 h of light from day 8 onwards.

**Table 1** Ingredients and nutrient composition of the diet for the provision of citric acid (CA) to Venda chickens

| | Starter | | | | Grower | | | | Finisher | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CA_0$ | $CA_{12.5}$ | $CA_{25}$ | $CA_{50}$ | $CA_0$ | $CA_{12.5}$ | $CA_{25}$ | $CA_{50}$ | $CA_0$ | $CA_{12.5}$ | $CA_{25}$ | $CA_{50}$ |
| 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 |
| $AME_N$ (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 |
| Citric acid | 0.81 | 0.90 | 0.79 | 0.63 | 0.71 | 0.71 | 0.71 | 0.66 | 0.64 | 0.65 | 0.65 | 0.66 |
| Phosphorus | 0.67 | 0.66 | 0.65 | 0.64 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Sodium | 0.19 | 0.19 | 0.17 | 0.13 | 0.15 | 0.15 | 0.15 | 0.15 | 0.13 | 0.13 | 0.13 | 0.13 |
| Chloride | 0.28 | 0.28 | 0.25 | 0.19 | 0.22 | 0.22 | 0.22 | 0.22 | 0.19 | 0.19 | 0.19 | 0.19 |

MCP = Monocalcium phosphate, Lysine HCL = Lysine hydrochloride.
Active ingredients contained in the vitamin premix were as follows (per kg of diet): vitamin A 11.25 MIU, vitamin $D_3$ 4.5 MIU, vitamin E 57.5 g, vitamin $K_3$ 3.0 g, vitamin $B_1$ 3.0 g, vitamin $B_2$ 7.0 g, vitamin $B_3$ 50.0 g, vitamin $B_5$ 15.0 g, vitamin $B_6$ 4.0 g, vitamin $B_{10}$ 2.0 g, vitamin $B_{12}$ 30.0 mg, vitamin H 215.0 mg, Antioxidant 200.0 g, Manganese 100.0 g, Iron 470.0 g, Zinc 600.0 g, Copper 250.0 g, Iodine 1.5 g, Inorganic selenium 150.0 mg, Organic selenium 150.0 mg, Organic zinc 20.0 g, $AME_N$ = apparent metabolizable energy corrected for nitrogen balance

**Table 2** Citric acid (CA) supplementation in the diet for provision to male Venda chickens

| CA levels | Description |
|---|---|
| **$CA_0$** | 0g of CA per kg |
| **$CA_{12.5}$** | 12.5g of CA per kg |
| **$CA_{25}$** | 25g of CA per kg |
| **$CA_{50}$** | 50g of CA per kg |

At the age of 90 d, 20 chickens per treatment were randomly selected, separately weighed, and slaughtered by cervical dislocation method as it is one of the most prevalent methods for slaughtering individual birds and it is perceived to be humane by users, easy to learn and perform, and does not require equipment and help of veterinarian (Mason *et al.,* 2009; Martin *et al.,* 2016). They were allowed to completely bleed out, then de-feathered, eviscerated, weighed, and recorded. The carcass was sliced on the joints into drumsticks, wings, breast, and thighs, as well as across the shoulder area to remove the backbone from the breast. The cuts were then weighed using an electronic weighing balance (AE ADAM, United Kingdom, Milton Keynes). The live and carcass weights were recorded and the meat piece weights (drumsticks, wings, breasts, and thighs) were also recorded. The carcass yield was calculated from the ratio of the eviscerated carcass weight to the live carcass (Mendes *et al*., 2004).

Carcass yield (%) = carcass weight (kg) × 100/ live body weight (kg) (1)

Only breast muscles without skin were put in trays as per dietary treatment and stored at 4 °C until further analysis. The day after slaughter, two trays from each treatment were removed from the refrigerator to measure physico-chemical attributes, i.e., pH after 24 h ($pH_{24}$), pH after 48 h ($pH_{48}$), pH after 72 h ($pH_{72}$), cooking loss, and shear force. After being chilled at 4 °C for 24 h, breast meat pectoralis major (p. major) of the left side was examined for meat pH. A digital pH meter (CRISON pH 20, Crison Instrument SA, Spain, Barcelona) was used to measure pH after calibration with pH 4.00 and 10.00 phosphate buffers.

For cooking loss determination, chilled chicken breast samples were weighed, placed into sealed polyethylene bag, and cooked on an electronic stove at 80 °C for 30 min to calculate the cooking loss. Internal chicken breast temperatures were determined using thermocouples and a data logger (Model UWTR data logger, Omega Engineering, Stamford, CT). Cooked meat samples were cooled down to ambient temperature (20 °C), dried with a paper towel (1-ply), then reweighed. Cooking loss (%) was calculated as (Ngambu *et al.,* 2013):

Cooking loss (%) = (weight before cooking - weight after cooking) / weight before cooking) ×100 (2)

After the determination of cooking loss, cooked breast meat samples were used for shear force measurement. The shear force was measured using a Warner–Bratzler Shear machine (Tallgrass Solutions, Manhattan, Kansan) (Novaković & Tomašević, 2017).

Data were subjected to the analysis of variance (ANOVA) procedure of the Statistical Package for the Social Sciences (SPSS) software, version 26 (SPSS, 2019), using a one-way procedure based on the following model:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

where: $Y_{ij}$ = value observed for the response variable, T, in treatment, I, and its repetition, j; μ = general average of all observations; $T_i$ = treatment effect (0, 12.5, 25, and 50g C/kg); and $e_{ij}$ = experimental error associated with the observation, $Y_{ij}$. Comparisons among means were made using Tukey's (HSD) test. The level of probability was set at $P$ <0.05 and means ± standard deviations were reported.

## Results and discussion

Carcass characteristics of male Venda chickens are given in Table 3. Live, carcass, breast, drumstick, and thigh weights and carcass yield were not statistically different between groups. Wing weight at 12.5 g CA/kg was higher than the other groups ($P$ <0.05). Similarly, it was reported that supplementation of CA in the diets increased/improved the carcass features of broiler chickens (Fascina *et al*., 2012). The current findings are comparable to those of Aksu *et al*. (2007), who found a marked improvement in carcass weight and some carcass parameters such as thigh, breast, and neck with a lower CA supplementation. Chowdhury *et al*. (2009), Hassan *et al*. (2010), and Hudha *et al*. (2010) discovered a considerable increase in carcass yield by using CA in the feed. Islam *et al*. (2008) found that dietary regimens with CA supplementation did not affect carcass parameters. This discrepancy could be due to different supplemented feeds containing different percentages of nutrients.

**Table 3** Effects of dietary citric acid (CA) supplementation on carcass characteristics of male Venda chickens

| Treatments[1] | Live weight (g) | Carcass weight (g) | Breast weight (g) | Wing weight (g) | Drumstick weight (g) | Thigh weight (g) | Carcass yield (%) |
|---|---|---|---|---|---|---|---|
| **$CA_0$** | $1618.0^b \pm 82.97$ | $1068.0^b \pm 61.79$ | $254.9^a \pm 15.24$ | $76.6^b \pm 4.54$ | $78.9^a \pm 5.72$ | $89.0^b \pm 4.00$ | $66.0^b \pm 1.60$ |
| **$CA_{12.5}$** | $1627.0^b \pm 81.93$ | $1077.0^b \pm 65.50$ | $261.7^a \pm 26.31$ | $82.6^a \pm 6.73$ | $80.8^a \pm 8.33$ | $85.4^b \pm 5.92$ | $66.2^b \pm 1.08$ |
| **$CA_{25}$** | $1723.0^a \pm 74.84$ | $1184.0^a \pm 66.03$ | $253.4^a \pm 20.60$ | $78.6^{ab} \pm 4.79$ | $83.1^a \pm 4.53$ | $99.4^a \pm 2.65$ | $68.7^a \pm 1.49$ |
| **$CA_{50}$** | $1345.0^c \pm 59.86$ | 871.0c ± 52.59 | $201.6^b \pm 9.69$ | $66.0^c \pm 2.45$ | $67.0^b \pm 5.99$ | $74.2^c \pm 3.00$ | $64.7^b \pm 2.18$ |
| ***P*-value** | 0.445 | 0.513 | 0.132 | 0.026 | 0.338 | 0.705 | 0.689 |

[a- c,]- means within a row with different superscripts differ significantly at $P$ <0.05

The amount of moisture released while cooking is determined by the pH of the meat (Yusop *et al.*, 2010). Meat products are deemed dry because meat with a high pH loses less water while cooking than meat with a low pH (Warriss, 2010). Cooking loss and shear force reflect the tenderness of the meat (Li *et al*., 2019). The effect of CA supplementation on physico-chemical attributes of male Venda chickens is given in Table 4. In the present study, there was a difference in the $pH_{24}$, $pH_{48}$, $pH_{72}$, cooking loss, as well as shear force values of the meat between groups ($P$ <0.05).

All meat samples supplemented with CA had lower pH values ($pH_{24}$, $pH_{48}$, and $pH_{72}$) compared to the control group ($P$ <0.05). The same trend was observed in cooking loss and shear force, which decreased as a response to increasing levels of CA supplementation ($P$ <0.05). Honikel (2004) reported that elevated levels of CA lowered the pH of the meat resulting in less red and more yellow meat color with less shear strength (shear force) or tenderness. This researcher indicated that the pace at which pH drops will affect the color, tenderness, loss of moisture during cooking, juiciness, and the microbiological stability of a food product.

CA can also be added to drinking water. Aclkgoz *et al*. (2011) found that CA in drinking water did not have a beneficial effect on the productivity of male broilers. Aktas *et al*. (2016) reported that low pH after CA dietary supplementation had a positive effect on texture and resulted in increased water-holding capacity, moisture content, and deceased cooking losses. In a study by Islam (2012), the addition of increasing levels of CA in drinking water decreased broiler chicken pH. The low pH value of meat products is known to affect several factors such as loss of redness, shear force, and texture (Sammel & Claus, 2003). The drop in shear force of meat samples as CA content increases was attributed to a fall in pH, which affects the water binding capacity (Burke & Monahan, 2003).

**Table 4** Effects of dietary citric acid (CA) supplementation on physico-chemical attributes of male Venda chickens

| Treatments[1] | $pH_{24}$ | $pH_{48}$ | $pH_{72}$ | Cooking loss (%) | Shear force (N) |
|---|---|---|---|---|---|
| **$CA_0$** | $5.9^a \pm 0.08$ | $6.0^a \pm 0.13$ | $6.2^a \pm 0.12$ | $35.1^a \pm 0.65$ | $21.3^a \pm 1.43$ |
| **$CA_{12.5}$** | $5.8^b \pm 0.05$ | $5.9^b \pm 0.05$ | $6.0^b \pm 0.04$ | $34.2^a \pm 2.04$ | $17.8^b \pm 0.60$ |
| **$CA_{25}$** | $5.8^{bc} \pm 0.02$ | $5.9^b \pm 0.05$ | $6.0^b \pm 0.05$ | $30.0^b \pm 0.61$ | $17.4^b \pm 0.54$ |
| **$CA_{50}$** | $5.7^c \pm 0.01$ | $5.8^b \pm 0.06$ | $5.9^b \pm 0.61$ | $29.2^b \pm 1.24$ | $15.1^c \pm 1.12$ |
| ***P*-value** | 0.050 | 0.050 | 0.050 | 0.044 | 0.042 |

$pH_{24\ h}$= pH after 24 h, $pH_{48}$ = pH after 48 h, $pH_{72}$= pH after 72 h, N = Newton, [a- c] = means within a row with different superscripts differ significantly at $P$ <0.05

## Conclusions

According to the findings of the study, the dietary supplementation of CA, especially at 12.5 and 50 g per kg, could improve the carcass characteristics of male Venda chickens. The physico-chemical attributes could also be improved with CA supplementation by improving the pH, shear force, and decreasing cooking losses.

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**Author contributions**

Conceptualization, BG and JWN; validation, BG and MVM; Writing original draft preparation, MMV; Writing review and editing, BG and MVM; supervision, BG and JWN. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest declaration**

The authors declare that they have no conflicts of interest relative to the content of this paper.

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