Evaluation of Apple (*Malus domestica*) Cider Vinegar and Garlic (*Allium sativum*) Extract as Phytogenic Supplements in Broiler Chickens

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DEDICATION

This dissertation is dedicated to my wife Blessing, I am forever grateful for the love, encouragement, and support. I also dedicate this work to my parents, Thomas and Annah Chabalala and my siblings, Mikateko, Evans and Kuhlula.

DECLARATION

I, Oscar Chabalala of student number 19022488, hereby declare that this dissertation for Master of Science in Agriculture (MSCANS) submitted to the Department of Animal Science, Faculty of Science, Engineering and Agriculture at the University of Venda has not been submitted previously for any degree at this or another university. It is original in design and in execution, and all reference material contained therein has been duly acknowledged.

Signature Chabalala **Date 21-04-2022**

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ABSTRACT

The use of antibiotic growth promoters (AGPs) in poultry may induce antibiotic resistance with risk of accumulation of harmful residues in poultry products, thereby compromising poultry and human health. Apple cider vinegar (ACV) and garlic are phytogenic substances which have been gaining considerable interest due to their ability to improve performance. This study investigated growth promoting effects of drinking-water supplemented with ACV and garlic extract on broilers. A total of 390, day-old Ross broiler chicks were housed in an open, deep litter house divided into 30 pen partitions, each measuring 2.3 m^2, to which treatments were allocated in a randomized 2 (sex) x 5 (additives) factorial experiment. Experimental units were allotted to antibiotic free diet plus untreated drinking water (Negative control (NC), antibiotic free diet plus ACV treated drinking water (T1), antibiotic free diet plus garlic treated drinking water (T2), antibiotic free diet plus ACV+garlic treated drinking water (T3) or antibiotic fortified diet plus untreated drinking water (positive control (PC). The experiment was replicated three times. The test period started at the age of 15 days and consisted of two phases: grower (15-28 days of age) and finisher (29-42 days of age). All data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedures of SPSS. Where main effects were significant, Tukey's test was used to separate the means. Males had higher feed intake than females (P<0.05) in all growth phases. Weight gain, FCR and mortality of the birds was not affected by sex (P>0.05) in all growth phases. Birds on the PC gained more weight (P<0.05) than birds on T1, T2, T3 and NC during all growth phases. Grower-phase feed intake was similar across treatments (P>0.05), while birds on the PC consumed more feed (P<0.05) during finisher phase than birds on the NC and on ACV and garlic additives. Birds on the PC had a lower grower-phase FCR (P<0.05) than birds on T1, T2, T3 and NC, though with lower FCR during finisher phase to birds on T1 and T3. Mortality was similar for all treatment (P>0.05) throughout the experiment. Dressing percentage was similar across sexes (P>0.05). Birds on the NC exhibited a lower dressing percentage (P<0.05) than all others. Meat

pH was higher in male birds (P<0.05), and was not affected by additives (P>0.05). Gut digesta pH, proventriculus weight, gizzard weight, gastrointestinal tract length across sexes were not affected by treatment (P>0.05). Birds on the PC had a higher liver weight (P<0.05) than all others, and higher spleen weights than birds on T2 (P<0.05). In conclusion, in the current study, ACV and or garlic additives did not benefit broiler performance.

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ABBREVIATIONS AND ACRONYMS

ACV Apple Cider Vinegar

AGP Antibiotic Growth Promoter

FCR Feed Conversion Ratio

GLM General Linear Model

CHAPTER 1 INTRODUCTION

1.1 Background

Antibiotic growth promoters (AGPs) are used to increase livestock productivity and efficiency of feeds (Suresh *et al.*, 2018). In poultry, AGPs are intended to improve growth performance and enhance gut health (David *et al.*, 2012). The AGPs mechanism of action is through control of gastrointestinal infections by alteration of the gut microbial composition (Singh *et al.*, 2013). A review of recent research on AGPs use in food-producing animals (Ronquillo and Hernandez, 2017) revealed a growing awareness of the risks they pose to both animal and human wellbeing as well as the environment, with increased search for safer alternatives (Mohamed *et al.*, 2014). Several alternatives have been proposed and tested in poultry production, among which are phytogenic feed additives (Amad *et al.*, 2011). Phytogenics are compounds derived from plant that are added into diets to improve performance of livestock (Banerjee *et al.*, 2013). Phytogenics contain complex mixtures of organic molecules and many active ingredients that have different antibacterial functions, making it harder for bacteria to gain resistance (Suresh *et al.*, 2018). The mode of action involves altering the gut microbiota, increased nutrient digestibility and absorption in the gut, as well as antioxidant and immunomodulatory activities (Ahmed *et al.*, 2013). Phytogenics have recently attracted considerable interest due to their ability to improve performance by maintaining a healthy gut environment (Murugesan *et al.*, 2015) and they have been accepted by consumers as natural food additives (Toghyani *et al.*, 2010).

Many natural feeds have beneficial multifunctional properties derived from specific bio-active ingredients (Huyghebaert *et al.*, 2011). One of the natural food condiments known to possess such functional properties is apple cider vinegar (ACV) (Bárdos and Bender, 2012). ACV is a low acidic fermented apple juice (Ahmadifar *et al.*, 2019). It's a major source of flavonoids, minerals, organic acids and several vitamins (del Campo *et al.*, 2008). Organic acids eliminate harmful gut bacteria and are safe for supplementation (Yagnik *et al.*, 2018). The growth-enhancing effects of ACV are attributed to its function in nutrient digestion and its high content of vitamins, minerals and organic acids (Pourmozaffar *et al.*, 2017). Organic acids are simple monocarboxylic acids such as propionic, butyric, acetic and formic acids (Mehdi *et al.*, 2018). Supplementing organic acids to drinking water or food reduces the digestive system pH and pathogens accumulation in the gut. This stimulates the improvement of useful intestinal microbial flora, supporting useful bacterial populace to triumph over pathogenic bacteria in addition to a reduction of poisonous metabolites produced through dangerous bacteria (Zarghi, 2018). Acetic acid is the predominant acid in ACV (Hayajneh, 2019) and acts as a natural antibiotic with the ability to kill pathogenic bacteria and at the same time to foster the growth of probiotics (Akanksha and Sunita, 2017). Drinking water supplemented with ACV reduces visceral abdominal fat content (Allahdo *et al.*, 2018). The ACV components contribute to a variety of pharmacological functions (Naziroğlu *et al.*, 2014). They reduce intestinal colonisation of pathogenic and non-pathogenic bacteria which decrease inflammatory process in the intestinal lining which improves the height of the villi and the functions of nutrients digestion, secretion and absorption (Khan and Iqbal, 2016).

Similar to ACV, garlic plant has important dietetic and medicinal functionality *(Hindi, 2013).* In poultry nutrition, garlic reduces the growth of pathogens in the gut, improves the functioning of the pancreas, increases weight gain and improves meat quality (Mahmood *et al.*, 2009). Garlic possesses antibacterial, antifungal, antiparasitic, antiviral, antioxidant, antithrombotic, anticarcinogenic properties and exhibits vasodilator characteristics (Issa and Omar, 2012).

Consumer concern on the harmful effects of sub-therapeutic use of antibiotics and the ban antibiotic growth promoters in Europe has prompted researchers to search for safer alternatives (Diarra and Malouin, 2014). Limited experiments have been done on the performance of broilers on ACV and garlic treated drinking water and some results are contradictory. Therefore, this study determined whether these additives (ACV and garlic) have a specific, synergistic/complementary effects on broiler performance, macro- morphometry of digestive organs weights, digesta pH, dressing percentage and meat pH.

1.2 Statement of the research problem

Antibiotic growth promoters have been used to enhance performance in poultry, with increased usage tied to the intensification of livestock production to meet consumer demand and improvement in the efficiency of conversion of natural resources to food animal products (Costa *et al.*, 2017; EL-Faham *et al.*, 2014). However, the use of antibiotics in wrong doses can lead to the development of resistant pathogens, with the risk of residual active metabolites of growth promoting additives in animal products (Ronquillo and Hernandez, 2017; Hao *et al.*, 2014). A range of potential phytogenic alternatives have been proposed leading to the need for research to evaluate the potency, modes of action, and to determine optimum dosages. Thus, this study investigated the potential of ACV and/or garlic extract as safe phytogenic supplement which can replace dietary antibiotic additives in broiler chickens.

1.3 Justification of the study

The health risks associated with AGPs have prompted the poultry industry to search for viable alternatives (Carrasco *et al.*, 2016), with interest increasingly focused on phytogenic products (Sadek *et al.*, 2014). Compared to AGPs, Phytogenics are less expensive and deemed less toxic with no residual effects if properly administered (Oladeji *et al.*, 2019). They also have multiple beneficial applications, improve the digestive enzyme activity and nutrient absorption and food palatability (Bhagwat *et al.*, 2021). ACV is a readily accessible potential phytogenic products with multi-biofunctional properties are (Budak *et al.*, 2014) and garlic extract (Hindi, 2013) and they have been demonstrated to be beneficial to human health (Tripathi and Mazumder, 2020). However, despite extensive literature suggesting beneficial effects of both ACV and garlic, there are few studies which conclusively demonstrate their beneficial effects in broiler chickens. Therefore, there is a need to investigate the potential of ACV and garlic extract as safe phytogenic supplements in broiler chickens to inform the feed processing industries and farmers in the poultry industry.

1.4 Research objectives

1.4.1 Major objective

To evaluate the potential safety of ACV and garlic extract as phytogenic supplements in male and female Ross 308 broiler chickens.

1.4.2 Specific objectives

To determine the effect of supplementing the drinking water of male and female Ross 308 broiler chicken with ACV and/or garlic on:

a) performance
   - weight gain.
   - feed intake.
   - feed conversion ratio.
   - mortality.

b) macromorphometry of digestive organs
   - gut digesta pH.
   - weight of digestive organs.
   - gastrointestinal tract length.

c) carcass and meat characteristics
   - dressing percentage.
   - breast meat pH.

1.5 Research hypotheses

Supplementing male and female Ross 308 broiler chickens drinking water with ACV and/or garlic extract has no significant effects on:

- weight gain.
- feed intake.
- feed conversion ratio.
- mortality.

- gut digesta ph.
- weights of digestive organs.
- gastrointestinal tract length.
- dressing percentage.
- breast meat pH.

CHAPTER 2 LITERATURE REVIEW

2.1 Use of antibiotic growth promoters in poultry

The AGPs have been used for decades to improve the production of meat through increased growth rate, FCR and disease prevention in poultry (Sugiharto, 2016; Huyghebaert et al., 2011). The administration of subtherapeutic doses of AGPs to poultry stabilizes the intestinal microbial flora to improve performance by suppressing pathogenic microbiota that compete for nutrient with beneficial bacteria of the gut (Hassan *et al.*, 2010). However, this practice has lately raised worries about drug resistant pathogens emergence that results in harmful impact to humans and the pollution of the environment (Ronquillo and Hernandez, 2017; Maron *et al.*, 2013). Due to these problems, antibiotics use as growth promoters have been banned by the European union since 2006, and has since been banned in other areas (Yegani and Korver, 2008). Since the ban on AGPs, search for natural alternatives that are safe and have better beneficial effects have been intensified. Phytogenic feed additives (PFAs) are the possible alternative and can have a positive impact on the health and performance of poultry (Amad *et al.*, 2011).

2.2 Potential phytogenic additives

Research to discover suitable substitutes to AGPs has increased dramatically in domestic animals in recent years (Huyghebaert *et al.*, 2011). The aim is to maintain low chick mortality rate and increase productivity while protecting consumer health and the environment. A lot of research has been carried out to find safer natural alternative with the same benefits as AGPs (Mehdi *et al.*, 2018). Apple cider vinegar is an acidic liquid produced by apples fermentation. It has long been known to be phytogenic (Pourmozaffar *et al.*, 2017). For example, Hippocrates prescribed ACV as a treatment for different diseases (Beheshti *et al.*, 2012). Bio-functional compounds in ACV include different flavonoids such as uercetin, cyanidin-3-glucoside, anthocyanin, catechins, camproforol, apicotin and organic acids (Zarghi, 2018).

Garlic, a plant widely used as a medicinal herb (Mansoub, 2011), has similarly been a subject of considerable interest since ancient times (Toghyani *et al.*, 2011b). It is reported to have many benefits on broiler performance (Khan *et al.*, 2012). Anti-inflammatory, anti-bacterial and anti-microbial effects have been reported with garlic (Mansoub, 2011). Garlic powder as feed additive reduces mortality, increases growth and improves FCR in broiler chickens (Puvača *et al.*, 2014), and stimulates the immune system and lowers blood cholesterol levels (Khan *et al.*, 2012). Garlic is used in animal production as puree, oil extracts, liquid extracts, powder and in mixtures with other herbs (Puvača *et al.*, 2013).

2.3 Phytogenic effects in broilers

2.3.1 Weight gain

2.3.1.1 Effects of apple cider vinegar

Compounds contained in ACV which could account for reported phytogenic activity include flavonoids, polyphenols, organic acids, vitamins and minerals (Pourmozaffar *et al.*, 2017). Acidification of drinking water is generally believed to improve broiler performance by decreasing pathogenic bacteria (Allahdo *et al.*, 2018). Broiler growth increased with water supplemented with mixed organic acids (Khan and Iqbal, 2016; Ghazalah *et al.*, 2011) and acetic acid (Abbas *et al.*, 2011). There are limited publications on the benefit of supplementary ACV on broiler chickens weight gain.

2.3.1.2 Effects of garlic extract

Previous reports suggested beneficial effects of garlic on broiler feed utilization efficiency (Khan *et al.*, 2012). Garlic contains organosulfur compounds that play a major role in anti-inflammatory and antioxidant effects (Pourali *et al.*, 2014). In contrast, garlic had no effect on broiler weight gain (Fayed *et al.*, 2011; Choi *et al.*, 2010; Fadlalla *et al.*, 2010). The dosage could be important, for example, Elagib *et al.* (2013) reported increased weight gain of broiler chickens supplemented at 3% feed garlic powder, but decreased gain at 5% garlic power. In contrast, 10 g and 15 g/kg feed did not affect broiler growth (Jakubcova *et al.* (2014).

2.3.2 Feed intake

2.3.2.1 Effects of apple cider vinegar

ACV is rich in organic acids such as propionic, lactic, acetic, citric, butyric, malic, formic and sorbic acids which are known to enhance growth performance (Pourmozaffar *et al.*, 2017). Organic acids maintain the cellular integrity of the intestinal mucosa and improve digestion by maintaining optimal intestinal flora and can therefore be used as AGPs alternative (Sultan *et al.*, 2015). Lilly *et al.* (2011) found that adding ACV to drinking water did not influence feed intake by broiler chicken. In contrast, Allahdo *et al.* (2018) reported that ACV supplementation in drinking water lowers broiler feed intake. The decrease in feed intake was caused by strong taste of added organic acids in the water, which would have dropped the appetite of the broilers, thus intake of feed reduction. Esmaeilipour *et al.* (2011) reported that the organic acids significantly decreased feed intake of the broiler chickens.

2.3.2.2 Effects of garlic

Yin and Cheng (2003) indicates that garlic improves broiler chickens productive performance) and Choi *et al.*, (2010) states that it increases feed palatability which in turn increases the birds' appetite (Choi *et al.*, 2010). Similarly, Brzóska *et al.* (2015) reported that liquid garlic extract stimulated the chickens appetite and this resulted in greater intake of feeds. Elagib *et al.* (2013) also reported that diets fortified with garlic significantly increased feed intake. In contrast, dietary garlic incorporation did not influence broiler feed intake (Toghyani *et al.*, 2011a; Onibi *et al.*, 2009).

2.3.3 Feed conversion ratio

2.3.3.1 Effects of apple cider vinegar

Organic acids are alternatives to antibiotics due to their growth-promoting properties (Fascina *et al.*, 2012). Adding organic acids (a component of ACV) to water for drinking helps to regulate the intestinal microflora, increases digestion, lowers the pathogen levels in the proventriculus and improves growth performance (Khan and Iqbal, 2016). There is limited research on the effect of ACV on broiler FCR. Notably, Allahdo *et al.* (2018) reported that dietary ACV supplementation in drinking water improved broiler FCR. Khan and Iqbal (2016) argue that the improvement in FCR can be attributed to better nutrient utilization with the addition of organic acids.

2.3.4 Mortality

2.3.4.1 Effects of apple cider vinegar

Worldwide, the poultry industry spends significant amounts of money on the treatment and prevention of several orally transmitted parasitic diseases (Castañeda and González, 2015). These parasites infect and reproduce within the mucosal epithelium of various sections of the avian gastrointestinal tract, causing intestinal damage with significant morbidity and mortality in poultry (Chapman, 2014). ACV is effective against these parasitic infections (Castañeda and González, 2015). Hayajneh *et al.* (2018) suggests that ACV is effective against coccidiosis in broiler chickens. However, supplementary citric acid did not decrease mortality (Sultan *et al.*, 2015). There is limited research on the effects of ACV on broiler mortality.

2.3.4.2 Effects of garlic extract

Garlic contains multiple sulphur compounds which have beneficial health-promoting effect (Kim *et al.*, 2013). Brzóska *et al.*, (2015) states that the antibacterial effect of garlic on gastrointestinal pathogenic bacteria and immune system activation reduces broiler mortality. Thus, adding garlic in the diet of broiler chickens decrease mortality (Al-Massad *et al.*, 2018;

Puvača *et al.*, 2015; Arczewska-Wlosek and Swiatkiewicz, 2013). However, Toghyani *et al.* (2011a) reported that the inclusion of garlic in the diet did not affect broiler mortality.

2.3.4.3 Effects of garlic

Mansoub (2011 notes that garlic has different functions some of which have been scientifically proven). Garlic increases small intestines villi height and activates the absorption processes, and this increases growth and improves FCR (Rehman and Munir, 2015). Incorporating garlic in the diet improved the FCR of broiler chickens (Puvača *et al.*, 2015; Fayed *et al.*, 2011; Canogullari *et al.*, 2010). By contrast, Issa and Omar (2012) and Onibi *et al.* (2009) report that dietary garlic supplementation has no effect on the FCR of broiler chicken.

2.3.5 Gut digesta pH

2.3.5.1 Effects of apple cider vinegar

A determinant in poultry performance and better profit in poultry production is the healthy gut of the bird (Samik *et al.*, 2007). The health of chickens and the kind of nutrients consumed affect the pH level in the digestive system (Guluwa *et al.*, 2020). Digesta pH in the digestive tract compartments affects the growth of microorganisms, which in turn affects the breakdown of food and absorption of nutrients (Mabelebele *et al.*, 2017). ACV is a natural health promoting phytogenic with various potential health benefits (Tripathi and Mazumder, 2020). It is a major source of vitamins, organic acids, flavonoids as well as minerals (del Campo et al., 2008). Organic acids, particularly acetic acid present in ACV inhibit microbial growth and modify the intestinal pH (Islam *et al.*, 2008). Nonetheless, information about the effect of ACV on the pH of the digesta in the intestines of broilers is limited.

2.3.5.2 Effect of garlic

A healthy gut is an important condition for improving broiler performance (Dono *et al.*, 2014). The inulin in garlic mediates decreases in digesta pH in birds (Grajek *et al.*, 2005). A low pH in the gut of birds that consumed garlic added meal could be a sign of the effects of prebiotic

(Yang *et al.*, 2019). Olukosi and Dono (2014) reported that garlic powder combined with turmeric reduced the pH of the digesta in the proventriculus, crop and caeca. Significantly, there is limited research about the effects of garlic on broiler gut digesta pH.

2.3.6 Digestive organs weight

2.3.6.1 Effects of apple cider vinegar

ACV is an acidic juice produced from apple fermentation (Iman *et al.*, 2015) and has numerous antimicrobial attributes on diverse species of microbes (Yagnik *et al.*, 2018). It contains polyphenols, organic acids such acetic acid which is the main ingredient in ACV (Naziroğlu *et al.*, 2014). Acetic and citric acid (Organic acids) have been incorporated in diets for their positive effects on bird growth and health (Islam *et al.*, 2008). Allahdo *et al.* (2018) reported that ACV with 5% acetic acid had no effect on relative weight spleen. There is little scientific research about the effects of ACV on the digestive organs and weight of chickens or other animals.

2.3.6.2 Effect of garlic

Garlic is t important in many fields, especially in the field of health and healing (Shetty *et al.*, 2013). In poultry nutrition, garlic as a natural additive, is beneficial and valuable to broiler farmers due to its anti-inflammatory, antibacterial, therapeutic, anti-parasitic and antiseptic properties (Rehman and Munir, 2015). Hossain *et al.* (2014) reported a significant increase in liver weight in broiler chickens supplemented with Korean aged garlic extract. Enoka *et al.* (2020) reported a rise in relative gizzard weight in garlic supplemented broilers. However, incorporating garlic in the diet of chickens did not influence relative weight of the liver, heart, and spleen (Enoka *et al.*, 2020; El-katcha *et al.*, 2016; Samanthi *et al.*, 2015; Mahmood *et al.*, 2009). Dieumou *et al.* (2009) reported that in broiler, the pancreas, heart and gizzard were unaffected by garlic oil treatment except for relative liver weight decrease. Lee *et al.* (2016) found that liver and spleen weights were significantly affected by dietary incorporation of fermented garlic but bursa fabricius and weight progressively declined with increasing fermented garlic levels. Dieumou *et al.* (2012) reported no differences among the relative weights of pancreas and gizzard on broilers supplemented with garlic extract. Elagib *et al.* (2013) also reported that both thymus and bursa exhibited no significant difference between the control and birds fed on garlic treated diet. However, the spleen weight was reduced significantly in birds that consumed garlic fortified diet compared to the control.

2.3.7 Length of gastrointestinal tract

2.3.7.1 Effects of apple cider vinegar

One important factor influencing the gut beneficial bacteria growth and growth performance of broiler is a healthy gastrointestinal tract (Heidari *et al.*, 2018). Acidifiers, for example, ACV, are important additives that can be incorporated into poultry feed as a suitable substitute for growth promoting antibiotics (Eftekhari *et al.*, 2015). Organic acid influences intestinal morphology (Cengiz *et al.*, 2012) of broiler chickens. Rehman *et al.* (2016) reported an intestinal length increase in birds that received acetic acid (ACV constituent). Awaad *et al.* (2018) revealed an increase in intestinal length of chickens that were supplemented with acidifier blend. Drinking water supplemented with ACV improved intestinal growth of broiler chickens (Allahdo *et al.*, 2018). Adil *et al.* (2011) also reported that acidifiers lead to an increase in both the intestinal length and weight of broilers.

2.3.7.2 Effect of garlic

The development and health of the digestive tract is the key to the performance of all livestock and poultry (Adibmoradi *et al.*, 2006) and is greatly influenced by the diet of animals (Santin *et al.*, 2001). Garlic has blanket activities which include anti-inflammatory, antimicrobial, antioxidant, antiprotozoal, antifungal, anticancer and hepatoprotective effects (Dar *et al.*, 2014) and it exhibits antibacterial activity and improves intestinal morphology of broilers (Abdullah *et al.*, 2010). However, there are limited scientific studies on the effects of garlic on the gastrointestinal tract length in broiler chickens

2.3.8 Carcass Yield

2.3.8.1 Effects of apple cider vinegar

Supplementation of organic acids (components of ACV) (Zarghi, 2018) in broiler diets improved the utilization of nutrient, growth and feed efficiency (Denli *et al.*, 2003). Allahdo *et al.* (2018) reported that ACV supplementation in drinking water had no effect on carcass yield. Similarly, Kopecký *et al.* (2012) stated that the incorporation of organic acids in drinking water did not influence carcass yield, percentage of breast and thighs. Islam *et al.*, (2008) also reported that supplementing a broiler chicken basal diet with acetic acid did not affect carcass yield. Nevertheless, there are limited scientific studies about the effects of garlic on carcass yield and the available research lacks consensus.

2.3.8.2 Effect of garlic

Garlic is a medicinal plant with amazing effects on poultry (Rehman and Munir, 2015). Garlic supplementation increases dressing percentage of broiler chickens (Fayed *et al.*, 2011). Raeesi *et al.* (2010) revealed that the supplementation of garlic significantly increased carcass yield of broiler chickens. Contrastingly, Onibi *et al.* (2009) reported that supplementary garlic in the diets of broilers does not influence carcass and organ characteristics. Likewise, Dieumou *et al.* (2011) reported that organ weights were not affected by garlic supplementation.

2.3.9 Meat pH

2.3.9.1 Effects of apple cider vinegar

Meat pH, juiciness, drip loss, tenderness, cooking loss and shelf life are quality attributes that are important to customers and processors in the production of quality meat products (Allen *et al.*, 1998). The pH of meat is mainly related to the biochemical state of the muscle at the time of slaughter, following rigor mortis development (Wattanachant, 2008). The water holding capacity of poultry meat is influenced by pH; and low pH of meat results in increased drip loss and cooking-loss (Northcutt *et al.*, 1994). Acidifiers (ACV) are used to maintain the pH of the broiler drinking water supply at less than 7 to reduce bacterial growth (El-Deeb *et al.*, 2020). Brzóska *et al.* (2015) reported that dietary acidifiers did not affect the breast muscles and leg chemical composition of fat, dry matter and protein content. There are limited scientific studies about the effects of ACV on broiler chicken meat pH.

2.3.9.2 Effect of garlic

Meat pH, tenderness and colour are important characteristics in evaluating meat quality that influence consumer opinion about palatability and product cost (Attia *et al.*, 2018). Meat with higher pH loses less water during storage and yield more juices after preparation and thus will give juicier, more tender and succulent eating experience (Rehman and Munir, 2015). Yet, there are few studies about the effects of garlic on breast meat Ph.

2.4 Summary of the literature review

Literature suggests that ACV has negative effects on feed intake of broiler chickens, while effects on weight gain and FCR are vague, perhaps given varied experimental conditions. On the other hand, although studies provide contradicting findings, garlic is widely reported to increase feed intake, weight gain, carcass yield and FCR, and to reduce mortality. These inconsistent findings about the phytogenic potency of ACV and garlic in broiler chickens demands further investigation.

CHAPTER 3 RESEARCH METHODOLOGY

3.1 Description of the study area

The study was conducted at the poultry facility of the experimental farm of the School of Agriculture, University of Venda, Thohoyandou, Limpopo Province, South Africa. Coordinates of the location are: 22.9761° S, 30.4465° E. The study site is characterized by arid and semi-arid conditions and temperature ranges from a minimum of 10°C during winter to a maximum of 40°C during summer (Odhiambo, 2011).

3.2 Ethical clearance

All the chickens were handled, weighed and slaughtered in accordance with the standards that comply with the animal welfare requirements of the University of Venda Animal Research Ethics Committee (Certificate number: SARDF/19/ANS/20/2011) and the South African Poultry Association Code of Practice (SAPA, 2012).

3.3 Material and Methods

3.3.1 Diets and preparation of phytogenic feed additives

Antibiotic-free (negative control) diets were custom made by Brennco® Feeds Broiler grower (Product 424-50 PROD 2021/08/30), Broiler Finisher (Product 428-50 PROD 2021/08/30] which were iso-nutrient to antibiotic treated (positive control) standard commercial Meadow® Feeds budget-range diets (Broiler Grower product 115-222-000, Broiler Finisher product 11523-000). The Meadow® Feed budget products are used to feed commercial broilers at the University of Venda Experimental Farm.

Table 1: Dietary nutrient targets

	Grower		Finisher	
	[1]Product 1	[2]Product 2	[3]Product	[4]Product 2
Nutrient composition	**AGP free diet**	**AGP fortified diet**	**AGP free diet**	**AGP fortified diet**
Protein(min)	190.0g/kg	180.0g/kg	165.0g/kg	160.0g/kg
Moisture(max)	120.0g/kg	120.0g/kg	120.0g/kg	120.0g/kg
Fat (Min)	-	25.0kg	-	25.0kg
Lysine (Min)	11.0g/kg	10.0g/kg	9.0g/kg	9.0g/kg
Fibre (Max)	50g/kg	60g/kg	70g/kg	70g/kg
Calcium (Min)	8g/kg	7g/kg	6g/kg	6g/kg
Calcium (Max)	12.0kg	12.0kg	12.0kg	12.0kg
Phosphorus (Min)	6.0kg	0.0kg	5.0kg	5.0kg

[1]Brennco® Feeds broiler grower (Product 424-50 PROD 2021/08/30), antibiotic free
[2]Meadow® Feeds budget broiler grower (Product 11522-000), antibiotic treated
[3]Brennco® Feeds broiler finisher (Product 424-50 PROD 2021/08/30), antibiotic free
[4]Meadow® Feeds budget broiler finisher (Product 11523-000), antibiotic treated

Aqueous garlic extracts were prepared according to Hayat *et al.*, (2016) with some modifications. Fresh garlic bulbs were purchased from the local market. The bulbs were separated into garlic cloves which were then washed with tap water. Fresh garlic bulbs were loaded into a centrifugal juicer (700W Russell Hobbs centrifugal juice maker Model no. RHJM01) and centrifuged at level 2. The extract was packaged in 750ml glass bottles and stored at 4°C.

To prepare ACV, apples (Golden delicious) were cut into small chunks (2-3 cm in diameter). A 3-litre glass jar was half filled with the apple chunks followed by the addition of 8 tablespoons of brown sugar and 1.3 litres of water. The mixture was stirred thoroughly to ensure homogeneity, and the glass jar was covered with a mutton cloth. The jar was kept at room temperature (21-27 °C) in a dark cupboard. The mixture was stirred daily for 4 weeks, after which the apple chunks were strained off and the cider retained in the glass jar and left to ferment for another 4 weeks, after which the vinegar was transferred into 750ml glass bottles with an airtight lid and stored at 4°C (Jahantigh *et al.*, 2021).

3.3.2 Experimental birds, management, and study design

3.3.2.1 Birds and management

A total of 390-day-old Ross 308 (195 Males and 195 Females) purchased from a commercial breeder were sexed at age of 14 days by feather sexing methods. The experiment was conducted in a 17.0 m x 9.0 m open broiler house with East and West walls (width) of the house made of face brick from the floor to the roof, while the South and North facing walls along the length were built using face brick to a height of 1.0 m, with the remainder covered by wire mesh to the ceiling. Heavy plastic sheeting on top of the wire mesh served as an adjustable curtain which could be raised or lowered to the desired level depending on the weather and desired temperature within the house. The house was divided into 30 partitions, each measuring 140 cm × 145 cm (2.3 m^2). Each pen had one tube feeder (Height 430mm, diameter 390mm) and one water fountain (Height 400 mm, diameter 360 mm, Poltek, Johannesburg, South Africa). The cement floor was covered with saw dust. The birds were exposed to continuous light throughout the test period and had *ad libitum* access to a flaked commercial starter feed and an *ad libitum* supply of clean drinking water.

3.3.2.2 Experimental design

The study design was a randomized 2 (sex) x 5 (additives) factorial experiment. During the test period, the birds were randomly allotted to 5 additive treatments. For each sex, there were 3 replicates per treatment with 13 birds per replicate, the test period started at the age of 15 days and consisted of two phases: grower (15-28 days of age) and finisher (29-42 days of age).

The treatment regimens (additives) were as follows:

- NC (Negative control) - Antibiotic free diet plus untreated drinking water
- T1- Antibiotic free diet plus ACV treated drinking water
- T2- Antibiotic free diet plus garlic treated drinking water
- T3- Antibiotic free diet plus ACV+garlic treated drinking water
- PC (Positive control)- Antibiotic fortified diet plus untreated drinking water

3.3.3 Data collection

3.3.3.1 Weight gain

On arrival, day-old chicks were distributed to different pens according to the experimental design. Weights of the birds in all the treatment groups were measured every 7-days that is, day 15, day 21, day 28, day 35 and day 42. On the sampling day, 3 birds per replicate were randomly selected. The selected birds were weighed, and their weights were recorded. Sasco Africa WP-PW30 digital weighing scale was used to weigh the birds. Weight gain of birds per week was calculated by subtracting the average weight of the selected birds at the beginning of the week from the average weight of the selected birds at the end of that week.

3.3.3.2 Feed intake

Like weight gain, weights of feed in all the treatment groups were measured at 7-day intervals that is, day 15, day 21, day 28, day 35 and day 42. Feed intake was determined by subtracting the weight of the feed refusals from the total feed offered to each replicate during a particular week and then dividing the feed intake value by the number of birds in the pen. Sasco Africa WP-PW30 digital weighing scale was used to measure the weight of feed offered and the residual feed.

3.3.3.3 Mortality

The birds were monitored daily, and mortality was recorded whenever they occurred and dead birds were taken out and disposed.

3.3.3.4 Terminal procedure and determination of small intestines digesta pH

On day 42 of the experiment, 6 birds per treatment (1 bird per replicate) were randomly selected for slaughter. The slaughter protocol followed that described by Benyi *et al.* (2015). Birds to be slaughtered were subjected to 24 hours of feed withdrawal with free access to drinking water. After 24 hours, the chickens were slaughtered and then defeathered using warm water. Small intestines were removed to obtain the gut content samples. Contents from the intestines were emptied into a plastic cup and pH of the contents was measured using a calibrated portable pH (PH-009(I) a pen type pH meter) meter by inserting the pH electrode into the digesta contained in the plastic cup. Distilled water was used to thoroughly rinse the probe between each reading (Cherian *et al.*, 2013).

3.3.3.5 Carcass yield, digestive organ weights and morphometry

On day 42 of the experiment, 1 bird per replicate in each treatment was randomly selected and subjected to 24 hours of feed withdrawal with free access to drinking water. After 24 hours, the chickens were slaughtered by stunning using a swift blow to the back of the head followed by swift single cut to the throat, bleeding and then defeathered using warm water. The viscera (gizzard, heart, liver, small intestine, cecum and abdominal fat), head, shanks and offal were removed, cleaned and weighed using Sasco Africa WP-PW30 digital scale. The lengths of the small and large intestines (Mabelebele *et al.*, 2017) were measured by tape measure. The dressed carcass was chilled for 5 hours at 14^{o}C. After chilling the carcass was weighed using a Sasco Africa WP-PW30 digital scale, and the cold carcass yield (dressing percentage) calculated by dividing the carcass weight by the live body weight of birds multiplied by 100 (Issa and Omar, 2012).

3.3.3.6 Meat pH

Breast muscle tissue was subjected to pH analysis using about 1 g of sample which was cut into small pieces and homogenized with 9 mL of distilled water, and subjected to pH analysis using Mettler Toledo pH-meter equipped with a glass electrode (Choo *et al.*, 2014).

3.4 Mathematical computations

3.4.1 Feed conversion ratio

After determining the body weight gain per pen and feed intake per pen, feed conversion ratio was calculated using the equation;

FCR= $\frac{feed\ intake\ per\ bird/pen}{(Final\ weight\ of\ bird/pen)-(initial\ weight\ of\ birds/pen)}$

3.4.2 Mortality rate

The mortality rate of the chickens per week per pen or replicate was calculated as the total number of deaths divided by the total number of chickens in pen or replicate, multiplied by 100.

Mortality rate=$\frac{Total\ number\ of\ birds\ deaths\ per\ pen}{Total\ number\ of\ birds\ per\ pen}$x100

3.5 Statistical analysis

The data was subjected to analysis of variance (ANOVA) for a 2 X 5 factorial experiment using the General Linear Model (GLM) procedures of IBM SPSS Version 26.0 (SPSS, 2019). Significant main effects means were separated using Tukey's post-hoc test, at p<0.05.

The statistical model that was used for the analysis of quantitative data was of the form:

$$Yijk = \mu + \alpha i + \beta j + \alpha\beta ij + \epsilon ijk$$

Where:

$Yijk$= the k^{th} observed value of the response variable (weight gain, feed intake, feed conversion ratio, mortality, gut digesta pH, digestive organs weight, gastrointestinal length, dressing percentage and breast meat pH) having the i^{th} additive effect and j^{th} sex

μ= overall mean

αi= effect of i^th Additive: (i=5; Negative control (NC), Positive control (PC), ACV (T1), Garlic (T2) and ACV+Garlic (T3)

βj= effect of the j^th Sex (j=2; Male or Female)

$\alpha\beta ij$= Effects of the interaction between Additives and Sex

ϵijk= the random error

Effects of ACV and or garlic extract on production performance of male and female Ross 308 are presented in Table 3. During the grower phase, male birds on the PC gained more weight ($P<0.05$) than birds on T1, and T3, but had a similar weight gain ($P>0.05$) with birds on the NC and T2. Male birds on the NC, T1, T2 and T3 had similar weight gain ($P>0.05$). Feed intake, FCR and mortality of male birds were not affected by dietary additives ($P>0.05$) during the grower phase. Female birds on the PC gained more weight ($P<0.05$) than those on the T1, T2 and T3 and had a similar weight gain ($P>0.05$) with birds on the NC. However, female birds on the NC, T1, T2 and T3 had similar weight gain ($P>0.05$). FCR of birds on the PC was lower ($P<0.05$) than of birds on the NC, T1, T2 and T3. Feed intake and mortality of female birds were not affected by dietary additives ($P>0.05$). Across sex, birds on the PC gained more weight and had a better FCR ($P<0.05$) than birds on the NC, T1, T2 and T3. However, feed intake and mortality of birds were not affected by additives ($P>0.05$). Male birds consumed more feed ($P<0.05$) and gained more weight ($P<0.05$) than female birds. However, FCR and mortality were not influenced ($P>0.05$) by sex.

During the finisher phase, male birds on the PC gained more weight ($P<0.05$) than male birds on the NC, T1, T2 and T3. However, male birds on the NC, T1, T2 and T3 had similar weight gains ($P>0.05$). Male birds on PC consumed more feed ($P<0.05$) than male birds on the NC, T1, T2 and T3. Feed intake of male birds on the NC was higher than of male birds ($P<0.05$) on the T3. However, feed intake of male birds on the NC, T1 and T2 was similar ($P>0.05$). FCR and mortality of male birds during the finisher phase were not affected by dietary additives ($P>0.05$).

Table 2: Effect of ACV and or garlic extract on production performance of male and female Ross 308 broiler chickens

		Grower (15 to 28 days of age)				Finisher (29 to 42 days of age)				Overall (15 to 42 days of age)			
		Weight gain (g/b)	Feed intake (g/b)	FCR	Mortality rate (%)	Weight gain (g/b)	Feed intake (g/b)	FCR	Mortality rate (%)	Weight gain (g/b)	Feed intake (Kg)	FCR	Mortality rate (%)
Sex	Additives												
Male	NC	903.33^{ab}	1672.05	1.87	0.00	853.33^{a}	1923.85^{b}	2.28	0.00	1757.67^{a}	3595.90^{ab}	2.06	0.00
	T1	776.67^{a}	1600.00	2.10	0.00	687.67^{a}	1711.28^{ab}	2.49	0.00	1462.33^{a}	3311.28^{a}	2.28	0.00
	T2	858.67^{ab}	1565.90	1.84	0.00	741.00^{a}	1792.56^{ab}	2.48	0.00	1599.00^{a}	3358.46^{a}	2.13	0.00
	T3	771.00^{a}	1547.18	2.01	0.00	569.00^{a}	1465.64^{a}	2.74	0.00	1340.67^{a}	3012.82^{a}	2.26	0.00
	PC	1051.00^{b}	1609.74	1.54	0.00	1398.67^{b}	2512.48^{c}	1.80	2.67	2449.00^{b}	4122.22^{b}	1.68	2.67
	SE	57.509	51.135	0.118	0.000	75.08	96.405	0.272	1.193	1.000	143.739	0.123	1.193
Female	NC	804.33^{ab}	1457.44	1.81^{b}	0.00	639.00^{a}	1450.51^{a}	2.35	0.00	1443.33^{a}	2907.95^{a}	2.02	0.00
	T1	722.33^{a}	1474.10	2.05^{b}	0.00	684.33^{a}	1574.62^{a}	2.35	0.00	1406.33^{a}	3048.72^{a}	2.17	0.00
	T2	681.00^{a}	1403.85	2.06^{b}	0..00	631.00^{a}	1456.56^{a}	2.31	2.67	1312.00^{a}	2860.41^{a}	2.19	2.67
	T3	746.67^{a}	1464.61	1.98^{b}	0.00	632.33^{a}	1439.74^{a}	2.31	0.00	1378.67^{a}	2904.36^{a}	2.12	0.00
	PC	1032.00^{b}	1520.77	1.48^{a}	0.00	1276.67^{b}	2234.10^{b}	1.76	0.00	2308.67^{b}	3754.87^{b}	2.06	0.00
	SE	54.205	73.505	0.069	0.000	70.239	66.773	0.234	1.193	77.979	134.236	0.087	1.193
Additives													
	NC	853.83^{a}	1564.74	1.84^{b}	0.00	746.17^{a}	1687.18^{a}	2.31	0.00	1600.00^{a}	3251.92^{a}	2.04^{ab}	0.00
	T1	749.00^{a}	1537.05	2.08^{b}	0.00	685.50^{a}	1642.95^{a}	2.42	0.00	1434.33^{a}	3180.00^{a}	2.22^{b}	0.00
	T2	769.33^{a}	1484.87	1.95^{b}	0.00	686.00^{a}	1624.56^{a}	2.42	1.33	1455.50^{a}	3109.43^{a}	2.16^{ab}	1.33
	T3	758.33^{a}	1505.90	1.99^{b}	0.00	600.67^{a}	1452.69^{a}	2.53	0.00	1358.67^{a}	2958.59^{a}	2.19^{b}	0.00
	PC	1041.50^{b}	1565.26	1.51^{a}	0.00	1336.67^{b}	2373.29^{b}	1.78	1.33	2378.33^{b}	3938.55^{b}	1.87^{a}	1.33
	SE	39.514	44.771	0.068	0.000	51.407	58.636	0.179	0.843	63.308	98.337	0.075	0.843
Sex													
	Male	871.73^{b}	1598.97^{b}	1.867	0.00	849.53	1881.16^{b}	2.36	0.53	1721.33	3480.14^{b}	2.08	0.53
	Female	797.07^{a}	1464.15^{a}	1.876	0.00	772.47	1631.11^{a}	2.23	0.53	1569.40	3095.26^{a}	2.11	0.53
	SE	24.991	28.316	0.043	0.000	32.512	37.084	0.133	0.533	40.040	62.194	0.048	0.533
Significance													
Additives (A)		*	NS	*	NS	*	*	NS	NS	*	*	*	NS
Sex (G)		*	*	NS	NS	NS	*	NS	NS	NS	*	NS	NS
A x G		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P<0.05).
NS=non-significant, SE= Standard error. *=P < 0.05
NC- Antibiotic free diet plus untreated drinking water,
T1- Antibiotic free diet plus ACV treated drinking water,
T2- Antibiotic free diet plus garlic treated drinking water,
T3- Antibiotic free diet plus ACV+garlic treated drinking water,
PC- Antibiotic fortified diet plus untreated drinking water

Female birds on the PC consumed more feed and gained more weight (P<0.05) than those on the NC, T1, T2 and T3. However, female birds on the NC, T1, T2 and T3 had a similar feed intake (P<0.05) and weight gain (P<0.05). FCR and mortality of female birds during finisher phase were not affected by dietary feed additives (P>0.05). Across sexes, birds on the PC consumed more feeds (P<0.05) and gained more weight (P<0.05) than birds on the NC, T1, T2 and T3. However, birds on the NC, T1, T2, and T3 showed similar feed intake (P>0.05) and weight gain (P>0.05). FCR and mortality of birds across sexes was not affected by additives (P>0.05). Male birds consumed more feed (P<0.05) than female birds. However, weight gain, FCR and mortality were not influenced (P>0.05) by sex during finisher phase.

Cumulatively, male birds on the PC gained more weight (P<0.05) than those on the NC, T1, T2. However, male birds on the NC, T1, T2 and T3 had similar weight gain (P>0.05). Male birds on the PC consumed more feeds (P<0.05) than birds on the T1, T2 and T3 and had a similar feed intake with birds on the NC. However, birds on the NC, T1, T2 and T3 had similar feed intakes (P>0.05). FCR and mortality of male birds were not influenced (P>0.05) by dietary feed additives. Female birds on the PC consumed more feeds and gained more weight (P<0.05) than birds on the NC, T1, T2 and T3. However, female birds on the NC, T1, T2 and T3 had a similar feed consumption (P>0.05) and weight gain (P>0.05). FCR and mortality of female birds were not influenced by additives (P>0.05). Across sexes, birds on the PC consumed more feeds and gained more weight (P<0.05) than birds on the NC, T1, T2 and T3. However, birds on the NC, T1, T2, and T3 showed similar feed intake and weight gain (P>0.05). Mortality of birds across sexes was not affected by additives (P>0.05). Birds on the PC had a lower FCR than birds on the T1 and T3 (P<0.05), and had similar FCR with birds on the NC and T2. However, birds on the NC, T1, T2 and T3 has a similar FCR (P>0.05). Male birds consumed more feed (P<0.05) than female birds. However, weight gain, FCR and mortality of the birds were not affected by sex (P>0.05).

Table 3: Effect of ACV and or garlic extract on viscera mass and length, digesta pH, dressing percentage and meat pH of male and female Ross 308 broiler chickens

		Small intestines digesta pH	Liver weight (g)	Spleen (g)	Proventriculus (g)	Gizzard (g)	Gastrointestinal tract length (m)	Dressing %	Meat pH
Sex	Additives								
Male	NC	5.50	41.53[ab]	2.21	8.67	28.61	1.77	56.11[a]	5.16
	T1	5.37	35.80[ab]	2.47	9.61	29.13	1.94	69.88[b]	4.95
	T2	5.50	28.64[a]	1.86	8.37	36.53	1.84	76.96[b]	5.02
	T3	5.53	28.88[a]	1.98	7.73	33.25	1.65	66.94[ab]	4.81
	PC	5.73	46.45[b]	2.67	7.76	23.70	1.77	73.98[b]	5.07
	SE	0.155	3.202	0.236	0.645	3.155	0.110	2.652	0.081
Female	NC	5.53	31.09[a]	2.40	8.37	30.25	1.86	69.01[ab]	4.79
	T1	5.67	32.48[a]	2.10	9.30	30.21	1.66	70.93[ab]	4.86
	T2	5.43	27.78[a]	1.94	8.69	33.86	1.89	63.93[a]	4.89
	T3	5.47	31.47[a]	2.23	8.73	30.01	1.81	74.46[ab]	4.83
	PC	5.63	47.84[b]	2.72	8.64	30.68	1.65	75.98[b]	4.97
	SE	0.115	2.737	0.182	0.593	4.681	0.148	2.512	0.070
Additives									
	NC	5,52	36,31[a]	2,30[ab]	8,52	29,43	1,82	62,56[a]	4,97
	T1	5,52	34,14[a]	2,29[ab]	9,46	29,67	1,80	70,41[b]	4,90
	T2	5,47	28,21[a]	1,90[a]	8,53	35,19	1,86	70,45[b]	4,96
	T3	5,50	30,18[a]	2,11[ab]	8,23	31,63	1,73	70,70[b]	4,82
	PC	5,68	47,14[b]	2,70[b]	8,20	27,19	1,71	74,98[b]	5,02
	SE	0.096	2.106	0.149	0.438	2.822	0.092	1.826	0.054
Sex									
	Male	5.53	36.26	2.24	8.43	30.24	1.79	68.77	5.00[a]
	Female	5.55	34.13	2.28	8.75	31.00	1.77	70.86	4.87[b]
SE		0.061	1.332	0.094	0.277	1.785	0.058	1.155	0.034
Significance									
Additives (A)		NS	*	*	NS	NS	NS	*	NS
Sex (G)		NS	NS	NS	NS	NS	NS	NS	*
A x G		NS	NS	NS	NS	NS	NS	*	NS

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P<0.05).
NS=non-significant, SE= Standard error. *=P < 0.05
NC- Antibiotic free diet plus untreated drinking water,
T1- Antibiotic free diet plus ACV treated drinking water,
T2- Antibiotic free diet plus garlic treated drinking water,
T3- Antibiotic free diet plus ACV+garlic treated drinking water,
PC- Antibiotic fortified diet plus untreated drinking water

Effects of ACV and or garlic extract on viscera mass and length, digesta pH, dressing percentage and meat pH of male and female Ross 308 broiler chickens are presented in Table 3. Small intestines digesta pH, proventriculus weights, gizzard weights, gastrointestinal tract lengths and breast meat pH of both male and female Ross 308 broiler chickens were not affected by additives (P>0.05). Male birds on the PC had significantly heavier livers (P<0.05) compared to the liver weights of counterparts on the T2 and T3 but had a similar (P>0.05) liver weight as those from male birds on the NC and T1. However, male birds on the NC, T1, T2 and T3 had a similar (P>0.05) liver. Female birds on the PC had a heavier liver (P<0.05) than female birds on the NC, T1, T2 and T3. Across sexes, birds on the PC had a higher liver weight (P<0.05) than birds on the NC, T1, T2 and T3. Spleen weight was not affected by additives in male (P>0.05) and female birds (P>0.05). However, across sexes, spleen weights of birds on the PC were higher than of birds on T2, but was similar (P>0.05) with birds on the NC, T1, T3 and PC. Dressing percentage of male birds on the T2 was higher than of birds on the NC, however it was similar (P>0.05) with male birds on the T1, T3 and PC. However, male birds on the NC had a similar (P>0.05) dressing percentage with birds on the T3. Dressing percentage of female birds on the PC was higher than of female birds on the T2 but was similar (P>0.05) with birds on the NC, T1, T3 and PC. Across sexes, birds on the NC had a lower dressing percentage (P<0.05) than birds on the PC, T1, T2 and T3. A significant interaction (P<0.05) between sex and diet was noted for dressing percentage, whereby female birds on the negative control exhibited a higher dressing percentage than male birds. Gut digester pH, liver weight, spleen weight, gizzard weight, proventriculus weight, gut length and dressing percentage of Ross 308 broiler chickens was not influenced (P>0.05) by sex. Male broiler chicken exhibited a higher (P<0.05) breast meat pH compared to the females. Meat pH was not affected by additives (P>0.05).

CHAPTER 5 DISCUSSION

In the present study, male broiler chickens gained more weight than females during the grower phase. However, during finisher phase and throughout the trial, sex did not influence weight gain. Male broiler chickens typically have higher growth rates than female chickens (Benyi *et al.*, 2015; Eid and Iraqi, 2014; Osei-Amponsah *et al.*, 2012; Abdullah Y. Abdullah, 2010; Sam *et al.*, 2010). Contrary to the present study, Ikusika *et al.* (2020) reported a heavier and higher feed intake in females than male Aboaca, Ross and Anak chickens. The higher body weight gain for males reflects the male's genetic metabolic advantage due to a number of factors, (Madilindi *et al.*, 2018), including greater competition for food, aggressive behavior by the male, differences in nutritional requirements for growth and fatness (Zerehdaran *et al.*, 2005).

Similar to the present findings, Madilindi *et al.* (2018) did not detect significant sex effects on FCR and mortality in Cobb Avian48 broiler chickens. Novele *et al.* (2008) and Trocino *et al.*'s (2015) findings are different to those of the current study since they reported a better FCR in female than male broiler chickens. The disparity in experimental findings can be attributed to different or interaction of environmental and genetic effects. In the present study, across sexes, birds on the positive control gained more weight than birds on the negative control, and all the test treatments, with the birds on negative control achieving similar weight gain to the test treatments in all the growth phases. Similar findings were previously reported for garlic by Fayed *et al.* (2011) and Jakubcova *et al.* (2014). The results of the present study imply that ACV and/ or garlic extract supplementation does not influence weight gain of broiler chickens. In contrast, Mahmood *et al.* (2009), Dieumou *et al.* (2012) and Al-Rabadi *et al.* (2020) reported a higher weight gain in birds supplemented with garlic extract. Garlic supplementation of the diet of broiler chickens resulted in higher live weight compared to control diet (Stanacev *et al.*, 2011; Onibi *et al.*, 2009). Different findings may be caused by additive preparation in relation to functional characteristics (powder, flour, extracts, concentration of active compounds) and or from the dosage.

Similar to the present finding, Allahdo *et al.* (2018) did not detect significant difference in weight gain of broiler chickens supplemented with ACV in drinking water compared to the control. Contrary to the present study, broiler chickens supplemented with ACV had a higher weight gain than the control from 1-28 days of age (Jahantigh *et al.*, 2021).

The results of this study indicate that across sexes, positive control had a higher feed intake than birds on other treatments, with the birds on negative control achieving similar feed intake to the test treatments during the finisher and throughout the trial which implies that ACV and/ or garlic extract supplementation does not influence feed intake of broilers. During the grower phase, feed intake was not influenced by additives, these results mean that ACV and/ or garlic extract supplementation does not have an effect on broiler feed intake. Like the present study, dietary garlic supplementation did not significantly influence the feed intake of the broiler chickens (Umatiya *et al.*, 2018; Issa and Omar, 2012; Dieumou *et al.*, 2009; Onibi *et al.*, 2009). The present results are different to the findings of Eid and Iraqi (2014) who reported a significant decrease in cumulative feed intake in broiler chickens that received a diet containing garlic compared to the control. Additionally, Raeesi *et al.* (2010) found that food consumption was significantly higher for the control group than for other groups fed on the garlic-containing diet.

Contrary to the present study, Allahdo *et al.* (2018) reported lower feed intake by birds which consumed water supplemented with ACV compared to birds that consumed additives free water during the starter (1-10 days of age) and grower (11-24 days of age) periods.

The results of the present study indicate that across sexes, birds on the positive control had a better FCR than birds on the negative control, and all the test treatments during grower and throughout the trial. This means that ACV and/or garlic extract fortified diet does not have a better effect on the FCR of broiler chickens. Birds on negative control achieved similar FCR to the test treatments during the grower, finisher and throughout the trial. This implies that ACV, garlic extract and the combination of ACV and garlic extract does not influence FCR of Ross 308 broiler chickens. During finisher phase, FCR was not affected by additives. These results are like those found by Dieumou *et al.* (2012) and Umatiya *et al.* (2018) who reported no significant difference between the FCR of birds supplemented with garlic extract and birds on the control. Contrastingly, Raeesi *et al.* (2010) reported that supplementation of garlic powder had a considerable effect on improvement of FCR. Fayed *et al.* (2011) also reported a significant difference in birds on control diet and birds supplemented with garlic, birds fed on a ration supplemented with garlic had lower FCR. The authors postulated that better FCR by birds on the garlic fortified diet could be attributed to garlic antibacterial properties which lead to a better nutrients absorption and to an improved FCR.

The current study found no significant differences in FCR between birds receiving ACV in their drinking water and the other treatment groups. Similarly, Jahantigh *et al.* (2021) reported no significant changes in the FCR between the ACV treated group and control groups. However, Allahdo *et al.* (2018) reported that during the starter (1 to 10 days) and grower phase (11 to 24 days) birds which consumed water supplemented with ACV had significantly lower FCR compared to those that consumed water without ACV. The growth enhancement effect of ACV is attributed to its high contents of certain nutrients, such as vitamins, organic acids and minerals and its role in nutrient digestibility (Pourmozaffar *et al.*, 2017). In the present study, male and female broiler chickens had similar mortality rate. This agrees with Beg *et al.*'s (2016) findings who reported similar mortality rate in male and female chickens.

The current study showed no statistically significant differences in mortality of birds supplemented with garlic extract and the combination of ACV and garlic extract compared to those on the negative and positive controls. This implies that garlic extract and the combination of ACV and garlic extract supplementation does not affect mortality of broiler chickens and therefore they are not a suitable alternative for antibiotics. Dieumou *et al.* (2012) and Fayed *et al.* (2011) reported similar findings indicating no significant difference between the mortality rate of birds supplemented with garlic and birds on the control diet. Al-Rabadi *et al.* (2020) reported that feeding different garlic powder levels at different feeding stages significantly eliminated any mortality incidence compared to broilers fed control diets during the same stages. Notably, Eid and Iraqi (2014) and Onibi *et al.* (2009) reported significant reduction in mortality for birds fed feed containing garlic whereas the current study did not find any significant differences in mortality between the treatments. Eid and Iraqi (2014) claims that garlic powder had a positive effect on the immune response of chickens which might have decreased infection with diseases which is then reflected on livability and performance of birds which received garlic powder.

The current study found no significant differences in mortality between birds receiving ACV in their drinking water and the other treatment groups. This implies that ACV and the combination of ACV and garlic extract supplementation does not affect mortality of broiler chickens and therefore they are not a suitable alternative to antibiotics. Similarly, Allahdo *et al.* (2018) reported that mortality of birds which consumed water supplemented with ACV was not significantly different from that of the control and other treatment groups.

Sex had no significant effect on intestine digesta pH in Ross 308 broiler chicken. There is limited research on the effect of broiler sex in relation to intestine digesta pH. The present study showed a statistically no significant difference between the intestine digesta pH of birds supplemented with ACV, garlic extract and ACV and garlic extract combination and those on negative and positive control. Sunu *et al.* (2021) reported different results indicating that garlic symbiotic significantly reduces the pH of the duodenum, jejunum, and ileum. Garlic symbiotic benefits the host as it provides a specific substrate for fermentation, promoting the growth of probiotics which improve the health of lactic acid bacteria (Adil and Magray, 2012). The fermentation of probiotics in the intestine produces a high concentration of lactic acid that causes a drop in pH (Nkukwana *et al.*, 2015). Dono *et al.*, (2014) suggests that any condition that promotes a lower pH in the gut, often associated with the colonization of beneficial microbes, may also be correlated with a higher efficiency of energy and nutrient use and growth.

The current study found no significant differences in gut digesta pH between birds receiving ACV in their drinking water and the other treatment groups. On the other hand, Abbas *et al.*

(2011) found that adding various levels of acetic acid (Organic acid found in ACV) to drinking water reduced the pH of the intestine in broiler chickens. In addition, Ndelekwute *et al.*, (2019) reported that acetic acid reduces the pH in the duodenum. Broiler chickens utilize minerals better because of organic acid which reduce intestinal pH that leads to an increase in the activity of digestive enzymes (Swiatkiewicz *et al.*, 2010).

The results of the present study indicate that sex does not influence the weight of the liver in Ross 308 broiler chickens. Similarly, several studies found that liver weights of male and female broiler chickens did not differ significantly between male and female birds (Dieumou *et al.*, 2012; Pires *et al.*, 2007; Peebles *et al.*, 1997; Plavnik and Hurwitz, 1982). Other researchers obtained different findings reporting that liver weight of male broiler chickens was significantly higher than that of female chickens (Madilindi *et al.*, 2018; Benyi *et al.*, 2015; Novele *et al.*, 2008). However, Brake *et al.*, (1993) found that female birds had a greater percentage of liver weight compared to male birds.

The results of this study indicate that birds on positive control had a higher liver weight than birds on negative control and all the test treatment, with the birds on negative control achieving statistically similar liver weight to the test treatments. This implies that ACV and/or garlic extract does not influence liver weight of broiler chickens that antibiotic growth promoters remain the best supplementation option. Several researchers agree with the findings of the current study indicating that liver weights of broiler chickens were unaffected by garlic dietary supplements (Aydogan *et al.*, 2020; Enoka *et al.*, 2020; Samanthi *et al.*, 2015; Dieumou *et al.*, 2012; Issa and Omar, 2012; Mahmood *et al.*, 2009). Heidari *et al.* (2018) also reported that liver weights of broiler chickens were not significantly affected by Acidifier supplementation. ACV has a protective effect on the liver and improves liver function (Bouazza *et al.*, 2016; Naziroğlu *et al.*, 2014). There is limited to no pre-existing research regarding the effect of ACV on the weight of the liver in broiler chickens.

In the present study, the weight of the spleen was not influenced by sex. A similar finding was reported by Pires *et al.* (2007) who found that sex did not affect spleen weights. There is limited research about the effect of sex on spleen weight. The results of this study indicate that birds on positive control had significantly higher spleen weights than birds on garlic supplemented diet, and spleen weight of birds on garlic supplemented diet was like that of birds on negative control and all the additives. Other researchers have obtained similar findings indicating that the relative weight of spleen was unaffected by garlic supplementation in broiler chickens (Aydogan *et al.*, 2020; Enoka *et al.*, 2020; El-katcha *et al.*, 2016; Lee *et al.*, 2016). However, Elagib *et al.* (2013) reported that spleen weights decreased significantly with feeding diets containing garlic powder compared to control diet. The current study shows no significant differences in spleen weights between birds that received ACV in their drinking water and the other treatment groups. Like the current study findings, Jahantigh *et al.* (2021) reported that ACV intake through diet did not change the weight of spleen.

Similar to the present study finding, sex did not have an effect on the weight of proventriculus (Stęczny and Kokoszyński, 2020; Tesfaye *et al.*, 2013). In disagreement to the present study, Leksrisompong *et al.*, (2007) found different results stating that the proventriculus weight was significantly larger in female than in male birds. Zhao *et al.* (2012) also reported that female birds have higher proventriculus weights than males. Similar to the findings of the present study, Vishwas *et al.*, (2020) and Kırkpınar *et al.*, (2011) found that the supplementation of garlic in broiler diet did not have significant effects on proventriculus weights (. In contrast, Al-Massad *et al.*, (2018) and Kafi *et al.*, (2017) state that garlic supplementation in broiler diet increased proventriculus weight. The current study found no significant differences in proventriculus weight between birds that received ACV in their drinking water and the other treatment groups. There is a dearth of information about the effect of ACV on proventriculus weight.

The results of the present study reveal that gastrointestinal tract length was not influenced by sex. These results suggest that the sex of broiler chicken in question does not affect the gastrointestinal tract. Nevertheless, Mabelebele *et al.* (2017) found that male chickens exhibited longer gastrointestinal tract than female chickens. Gonzales *et al.* (2003) and Novel *et al.* (2009) also reported longer intestines in males than female broiler chickens. However, other researchers obtained results that confirm this study indicating that garlic supplementation does not have an effect on gastrointestinal tract length of broiler chickens (Patel *et al.*, 2017; Daneshmand *et al.*, 2012; Tatara *et al.*, 2008). In contrast, Al-Massad *et al.* (2018) reported that powdered garlic promotes small intestine length compared to control groups. Like the present study results, Jahantigh *et al.* (2021) reported that dietary ACV did not significantly affect the length of intestines.

The current study shows no significant differences in the dressing percentages between male and female broilers. This means that the dressing percentage was not affected by the sex of the broiler chickens. Similarly, Novel *et al.* (2009) and Olawumi and Fagbuaro (2011) reported no significant differences in dressing percentages of males and female broiler chickens. However, Hussein *et al.* (2019) reported a higher dressing percentage in male than female broiler chickens. The results of the present study indicate that birds on the negative control had a lower dressing percentage than birds on the positive control, and all the test treatments. Birds on positive control achieved statistically similar dressing percentage to all the test treatments. The result implies that ACV and or garlic extract is not a suitable alternative for antibiotic growth promoters in broiler chickens. Regarding the increased dressing percentage of garlic extract, garlic supplementation in diet of broiler chickens several researchers obtained results that confirm the current study(Eltazi *et al.*, 2014; Oleforuh-Okoleh *et al.*, 2014; Fayed *et al.*, 2011). This notwithstanding, there are other researchers who indicate that garlic supplementation does not influence the dressing percentage of broiler chickens (Patel *et al.*, 2017; Kharde and Soujanya, 2014; Fadlalla *et al.*, 2010; Dieumou *et al.*, 2009). The current study shows that garlic extract supplementation negatively affected dressing percentage of broiler chickens by decreasing it. Notably, there is limited research regarding the effect of ACV on the dressing percentage in broiler chickens.

As in the present study, Schneider *et al.*, (2012) found that male birds had higher breast meat pH than female birds. However, Hussein *et al.* (2019) reported that muscles of female birds had a higher pH than that of male birds, implying that the sex of the Ross 308 broiler had influenced the pH of the breast meat pH. Other researchers found no significant difference in the breast meat pH value of male and female broiler chickens (Kirkpinar *et al.*, 2014; Lopez *et al.*, 2011; Musa *et al.*, 2006).

Kirkpinar *et al.*, (2014) found results like those of the current study stating that garlic supplementation had no effect on the breast meat pH of broiler chickens.) This implies that garlic supplementation on broiler diet does not influence the pH of breast meat. However, Choi *et al.* (2010) and Abdullah *et al.* (2010) reported a decrease in the breast meat pH in birds supplemented with garlic. There is limited or no pre-existing research regarding the effect of ACV on breast meat pH in chickens. Significantly, the current study established that ACV supplementation in the feeds of broiler chickens does not affect breast meat pH.

CHAPTER 6 CONCLUSION

In conclusion, weight gain, FCR and mortality of Ross 308 broiler chickens were not affected by sex in all growth phases. However, the feed intake of the male broiler chickens was higher during the grower phase, and cumulatively compared to that of female broiler chickens. Higher feed intake by males was expected since male Ross 308 broiler chickens are generally aggressive, have greater competition for feed, greater nutritional requirements and are dominant than females. Dietary supplementation of ACV, garlic extract and the combination of ACV and garlic extract did not influence weight gain, feed intake, FCR and mortality. This implies that ACV, garlic extract and the combination of ACV and garlic extract does not influence the performance of broiler chickens. The sex of broiler chicken does not affect viscera mass and length, small intestines digesta and dressing percentage. The breast meat pH is influenced by sex of the broiler chicken and male chickens have high meat pH than females. Dietary supplementation of ACV, garlic extract and the combination of ACV and garlic extract does not affect small intestine digesta pH, proventriculus weight, gizzard weight and length of gastrointestinal tract. ACV, garlic extract and the combination of ACV and garlic extract supplementation decrease the weight of liver and spleen of Ross 308 broiler chickens. The ACV, garlic extract and the combination of ACV and garlic extract increased the dressing percentage.

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