Effects of Supplemental Zingerone on Cobb 500 Broiler Chicken (*Gallus gallus domesticus*) Growth Performance, Health and Meat Quality

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A thesis submitted to the Faculty of Health Sciences, University of Witwatersrand, School of Physiology, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2023

DECLARATION

I. **Bayanda Mdoda**, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

…….

(Signature of Bayanda Mdoda)

Signed on the …18th of July 2023.

DEDICATION

To the Mdoda and Marau families

RESEARCH OUTPUTS

CONFERENCE PRESENTATIONS

Bayanda Mdoda, Faith Machabi, Busisani W. Lembede, and Eliton Chivandi, 2022. "Effects of zingerone on growth performance, feed intake and utilisation efficiency, carcass yield and viscera macromorphometry of Cobb 500 broiler chicken". Virtual presentation, 53rd South African Society of Animal Science Congress, 27-29 September 2022, Pietermaritzburg, KwaZulu Natal, South Africa.

PUBLICATIONS

Article 1: Bayanda Mdoda, Faith Machabi, Busisani W. Lembede, and Eliton Chivandi (2023). Effects of zingerone on growth performance, feed intake and utilisation efficiency, carcass yield and viscera macromorphometry of Cobb 500 broiler chicken [**Manuscript accepted by Journal of Animal Health and Production, see appendix 5**]

Article 2: Bayanda Mdoda, Pilani Nkomozipi & Eliton Chivandi (2023). Dietary zingerone increases the redness, total monounsaturated, polyunsaturated, oleic and linoleic fatty acids content of Cobb 500 broiler chickens breast meat **[Manuscript under review with Heliyon (Manuscript ID: HELIYON-D-23-05956)]**

Article 3: Bayanda Mdoda, Busisani W. Lembede, Pilani Nkomozepi and Eliton Chivandi (2023). Zingerone as a substitute to zinc bacitracin: effects on oxidative stress, systemic antioxidant status, liver fat content, function and histomorphometry of Cobb 500 broiler chicken **[Manuscript under review with Journal of Advanced Veterinary and Animal Research (Manuscript ID: JAVAR-2023-05-062)]**

GENERAL ABSTRACT

Commercial broiler and pullet chicken producers supplement chicken diets with sub-therapeutic doses of antibiotics such as zinc bacitracin that act as growth promoters to enhance production performance, meat and egg quality. Use of these antibiotics as growth promoters, in addition to causing environmental pollution, causes the public health challenge of antibiotic resistance which compromises poultry and consumer, hence the need to search for environmentally friendly and health-friendly alternatives to antibiotics. Phytochemicals, zingerone included, display biological activities similar to those of antibiotics. This study evaluated zingerone`s potential to replace bacitracin (ZnBcn) as a growth-promoting diet supplement in broiler feed specifically determining its effects on growth performance, meat quality and bird health.

One hundred and twenty unsexed 1-day-old Cobb 500 broiler chicks (10 chicks per replicate with 3 replicates per diet) were randomly assigned to four dietary treatments where zingerone replaced ZnBcn at: diet 1 – 0 mg kg^{-1} (control: 500 mg akg^{-1} of zinc bacitracin); diet 2 – 40 mg kg^{-1}; diet 3 – 80 mg kg^{-1} and diet 4 – 120 mg kg^{-1} in the starter, grower and finisher diets. The broiler chicks were fed *ad libitum* for 6 weeks: starter (week 1-2), grower (week 3-4), and finisher (week 5-6). Initial and weekly body mass, daily feed intake (FI), and terminal body mass (TBM) were measured. Body mass gain (BMG), average daily gain (ADG), and feed conversion ratio (FCR) were computed. On day 42, the chickens were humanely slaughtered, blood collected and carcasses dressed. The gastrointestinal tract (GIT) and accessory GIT viscera organs were weighed and small and large intestine lengths were measured. Empty carcass masses were measured and the dressing percentages were computed. Viscera macromorphometry, long bone indices and carcass traits, the meat's physical quality [initial and ultimate pH (pH_i and pH_u), colour, thawing loss (TL), cooking loss (CL), and tenderness] traits, proximate and amino acid content and fatty acid profiles were measured. Plasma malonaldehyde (MDA) concentration, glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities, surrogate markers of liver and kidney function, liver fat content and histology were determined. Across growth phases and overall, dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on the chicken's TBM, BMG, ADG, FI, and FCR. It also had similar effects ($p > 0.05$) as ZnBcn on the chicken's empty carcass mass, dressing percentage, long bone indices and viscera macromorphometry.

Dietary zingerone had similar ($p > 0.05$) effects as ZnBcn on the broiler chicken meat's pH_i, pH_u, CL, TL and tenderness. However, at 40 mg kg^{-1} of feed (diet 2) it increased the meat's redness (a^*) compared to that of counterparts fed the ZnBcn-fortified control diet. Furthermore, supplemental zingerone had a similar effect to that of ZnBcn on the meat's crude protein content but it significantly increased the meat's ash and fat contents ($p < 0.01$; $p < 0.0001$). Meat from chickens fed diet 2 (40 mg kg^{-1} of feed zingerone) had the highest concentration of essential amino acids ($p < 0.05$) and that from chickens fed diets 3 (80 mg kg^{-1} of feed zingerone) had the lowest ($p > 0.001$) total amino acid content. Dietary zingerone had a similar ($p > 0.05$) effect as ZnBcn on the chicken meat's total saturated fatty acids, but breast meat from chickens fed diets 3 (80 mg kg^{-1} of feed zingerone) had significantly increased ($p < 0.0001$) total monounsaturated fatty acid and oleic acid content. Meat from chicken-fed diet 4 (120 mg kg^{-1} of feed zingerone) had the highest total polyunsaturated fatty acid and linoleic acid content and a higher PUFA:SFA ratio compared to that from counterparts fed diets 1, 2 and 3.

Supplemental zingerone had similar effects ($p > 0.05$) as ZnBcn on the chickens' liver masses and fat contents, plasma MDA concentration, GSH-Px, GST, SOD, CAT, alkaline phosphatase, alanine transaminase activities, albumin, total bilirubin, creatinine and urea concentrations. Chickens' hepatic inflammation and steatosis scores were similar across diets ($p > 0.05$). At 120 mg kg^{-1} of feed zingerone, though similar to the control, supplemental zingerone decreased the chickens' plasma globulin and total protein concentration ($p < 0.01$; $p < 0.05$) compared to counterparts supplemented at low and medium dose of zingerone.

Zingerone can be used as a growth promoter in place of zinc bacitracin in broiler chicken diets without compromising growth, feed use efficiency, carcass yield, long bone and GIT viscera growth and development, the meat's pH, CL, TL and tenderness. Furthermore, it can be used without eliciting oxidative stress in the birds and with no risk to kidneys, liver and general health of the birds. Importantly, zingerone, as a dietary supplement, can be used to enhance broiler chicken meat's redness, positively impacting its acceptability and meat's total monounsaturated, oleic acid, total polyunsaturated and linoleic acid fatty acid profile; thus improving its nutritional value.

ACKNOWLEDGEMENTS

"*Tell me and I forget, teach me and I may learn, involve me and I learn*" Benjamin Franklin – I would like to express my profound gratitude to my supervisor **Associate Professor Eliton Chivandi** and **Doctor Busisani "Wiseman" Lembede** for their helpful guidance, constructive feedbacks and encouragement. Your professional supervision helped me to achieve my best ability during the entire period of my research and writing of this thesis.

I am sincerely thankful to:

- **Professor Kennedy H. Erlwanger** for his valuable technical input during termination and sample collection.

- **Mrs Nkele Maseko**, **Miss Faith Machabi** and my postgraduate colleagues: **Dr Nasiru Muhammed, Mr. Khaalid Khan, Mrs Mmahinne Mosana and Miss Malebogo Bopape** for their assistance during termination and being part of my journey.

- Wits Research Animal Facility staff members for their assistance with the care and welfare of the chickens as well as termination.

- **Dr Monica Gomes, Dr Thuthuzelwa Stempa and Miss Amelia Rammekwa** for their technical assistance with the histology, meat tenderness assay and IDEXX chemistry clinical chemistry assays, respectively.

- The Wits School of Physiology, Faculty of Health Sciences Research Committee and the National Research Foundation of South Africa for funding this study.

- **Miss Anathi Kupiso** and **Mr. Bahle Mdoda** for their endless love, support and generosity throughout this journey.

"*Gratitude makes sense of our past, brings peace for today, and create vision for tomorrow*"
Melody Beattie

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
AGP	Antibiotic growth promoters
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASP	Aspartate aminotransferase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
BWG	Body weight gain
BUN	Blood urea nitrogen
CF	Crude fibre
CL	Cooking loss
CP	Crude protein
DAFF	Department of Forestry and Fisheries
DHA	Docosahexaenoic acid
DL	Drip loss
DM	Dry matter
EAAs	Essential amino acids
EE	Ether extract
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
FA	Fatty acids
FAO	Food and Agriculture Organisation
FI	Feed intake
FCR	Feed conversion ratio
GE	Gross energy
GIT	Gastrointestinal tract
IMF	Intramuscular fat
LDL	Low density lipoprotein
MDA	Malonaldehyde

n3PUFA	Omega-3 polyunsaturated fatty acids
n6PUFA	Omega-6 polyunsaturated fatty acids
ROS	Reactive oxygen species
SAPA	South Africa Poultry Association
SSA	Sub-Saharan Africa
TBARS	Thiobarbituric acid reactive substances
TFAs	Total fatty acids
TMUFA	Total monounsaturated fatty acids
TPUFA	Total polyunsaturated fatty acids
TSFA	Total saturated fatty acids
USDA	United State Department of Agriculture
ZnBcn	Zinc bacitracin

NOMENCLATURE

^{o}C	Degree Celsius
L*	Lightness
a*	Redness
b*	Yellowness
pHi	Initial pH
pHu	Ultimate pH

CHAPTER ONE: INTRODUCTION AND JUSTIFICATION

1.0 Preview of thesis structure

This thesis is structured in a divided block format. It consists of six chapters and a section of appendices. Chapter One gives a background on the demand for poultry meat and eggs in sub-Saharan Africa (SSA) and South Africa. It also gives a narration on the nutritional value of poultry meat and eggs and highlights the importance and contribution of poultry to rural households' food security and the economy of the SSA. The drivers of the increase in demand for poultry meat and eggs: a growing human population, urbanisation and an improvement in incomes, are discussed. The chapter also provides a discourse on the use and impact of the incorporation of antibiotics as growth Promoters (AGPs) in poultry diets on poultry productive performance, the environment, poultry and consumer health. A discussion justifying the search and use of "natural alternative" to antibiotics in the fortification of poultry diets, with a focus on the potential of phytochemicals, is given. Lastly, the chapter concludes by stating the study`s aim, objectives and hypothesis. Chapter Two provides a critical review of the literature pertinent to the study. It gives a detailed discussion on the application of fortifying poultry and livestock feeds with antibiotics as growth promoters. The mechanisms of action of antibiotics added to poultry diets are discussed and the detrimental effects these antibiotics' residues in poultry wastes and products (eggs and meat) on the environment, poultry and consumer health are highlighted. The Chapter then goes on to highlight the dire need to search for and develop "greener alternatives" to antibiotics for use as growth-promoting supplements in poultry diets. In this discourse, the importance of using plant-bioactive compounds in animal/poultry diets and the health-beneficial biological properties of zingerone are discussed creating a basis for justifying the evaluation of its (zingerone) as a potential growth promoter in broiler chicken diets.

Chapter Three is the first of a series of experimental chapters of the study. Essentially it gives an interrogation of the potential of zingerone to substitute the antibiotic in Cobb 500 broiler chicken diets by determining its effects on feed intake and utilisation efficiency, growth performance (body mass and long bone-based indices) and impact on gastrointestinal tract (GIT) morphometry. The chapter is written in a journal paper format with the "introduction section" giving a brief background, problem statement and study justification, the aim and objectives of the experiment. It then gives a detailed description of the methodology, statistical analysis and presents the findings on the effects of zingerone on productive performance and GIT growth and

development of Cobb 500 broiler chickens. The chapter concludes with a discussion of the study findings and key points to the reader the key take-home message.

Chapter Four is the second experimental chapter of the study. Like the third chapter, it is also in a journal paper format. It contains a narrative that evaluates the effects of zingerone as a substitute of zinc bacitracin in Cobb 500 broiler chicken diets, the physico-chemical properties of broiler chicken meat and in the discussion, it highlights the implications of the effects of dietary zingerone on meat quality and on the nutritional value of the meat and its potential effects on consumer health.

Chapter Five, the third experimental chapter, gives, in a journal paper format, an account of the effects of dietary zingerone on Cobb 500 broiler chicken`s health. Dietary interventions impact poultry health, thus the chapter interrogates and narrates findings on the effects of zingerone on the chickens' oxidant and antioxidant status, liver and kidney health. In fulfilling this objective, the chapter describes the determination of the effects of supplemental zingerone on the Cobb 500 broiler chickens' plasma malondialdehyde concentration, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, and catalase activities, surrogate plasma markers of liver and kidney function, hepatic fat content and histology. The chapter concludes with a detailed discussion of the findings and draws key inferences as the take-home message.

Chapter Six, the last of the series of chapters, summarises the major study findings and gives key conclusions drawn from the study. It then gives, briefly, some limitations of the study and then makes a proposal recommending what future studies should focus on in their evaluations. The last chapter, Chapter Seven, gives a list of all the references cited throughout this thesis. Attached to this last chapter is also a list of appendices.

1.1 Introduction

Poultry products (meat and eggs) are the most affordable sources of animal-derived protein in rural households with approximately 98% of the poor and undernourished populace depending on these products for human consumption (Achilonu et al., 2018). In sub-Saharan Africa (SSA), poultry farming has emerged as the most profitable business not only for the self-employed (Bamidele et al., 2019) but also on a commercial level (Abraham & Pingali, 2020). While

commercial poultry production gives employment to 421 664 people in SSA and 57 000 in South Africa (SA) [South African Poultry Association (SAPA, 2019)], about 80% of SSA rural households are actively involved in poultry production (Kryger et al.. 2010). Poultry production for subsistence purposes is generally characterised by low productive performance. The reason for this is that the majority of disadvantaged poultry farmers practise subsistence production in the overcrowded and semi-arid areas in the former homelands. The demand for broiler chicken meat has and continues to increase especially in SSA and SA (OECD-FAO, 2016). This demand is envisaged to increase by 81 -121% and 78 - 89% for SSA and SA, respectively by 2050 (OECD-FAO, 2016). The demand for chicken eggs is expected to increase by 70 - 78% and 63 - 70% for SSA and SA, respectively by 2050 (Mottet & Tempio, 2017). Compared to red meats (beef, lamb and pork), broiler chicken meat is relatively more affordable to the general (poor) public as its price per unit weight is lower (Tan et al., 2018). When compared to red meats, poultry meat has a healthier, more beneficial nutrient profile: high-quality protein and low-fat content (Kim et al., 2017; Qi et al., 2018). In addition, it also has higher essential amino acid (EAA) as well as essential fatty acids (EFA) content when compared to red meats (Kim et al., 2017; Alagawany et al., 2019). The EAAs are required for the early development of the neonatal brain (Mazza et al., 2007) while EFAs lower the risk of cardiovascular disease occurrence by 19% in adults (Bernstein et al., 2010). In addition to an increase in human population and expansion of urban settlements in SSA, the favourable nutrient profile of broiler chicken and poultry meat, in general, has added to the increase in demand and consumption (Özturk and Kose, 2017; Kralik et al., 2018). Despite the South African poultry industry being one of the largest commercial agricultural sectors in SSA, it contributes 64.4 to 88% of the total poultry protein consumed locally (SAPA, 2016). The deficit required to meet demand is made by imports from Brazil and European countries (Berkhout, 2019).

Both local and international commercial poultry production makes use of antibiotics as dietary supplements in poultry feeds (Huyghebaert et al., 2011). These antibiotics, which are used as antibiotic growth promoters (AGPs) in intensive production operations, have been shown to boost poultry and livestock productive performance (Mehdi et al., 2018; Roth et al., 2019) and to enhance enterprise profitability (Muaz et al., 2018). The antibiotics added in the poultry and livestock feeds essentially serve two purposes: as therapeutics to improve poultry health and as prophylactics to prevent bacterial diseases (Khodambashi Emami et al., 2012; Chattopadhyay,

2014). Antibiotics used as AGPs reduce the growth of microbes that reside in the birds' gastrointestinal tract which drain vital dietary nutrients meant for the host animal (Torok et al., 2011; Singh et al., 2013). These AGPs also exert positive effects on the gut health of birds by reducing the immunological stress caused by enteric microbiota which compromises the growth performance and feed utilisation efficiency of the bird (Lee et al., 2012). In the poultry industry, these AGPs substantially contribute to its prosperity (Gadde et al., 2017) by improving feed utilisation efficiency and reducing time to slaughter weight (Chattopadhyay, 2014; Hossain et al., 2015;).

It has been demonstrated that more than 60% of the developed antibiotics end up in the poultry production chain either as prophylactics and or therapeutics (Manafi et al., 2019). Their prolonged use has been associated with the emergence of multi-drug resistance pathogenic bacteria (Salim et al., 2018). This antibiotic-use-induced antibiotic resistance has become a major public health concern as it negatively impacts poultry, livestock and human (consumer) health (Achilonu et al., 2018). The growing public health concern is worsened by the ease of transmission of pathogenic antibiotic-resistant bacteria in livestock and poultry to humans via the food chain (Manyi-Loh et al., 2018). Research has shown a potential link between the practice of using sub-therapeutic doses of antibiotics and the development of antimicrobial resistance among the microbiota (Lillehoj et al., 2018) in both poultry and poultry products (Achilonu et al., 2018). If current trends of antibiotic resistance persist, it is estimated that by 2050, drug (antibiotic) resistant infections are likely to cause the death of 10 million people annually (de Kraker et al., 2016; O'Neill, 2016). Thus, the addition of antibiotics in livestock and poultry feeds as supplements has come under serious scrutiny due to an increased societal and political demand for livestock and poultry products from green production systems (Valenzuela-Grijalva et al., 2017; Achilonu et al., 2018). While the debate on the benefits versus the harmful effects of using antibiotics as AGPs in livestock and poultry feeds has continued for some time (Abd El-Hack et al., 2022), it is now accepted that the benefits of their use are outweighed by their negative effects (Muaz et al., 2018).

In 2006, the European Union Commission (EC) imposed a ban on the marketing and use of antibiotics as growth promoters in poultry/livestock feed [EC Regulation No. 1831/2003] (Nunan, 2022; Wallinga et al., 2022). This declaration was taken with a view to mitigate

antibiotic resistance and contamination of poultry meat and eggs (Rafiq et al., 2022). Consumption of these residues in edible products puts the health of millions of consumers at risk including the development of antibiotic resistance, allergy, reproductive disorders, and hypersensitivity reactions (Falowo & Akimoladun, 2019; Arsène et al., 2022). While the ban holds in EU countries, withdrawal of antibiotics such as AGPs in poultry/livestock feeds has been shown to result in decreased growth rate, compromised feed utilisation efficiency and increased health challenges (Kirchhelle, 2018; Maria Cardinal et al., 2019), as well as poor quality edible poultry and livestock products (Rahman et al., 2022). These negative outcomes associated with the withdrawal of antibiotics from poultry feed have triggered a dire need to search for alternative substances and strategies that can be deployed to support poultry growth performance and health without compromising poultry productive performance, the quality of edible poultry products as well as consumer health (Salim et al., 2018; Roskam et al., 2020). It is crucial that the proposed alternatives resonate with consumer demands and expectations and that they also allay the problem of resistance by infectious bacteria.

Phytochemicals, natural bioactive compounds derived from plants, exhibit important biological activities among them antibacterial, antifungal, antiviral (Akbarian et al., 2016), antioxidant (Lee et al., 2017), hypoglycaemic (Xalxo et al., 2018), hypolipidaemic, reno- and hepato-protective (Bahadoran et al., 2013) activities as well as being nutrigenomic on the immune system development (Hashemi & Davoodi, 2011). These health-beneficial biological activities exhibited by phytochemicals can be exploited in poultry production (Gadde et al.. 2017) as they potentially mitigate microbial-induced negative effects on poultry productivity and product quality thus can potentially contribute to enhance feed utilisation efficiency (Kamboh et al., 2018). Due to their presence in plants, phytochemicals are natural and deemed safe (Zagga et al., 2018) for possible use as natural growth supplements in poultry and livestock feeds (Sethiya, 2016). Phytochemicals can possibly be used to replace AGPs resulting in “greener” poultry production (Salim et al., 2018) and mitigation of the challenge of multi-drug resistance brought about by the use of antibiotics as feed additives (Salim et al., 2018). Recently, research has started evaluating the potential of phytochemicals to be used as natural growth promoters (NGPs) in ruminant, swine and poultry feeds (Achilonu et al., 2018). However, although green alternatives have been used to replace AGPs in poultry and or livestock production, they are not yet commercially accepted for use in poultry animal feed. Literature shows that the use of phytochemicals as feed additives

resulted in stabilised intestinal microbiota, reduced microbial toxic metabolites in the gut (Kim et al., 2015) and reduced oxidative stress but increased systemic antioxidant activity (Settle et al., 2014) in addition to immunomodulatory effects (Kim et al., 2016; Lee et al., 2017). Despite their known and potentially positive effects on livestock and poultry productive performance, through antimicrobial activity and enhancement of the flavour and palatability of feed (Valenzuela-Grijalva et al., 2017), caution must be exercised as higher dietary phytochemical inclusion could cause reduced feed intake due to odour and flavour tainting (Yan et al., 2011).

Among the multiplicity of potentially useful phytochemicals zingerone, chemically vanillylacetone, is a non-toxic (Archana & Maheswari, 2016) phenolic compound constituting about 9.25% in ginger, *Zingiber officinale* Roscoe (Ahmad et al., 2015). Zingerone possesses antioxidant (Su et al., 2019), anti-inflammatory (Srinaath et al., 2019), hepato- nephro- and gastro-protective as well as growth and immune stimulating activities (Karampour et al., 2019). These health-beneficial biological activities can potentially be exploited in poultry production by supplementing broiler chicken diets with zingerone as a NGP in place of AGPs. While some research has demonstrated that when used as a dietary supplement in rat feed and as a traditional medicine in humans, zingerone improved general and gut health (Archana & Maheswari, 2016; Karampour et al., 2019). There is a dearth of information with regards to the efficacy of zingerone as a natural growth promoter in poultry feeds and its effects on the growth, feed use economy and health (oxidant and antioxidant status, general, liver, kidney, GIT and bone health) and meat quality of broiler chicken.

1.2 Study aim and objectives

This study, therefore, evaluated the potential of zingerone to substitute the antibiotic, an AGP, as a NGP, in broiler chicken starter, grower and finisher diets and evaluate its effects on performance, feed economy, health and product quality of broiler chickens. This was achieved by specifically determining the effects of supplemental zingerone on:

a) performance by evaluating effects on:
   - growth performance as measured by body mass indices [(terminal body mass (TBM), body mass gain (BMG), average daily gain (ADG)], long bone (tibiae and femora length, mass and mass:length ratio) indices.

- feed utilisation efficiency as measured by daily feed intake and feed conversion ratio during the brooding, grower and finisher stages.
- viscera macro- and micro-morphometry (masses and lengths where appropriate).

b) meat quality by evaluating zingerone's effects on meat colour, pH, thaw loss, cooking loss, and tenderness as well as on the proximate, amino acid and fatty acid content.

c) health profile by evaluating effects on:
   - surrogate markers of the liver [alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities as well as on plasma albumin, globulin and total protein concentration] and kidney (plasma blood urea nitrogen, creatinine and total bilirubin concentration) function.
   - oxidant and antioxidant status as measured by plasma thiobarbituric acid reactive substances concentration and superoxide dismutase (SOD), catalase (CAT), glutathione-peroxidase (GSH-Px) and glutathione-S-transferase (GSH-TS) activities.
   - liver histology (steatosis and inflammation)

1.3 Hypotheses

1.3.1 Experiment one: hypothesis

H_0: Supplemental zingerone does not affect the growth as determined by body mass and tibiae and femora indices, feed use economy, gastrointestinal tract and accessory GIT viscera organs macromorphometry of Cobb 500 broiler chickens.

H_1: Supplementary zingerone affects the growth as determined by body mass and tibiae and femora indices, feed use economy, gastrointestinal tract and accessory GIT viscera organs macromorphometry of Cobb 500 broiler chickens.

1.3.2 Experiment two: hypothesis

H_0: Supplemental zingerone does not affect the physico-chemical properties (pH, colour, TL, CL, tenderness, proximate and amino acid content and fatty acid profile) of the breast meat and physical properties (pH, colour, TL, CL and tenderness) of the thigh meat from Cobb 500 broiler chicken.

H_1: Supplementary zingerone affects the physico-chemical properties (pH, colour, TL, CL, tenderness, proximate and amino acid content and fatty acid profile) of the breast meat and physical properties (pH, colour, TL, CL, tenderness) of the thigh meat from Cobb 500 broiler chicken.

1.3.3 Experiment three: hypothesis

H_0: Supplemental zingerone does not affect the oxidant and antioxidant status, kidney and liver function and general health of Cobb 500 broiler chicken.

H_1: Supplementary zingerone affects the oxidant and antioxidant status, kidney and liver function and general health of Cobb 500 broiler chicken.

In the next chapter, the literature gives a detailed discussion on the importance of poultry meat production and its benefits to consumer health. It also describes antibiotic use in poultry production, the mechanism of action of antibiotic growth promoters when supplemented in poultry diets, the threat antibiotics pose to poultry and human health and the ban of AGPs in poultry production. In addition, it gives highlights on the use of plant-derived compounds as alternatives to antibiotics in poultry/animal production. Ginger-derived compound, zingerone and its health-beneficial biological compounds are also discussed.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

Poultry and livestock are important food sources for human consumption that play a role in combating poverty and food insecurity, especially in the developing world (Raza et al., 2019). Poultry meat or eggs are rich dietary protein sources containing all the essential amino acids and necessary essential and long-chain fatty acids (Mancinelli et al., 2022). The excessive consumption of diets rich in saturated fatty acids has been associated with increased risks of metabolic syndrome and cardiovascular diseases (Wali et al., 2020). Poultry meat, compared to red meat, contains less fat and thus is leaner (Kralik et al., 2018). Therefore, poultry meat is considered a healthier option compared to red meat. In addition to the preference for poultry meat for its health benefits, the meat, specifically broiler chicken, is more affordable and accessible compared to red meat (Kleyn & Ciacciariello, 2021). These elements of affordability and access to animal protein are crucial in resource-limited communities, which are dominant in sub-Saharan Africa (SSA).

The poultry industry is one of the most advanced meat industries in SSA, competing with those from developed countries (Nkukwana, 2018). This advanced state of the industry is due to the use of developed poultry strains with faster growth rates, better feed formulation and health management at the farms (Mehdi et al., 2018; Nkukwana, 2018). However, despite the South African poultry industry, particularly the broiler and pullet chicken industry, being the most advanced on the African continent, production is not sufficient to meet the demand for chicken meat and the country, South Africa, still relies on importing broiler chicken meat from other countries (South African Poultry Association, 2019) in order to meet the deficit. In addition to this, the demand for meat is increasing and is expected to continue increasing up until 2050 (Food and Agriculture Organisation, 2018). In SSA, for example, the consumption of meat is projected to increase by 2.5 kg per capita of the population by 2030 (Bruinsma, 2017). The increasing demand for poultry products is driven by the continued regional growth in human population, higher income and urbanisation (Mottet & Tempio, 2017). As a result of the sustained and increasing demand, farmers are under immense pressure to produce poultry, especially broiler chicken meat in a relatively shorter production cycle (Nkukwana, 2018). The shortening of the production cycle is facilitated by using genetically improved birds as well as the use of growth-promoting feed additives (Selaledi et al., 2020). One of the technologies deployed to increase poultry productivity is the routine addition of antibiotics in poultry feeds in order to enhance

growth performance and feed utilisation efficiency (Agyare et al., 2019). Some of the major antibiotics used as growth-promoting feed supplements and for prophylaxes against diseases in poultry production are summarised in Table 2.1 below.

Table 2.1: Common types of antibiotics used as growth promoters and prophylactic agents against disease in poultry production

Antibiotic class	Active compounds	Treatment objectives
Atoxyl	Arsanilic acid	Feed efficiency, growth promotion and pigmentation enhancement
Peptidomimetics	Bacitracin	Feed efficiency and growth promotion
Glycolipid	Bambermycin	Feed efficiency and growth promotion
Tetracyclines	Chlortetracycline and Oxytetracycline	Feed efficiency, growth promotion and disease control
Lincosamide	Lincomycin	Feed efficiency and growth promotion
β-lactams	Penicillin	Feed efficiency, growth promotion and disease control
Macrolides	Tylosin and Erythromycin	Feed efficiency, growth promotion and disease control
Peptides	Virginiamycin	Feed efficiency and growth promotion

(Regassa et al., 2009)

2.1 Use of antibiotics as growth promoters

Antibiotics destroy and inhibit the proliferation of bacteria. In poultry production they are used to treat bacterial infections (Mehdi et al., 2018). Furthermore, the antibiotics are used as prophylactic agents, therapeutics and growth-promoting feed supplements (Rahman et al., 2022). Use of antibiotics as poultry feed supplements emerged in the 1940s (Muaz et al., 2018; Elwinger et al., 2019). Thereafter, AGPs were used as prophylactic agents against pathogens and this improved growth rates and feed utilisation efficiency in broilers (Dhama et al., 2014). Zinc bacitracin is one of the many AGPs used by the poultry production industry.

2.2 Zinc bacitracin as a growth promoter

Zinc bacitracin (ZnBcn), a polypeptide antibiotic is produced by a strain of *Bacillus subtilis* and *Bacillus licheniformis* bacteria (Hassan et al., 2020). It has a strong antibacterial activity on Gram-positive bacteria (Kumar, 2017), thus it plays a significant role in both the pharmaceutical and livestock industries (Hong et al., 2017). In farm animals including poultry, ZnBcn is commonly used as a growth-promoting antibiotic (Li et al., 2017). The growth-stimulating effect of ZnBcn is mediated by the activity of the antibiotic against harmful intestinal bacteria (Engberg et al., 2000; Crisol-Martínez et al., 2017). Numerous studies have shown that the incorporation of ZnBcn and other antibiotics in poultry feed significantly improved chicken live weight and live weight gain, reduced time to market weight and improved feed use efficiency (Crisol-Martínez et al., 2017). Antibiotics, including ZnBcn, used as growth-promoting feed supplements employ several mechanisms in improving poultry productive performance.

2.3 Antibiotic as growth promoters: mechanisms

The avian gut is a barrier between the birds and the environment and it is characterised by functional complexity (Shini et al., 2021). It contains mostly gram-positive bacteria and any digestive imbalance may increase pathogenic gram-negative bacteria (Aruwa et al., 2021). An important part of the GIT is the small intestine, the segment where the majority of the digestion and absorption of nutrients takes place (Shini & Bryden, 2022). When antibiotics are used as growth promoters, they interact with the intestinal microbiota: they suppress the population of undesirable gut microbiota but promote the proliferation of favourable gut microbiota (Agunos et al., 2012; Landoni & Albarellos, 2015). This reduction in the population of undesirable gut bacterial population decreases the competition for nutrients between the gut bacteria and the

birds. Growth-promoting antibiotics used to fortify poultry feeds have been shown to increase small intestine villi density and length which (the increases) mediate increased nutrient digestion and absorption (Gadde et al., 2017) thus availing more nutrients for assimilation. Furthermore, by inhibiting the proliferation of undesirable gut bacteria, antibiotic feed supplements reduce GIT irritation and inflammation, which are characteristic of enteric diseases that negatively affect chicken growth (Adedokun & Olojede, 2019). Niewold (2007) reported that subtherapeutic levels of AGPs in poultry/animal diets reduce the weight and length of the intestines. Thus the thinner intestinal epithelium in AGP-fed animals enhances nutrient absorption and reduces the metabolic demands of the GIT system (Niewold, 2007). The reduction of GIT bacteria also alleviates the competition for vital nutrients between the host bird and microbes (Ferket & Gernat, 2006).

2.4 Antibiotic growth promoters use: threats to the environment and public health

Despite the substantial contribution made towards the efficient and increased production of the poultry industry through the use of antibiotics as growth promoters, their routine use has come under intense scrutiny (Gupta et al., 2021). The prolonged routine use of antibiotics as growth-promoting poultry feed supplements has been reported to result in the development of gut resident bacteria with resistance to treatment with antibiotics (Selaledi et al., 2020), a phenomenon termed antibiotic resistance which is a major public health challenge. Antibiotic resistance develops from bacterial gene mutations and horizontal gene transfers among bacterial species in the gut (Selaledi et al., 2020). The spread and persistence of antibiotic-resistant bacteria are escalated by the intensive and routine use of antibiotics as growth-promoting feed supplements in addition to treating poultry bacterial infections (Manyi-Loh et al., 2018). The spread of antibiotic-resistant bacteria circulates among poultry, poultry product consumers and the environment and has thus become a global health issue (Suresh et al., 2018). For example, poultry may spread the resistant bacteria among each other through faeces and wastewater at the farms (Hedman et al., 2020) while the faecal particles and wastewater may end up in the environment contaminating the soil and plants (Luiken et al., 2020). In humans, antibiotic bacterial resistance often causes diarrhoea and other gastrointestinal discomforts from consuming poultry products tainted with antibiotic-resistant bacteria residues in poultry meat and eggs (de Mesquita Souza Saraiva et al., 2022). Furthermore, live antibiotic-resistant bacteria shed in poultry wastewater and faeces find themselves in the environment posing a grave public health challenge. For these reasons, the Antimicrobial Resistance Collaborators have declared antibiotic

resistance as a global risk that kills about 1.27 million people every year worldwide (Murray et al., 2022). Importantly and worryingly, it is estimated that by 2050 on a global scale approximately 10 million people could die yearly from bacterial resistance to antibiotics (O'Neill, 2016). Therefore, if drastic measures are not implemented there could be a global burden of deaths resulting in a major economic loss due to increased sickness and reduced productivity (Dadgostar, 2019). The public health burden caused by fortifying poultry and other livestock feeds with antibiotics as growth promoters have led to some regions calling for the minimisation of their use and in other instances a total ban on their use.

2.5 The ban on antibiotic growth promoters

Several European Union (EU) countries and the United States promulgated regulations that banned the routine use of antibiotics as growth-promoting feed supplements in 2006 (Salim et al., 2018). However, not all developed countries have institutionalised this ban. In such countries and in developing world countries including South Africa, some antibiotic drugs banned by the EU are still incorporated for growth promotion in poultry feeds (Moyane & Aiyegoro, 2013). Although in South Africa a few antibiotics are made locally, there is poor regulation of antibiotic use in animal production compared to developed countries (Anomaly, 2020). As a result, antimicrobial resistance is prevalent and poses a threat to food safety and consumer health (Selaledi et al., 2020). In a study conducted in Kwa-Zulu Natal, South Africa, a negative relationship between the limited use of antibiotics in poultry feed and the occurrence of antibiotic-resistant strains was evident (Selaledi et al., 2020). Furthermore, a study by Hedman et al. (2020) reported that chicken obtained from small-scale farming communities, where little to no antibiotics were used, had the least antibiotic-resistant bacteria strains compared to intensive commercial and intensive free-range broiler chicken farming operations. Therefore, limiting the use of antibiotics in poultry feed may help to combat the public health and environmental challenges caused by antibiotic-resistant bacteria.

The complete withdrawal of supplementation of chicken feeds with antibiotics has been shown to result in a decrease in growth performance and feed utilisation efficiency (Moyane & Aiyegoro, 2013), compromised production efficiency and product quality (Laxminarayan et al., 2015). The reduction and or banning of antibiotics in South Africa is predicted to threaten food security in many South African communities as this would negatively impact production efficiency and

profitability of broiler and pullet chicken enterprises (Selaledi et al., 2020). In addition, a complete withdrawal without alternatives may not be possible in disease-burdened areas. Thus, to maintain "antibiotic-type-mediated" high poultry production efficiencies and enhance household food and nutrition security without polluting the environment and endangering public health, then there is a dire need to develop and promote safer growth-promoting poultry feed supplements as alternatives to antibiotics. Several compounds, among many, probiotics, prebiotics, synbiotics and phytochemicals, based on their health-beneficial and growth-stimulating activities, have become in the quest to develop and deploy environmentally more friendly and consumer-protecting poultry feeding technologies.

2.6 Plant-derived compounds as alternatives to antibiotic growth promoters

There are existing multiple microbial- and plant-derived compounds that potentially can be used as growth-promoting agents used to fortify poultry diets.

2.6.1 Probiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Vinayak et al., 2021). They are used in animal feeds as alternative feed additives and have been shown to modulate intestinal immune pathways through interactions with the gut microbiota (Quinteiro-Filho et al., 2015). These interactions are key to maintaining gut homeostasis and function and improving feed efficiency (Forkus et al., 2017). Recently, the use of probiotics in poultry production has gained momentum due to the banning and or reduction in the use of antibiotic growth promotants in poultry feeds (Shini & Bryden, 2022). Probiotics are used in poultry feeds mainly as Gram-positive bacteria, fungi and yeast strains (Smialek et al., 2018) to enhance the nutritional value of feed (Mishra & Jha, 2019; Neveling & Dicks, 2020). Supplementing poultry diets with probiotics as a substitute for AGPs has been shown to enhance the immune response, intestinal morphology, gastrointestinal tract health, feed utilisation and growth performance (Markowiak & Ślizewska, 2018; Abd El-Hack et al., 2020).

2.6.2 Prebiotics

Prebiotics are non-digestible fibre compounds (Richards et al., 2020) that improve the survival and growth of healthy microbial species in the digestive system (Ricke et al., 2020). They consist of short chains carbohydrates, predominantly oligosaccharides including fructo-, galacto- and

mannan-oligosaccharides and inulin (Abd El-Hack, et al., 2020). These short-chain carbohydrates with prebiotic activity, play an imperative role in the growth of beneficial gut bacteria such as *Lactobacillus* and *Bifidobacterium* bacterial species (Abd El-Hack et al., 2020; Azad et al., 2020). Fortifying broiler chicken feeds with prebiotics in place of antibiotic growth promoters has been shown to alter the intestinal microbes and modulate the immune system resulting in enhanced gut health, growth performance and carcass characteristics (Markowiak & Ślizewska, 2018).

2.6.3 Synbiotics

A combination of probiotics and prebiotics make up synbiotics (Mohammed et al., 2021). The synergistic mixture of *bifidobacteria* and fructo-oligosaccharides, lactitol and lactobacilli are used as synbiotics (Śliżewska et al., 2020). When used as poultry feed supplements as growth promoters and immune enhancers, synbiotics have been shown to enhance health and increase the growth performance of broiler chickens (Bogucka et al., 2019; Kridtayopas et al., 2019). Śliżewska et al. (2020) reported that fortifying broiler chicken diets with synbiotics improved the intestinal morphology and increased nutrient absorption in broiler chickens which resulted in improved productive performance. In addition, synbiotics have been shown to mediate improved flock health by controlling and mitigating the cellulitis caused by *E. coli* (Markowiak & Ślizewska, 2018). In addition to pre- and pro-biotics and synbiotics, secondary plant-derived metabolites, phytochemicals, can also potentially be used to fortify poultry feeds as growth-promoting agents.

2.6.4 Phytochemicals

Phytochemicals, which are produced by plants to protect themselves against herbivory, insect pest attack and infection by bacterial, protozoal and viral diseases (Yactayo-Chang et al., 2020; Zhao et al., 2022), are natural bioactive compounds (Makhuvele et al., 2020). These plant-derived compounds, exhibit antioxidant and antimicrobial activities similar to those of antibiotics (Gadde et al., 2017; Callaway et al., 2021) and are deemed safe to use in animals (Ghildiyal et al., 2020). Phytochemicals have been also shown to stimulate a stable intestinal environment by promoting the proliferation of favourable microbiota and decreasing the production of toxic microbial metabolites (Gadde et al., 2017; Lillehoj et al., 2018). In addition, these phytochemicals modulate the immune system and thus decrease the risk of cancers and autoimmune diseases (George et al.,

2021). Therefore, these phytochemical-mediated health-beneficial biological activities can be tapped into to promote the gut and overall health of poultry and livestock.

The major types of phytobiotics and their effects in poultry are summarised in Table 2.2 below.

Table 2.2: Major types of phytobiotics used and effects in poultry

Plant	Supplement form	Levels	Enhanced performance/production	References
Cinnamon (*Cinnamomum zeylanicum)*	Powder, oil extract	2 g/kg 250 mg/kg	BMG, FI, FCR, and carcass traits	(Abd El-Hack, et al., 2020; Qaid et al., 2022)
Garlic (*Allium sativum*)	Powder	7.5 kg/ton	BMG, FI, FCR, carcass traits, GIT organs and general health	(Al Massad et al., 2018; Omer et al., 2019)
Moringa (*Moringa oleifera*)	Powder	1000 g/ton	BMG, FI, FRC, GIT organs, meat quality traits and general health	(Nduku et al., 2020; Wahab et al., 2020)
Peppermint (*Mentha piperita)*	Powder, oil extract	250 mg/kg 0.6%	BMG, FI, FCR, carcass traits and plasma constituents	(Ahmed et al., 2016; Petričević et al., 2021)
Turmeric (*Curcuma longa*)	Rhizome powder, dried rhizome extract	250 mg/kg 0.2%	BMG, ADG, FI, FCR and immune responses	(Akhavan-Salamat & Ghasemi, 2016; Khodadadi et al., 2021)
Thyme (*Thymus vulgaris leaf*)	Powder, oil extract, essential oil	15 g/kg 0.5%	BMG, ADG, FI, FCR, blood parameters, immune status and antioxidant activities	(Abdel-Ghaney et al., 2017; Wade et al., 2018; Witkowska et al., 2019)
Ginger (*Zingiber officinale*)	Powder	5 g/kg 6%	BMG, FI, FCR, carcass traits, immunity and gut morphometry	(Ali et al., 2019; Asghar et al., 2021)

ADG – average daily mass gain, BMG – body mass gain, FI – feed intake, FCR – feed conversion ratio

2.7 Ginger

Ginger, *Zingiber officinale* Roscoe, indigenous to South-East Asian countries and the Indian continent (Hosseinzadeh et al., 2017; Alsherbiny et al., 2019) is a flowering plant. Its rhizomes are widely used for culinary and medicinal purposes (Gungor et al., 2020; Mehrzadi et al., 2021). In the history of therapeutic medicine, ginger has been used to treat yellow fever, obesity, type 2 diabetes mellitus, diarrhoea, inflammation, allergies, rheumatoid arthritis and cardiovascular diseases (Unuofin et al., 2021; Cerdá et al., 2022). Gingerols, shogaols and zingerone are the key phytochemicals that confer medicinal activities to ginger (Hosseinzadeh et al., 2017). Gingerols are responsible for ginger's pungent aroma (Ahmad et al., 2015). Although gingerols possess antioxidant, anti-diabetic, anticancer, antimicrobial and anti-inflammatory, they are highly unstable compounds (Alsahli et al., 2021; Mehrzadi et al., 2021). Gingerols are converted into shogaols, paradols and zingerone when ginger is dried or cooked (Alsherbiny et al., 2019; Wen et al., 2020). Studies on gingerol, shogaol and paradols have shown them to mediate an improvement in growth performance and feed efficiency when used as feed additives in broiler chicken feeds (Ali et al., 2019; Wen et al., 2020). However, no study has evaluated the effect of zingerone's effects on broiler chicken growth performance, feed use efficiency, meat quality and bird health have been reported. Zingerone has been identified as a key phytochemical in ginger that possesses numerous health-beneficial biological activities (Gungor et al., 2020; Alsahli et al., 2021).

Figure 2.1: Fresh ginger rhizomes [Source: https://www.gettyimages.com/photos/ginger-root (Accessed: 19-10-2022)]

2.8 Zingerone

Zingerone, chemical formula, 4-(4-hydroxy-3-methoxyphenyl) butan-2-one, is a major component of dry ginger root and thermal degradation of gingerols (Gungor et al., 2020). It is formed from gingerols through retro-aldol reactions (Alsahli et al., 2021). It is used as an additive flavouring in food owing to its sweet and spicy flavour (Kim et al., 2020). This phytochemical is a natural polyphenol and has been shown to possess numerous beneficial biological activities among many, antioxidant (Karampour et al., 2019), anticancer (Vinothkumar et al., 2014) anti-inflammatory, antimicrobial, antidiarrhoeic, and antiapoptotic (Ahmad et al., 2015). Experimental studies in animals have been conducted to investigate the protective health effects of zingerone including the anti-inflammatory, antioxidant, hepato-, nephro- and gastro-protective, appetite and growth-stimulating roles (Çağlayan et al., 2019; Wali et al., 2020; Mehrzadi et al., 2021). Figure 2.2 below shows the chemical structure of zingerone and figure 2.3 shows a schematic flow of its (zingerone) biological activities.

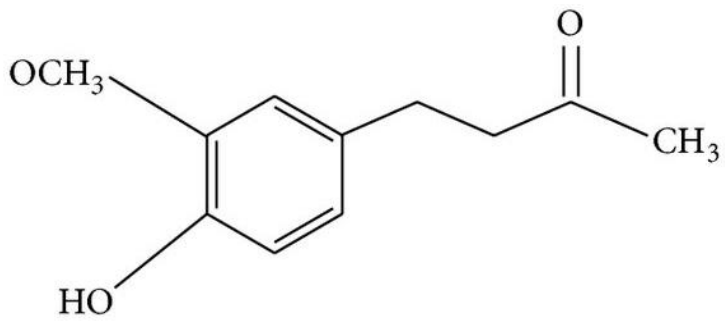

Figure 2.2: Chemical structure of zingerone from ginger (Source: Ahmad et al., 2015)

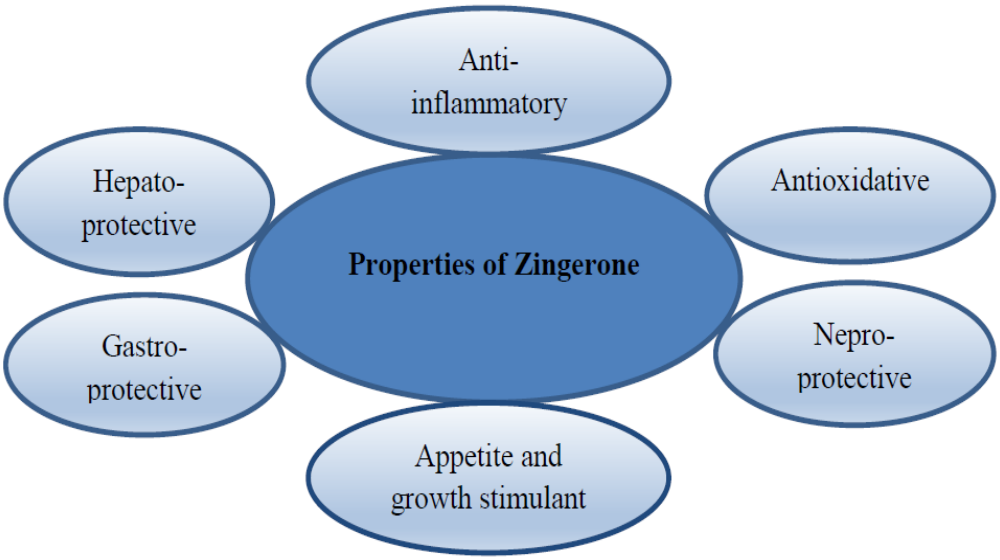

Figure 2.3: Pharmacological actions of zingerone (Source: Ahmad et al., 2015)

2.9 Pharmacological actions of zingerone

2.9.1 Anti-inflammatory activity

Inflammation is a reaction by the immune system against an injury with the aim to recover damaged tissue (Dal Pont et al., 2020). It is characterised by swelling, pain and redness. However, uncontrolled inflammatory processes are often involved in the pathology of many cardiovascular and intestinal disorders (Chen et al., 2018). Several conventional pharmacological agents are used to mitigate the effects of chronic inflammation, but often elicit severe side effects (Furman et al., 2019) hence continue to search for anti-inflammatories that induce fewer side effects (Lillehoj et al., 2018). Zingerone, in carrageenan-induced inflammation, decreased the levels of interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), cyclo-oxygenase-2 and prostaglandin (Mehrzadi et al., 2021). The IL-6 and TNF-α are pro-inflammatory cytokines that regulate the generation of inflammatory mediators. The cytokines increase the expression of cyclo-oxygenase which catalyses the conversion of arachidonic acid to prostaglandins that play a key role in inflammatory responses (Sellers et al., 2010; Wang et al., 2021). Zingerone also directly attenuated the gene expression of cyclo-oxygenase by suppressing the transcription factor, nuclear factor-kβ, that is involved in regulating the immune response (Hsiang et al., 2013). Furthermore, oxidative stress can cause inflammation and vice versa. This means that the anti-inflammatory effects of zingerone can also be effective in reducing oxidative stress.

2.9.2 Antioxidant activity

Oxidative stress occurs when the production of reactive oxygen species exceeds the processes that remove the oxidant species (Pizzino et al., 2017). It is recognised that as an activity, oxidative stress plays a central role in the progression of many diseases (Liguori et al., 2018). Oxidative stress is evidenced predominantly in biological systems by the generation of free radicals from oxygen at high concentrations which can be measured by lipid peroxidation and reactive oxidative species (Salehi et al., 2020). Zingerone has been regarded to possess antioxidant defences. It can remove free radicals that are released from the metabolism of fuel nutrients in the body (Ahmad et al., 2015). Antioxidants donate an electron to neutralize electron-gained reactions, however, antioxidants remain stable in their oxidised form, therefore are able to scavenge free radicals and protect against cell damage (Archana & Maheswari, 2016). Zingerone is believed to act as a reducing agent that donates its hydrogen atom, removes free radicals and decomposes peroxides (Mani et al., 2016). A study on rats supplemented with zingerone in their diets reported a marked decrease in lipid peroxidation but demonstrated increased activity of the antioxidant enzymes catalase, glutathione reductase and superoxide dismutase and non-enzyme antioxidants such as vitamin E which are important in removing reactive oxidative species (Mani et al., 2016). The antioxidant and anti-inflammatory properties of zingerone act to cause various protective roles in different organs.

2.9.3 Hepato-protective activity

The liver is an important organ involved in many bodily functions such as the production of bile, hormones, specific proteins and cholesterol (Kalra et al., 2022). It acts as a glucose store, it breaks down lipids and detoxifies harmful substances including drugs, alcohol and xenobiotics and conjugates bilirubin (Dutta et al., 2021). Therefore, it is important to keep a healthy liver. Liver damage is implicated in chronic alcohol abuse, non-alcoholic steatohepatitis and non-alcoholic fatty liver diseases. Zingerone has been shown in liver damage-induced rat models to decrease and reverse blood alanine aminotransferase (ALT) and aspartate aminotransferase (ASP) levels (Cheong et al., 2016; Narayanan & Jesudoss, 2016). These are liver enzymes produced in hepatocytes that are used to test liver function. Raised concentrations of these enzymes indicate that there is leakage into the systemic circulation of these enzymes from damaged hepatocytes. When zingerone was administered to carbon tetrachloride induced-liver injury in rats, it decrease AST and ALP activities and reversed the chemically-induced abnormal

liver histology (Narayanan & Jesudoss, 2016). Zingerone also decreased the concentrations of serum cholesterol and triglycerides while increasing HDL levels (Narayanan & Jesudoss, 2016) and it also downregulated the expression of TNF-alpha and TLR-4 (Kumar et al., 2014). Decreased hepatic inflammation, cholesterol and triglycerides mitigates liver fibrosis.

2.9.4 Nephro-protective activity

Kidney injuries greatly influence the welfare of chickens because the kidney carries out important functions including the excretion of metabolic wastes, regulating homeostasis and synthesis of calcitriol, renin and erythropoietin (Cook et al., 2001; de Jong et al., 2020). A common renal disease in chickens, urolithiasis, caused by uroliths, mediate progressive obstruction of the ureter (Wideman, 2016) resulting in kidney atrophy at the ureteral site and hypertrophy as compensation in the undamaged parts of the kidney (Hicham & Amine, 2021). Urolithiasis is known to increase the mortality of chickens (Hicham & Amine, 2021). Therefore, it is important to supplement poultry diets with substances that can prevent kidney injuries. Zingerone administered orally in rats has been shown to attenuate oxidative stress and inflammation in kidneys (Türk et al., 2020) and its administration decreased inflammatory mediators, caspase 3 and 9 activities and increased antioxidant enzyme activities (Alibakhshi et al., 2018). Zingerone possesses anti-inflammatory effects that can prevent oxidative stress and anti-apoptotic effects that prevent kidney atrophy.

2.9.5 Gastro-protective activity

Among the many biological activities of zingerone, is its gastrointestinal activity as shown in several studies (Karampour et al., 2019; Wu et al., 2019). It has been established that a healthy gut promotes poultry growth and development (Aruwa et al., 2021). Zingerone's antioxidant and anti-inflammatory activities are multi-effective in attenuating many diseases that can be induced by oxidative stress. Research has shown that zingerone plays a protective role against gastrointestinal diseases (Karampour et al., 2019; Wu et al., 2019). Irritable bowel syndrome (IBS) is characterised by the chronic occurrence of abdominal pain, discomfort and changes to bowel habits (Wu et al., 2019): it is an uncomfortable disorder often associated with embarrassment and stress. Water stress-induced diarrhoeal-IBS was reported to be mitigated in IBS patients treated with orally administered zingerone treatment for 21 days at 10 mg kg^{-1} and 20 mg kg^{-1} body weight (Banji et al., 2014). Similarly, oral treatment of diarrhoeal rats with

zingerone decreased faecal output and stimulated increased activities of catalase and glutathione but decreased inflammatory markers (Banji et al., 2014). These reported findings demonstrate the antioxidant and anti-inflammatory properties of zingerone that manifest its gastro-protective role in gastrointestinal disease. In addition, zingerone was shown to prevent enterotoxin from mediating the secretion of fluids into the ileum, which protected against possible fluid-mediated gut diarrhoea (Iwami et al., 2011). In the same study, zingerone inhibit colonic smooth muscles via blockage of sodium channels thus helping prevent osmotic-induced diarrhoea (Iwami et al., 2011). Research has thus clearly demonstrated the gastro-protective properties of zingerone through its anti-diarrhoeic, anti-inflammatory and antioxidant properties. Severe diarrhoea is known to cause dehydration and a concomitant loss in poultry body weight through diarrhoea thus through its anti-diarrhoeal properties, zingerone can potentially be utilised to combat diarrhoea in poultry.

2.9.6 Appetite and growth stimulating activities

Ginger is pungent and has an aromatic smell hence its use as a spice. Ginger`s taste is due to phenolic compounds it contains. Unlike whole ginger, zingerone is less pungent taste (Nutakor et al., 2020). Zingerone has appetite-stimulant properties (Ahmad et al., 2015) and is known to stimulate saliva secretion and the latter (increased salivation secretion) mediates stimuli that regulate appetite resulting in increased food consumption (Keesman et al., 2016). When supplementing feed with medicinal plants, it is important to take cognisance of the different taste preferences of different animals as there are species-dependent taste preferences (Kuralkar & Kuralkar, 2021). In studies where ginger was used as a feed supplement in broiler chicken, no significant change was reported on the feed intake of the chicken fed the ginger-based test diets compared to counterparts fed the control diet (Zhang et al., 2009). Unlike zingerone which has a less pungent, the gingerol in ginger is responsible for its more pungent taste (Kukula-Koch & Czernicka, 2021), hence the use of zingerone as a feed supplement is less likely to compromise the palatability of feed and impact feed intake.

However, despite its potential reflected in its array of health-beneficial biological activities that mirror antibiotics, no study has evaluated the effectiveness of zingerone as an alternative growth-promoting feed supplement to antibiotics in broiler chicken feeds. This study, therefore

characterised the effects of supplemental zingerone on the growth, feed use economy, meat physical properties and nutrient profile and bird health.

The following chapter focuses on the effects of supplemental zingerone on growth performance, feed utilisation efficiency, gastrointestinal tract organ macromorphometry, carcass yield and long bone indices of Cobb 500 broiler chickens.

CHAPTER THREE: EFFECT OF SUPPLEMENTAL ZINGERONE ON GROWTH PERFORMANCE, GASTROINTESTINAL TRACT ORGAN MACROMORPHOMETERY AND CARCASS YIELD OF COBB 500 BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*)

Abstract

Zingerone`s potential to substitute zinc bacitracin (ZnBcn) as a feed supplement in broiler chicken diets was evaluated by determining effects on growth, feed use efficiency, carcass yield, long bone and gastrointestinal tract viscera macromorphometry. One hundred and twenty unsexed 1-day-old Cobb 500 broiler chicks (10 chicks per replicate with 3 replicates per diet) were randomly assigned to four dietary treatments where zingerone replaced zinc bacitracin (ZnBcn) at 0 (control: 500 mg kg^{-1} of zinc bacitracin); 40; 80 and 120 mg kg^{-1} in the starter, grower and finisher diets. The broiler chickens were fed *ad libitum* for 6 weeks: 2 weeks for each of the starter, grower and finisher growth phases. Initial and weekly body mass, daily feed intake (FI), and terminal body mass (TBM) were measured. Body mass gain (BMG), average daily gain (ADG), and feed conversion ratio (FCR) were computed. On slaughter, the chickens were dressed, viscera organs extracted and their mass and the lengths of small and large intestines determined. Empty carcass mass and femora and tibiae indices were measured. The dressing percentage was computed. Across growth phases and overall, dietary zingerone had similar effects as ZnBcn on the chicken's TBM, BMG, ADG, FI and FCR, on empty carcass mass, dressing percentage, long bone indices and viscera macromorphometry. Zingerone at 40; 80 and 120 mg kg^{-1} of feed can be used as a growth promoter in place of ZnBcn in Cobb 500 broiler chicken diets without compromising growth performance, feed intake and utilisation efficiency, carcass yield, long bone and gastrointestinal tract organs growth and development.

3.0 Introduction

Growth performance is the central performance attribute that poultry breeders focus on in order to improve and increase production by commercial poultry producers (Tallentire et al., 2018). Poultry breeders intensively select poultry strains with faster growth rates, efficient feed conversation rates, and higher breast meat yield (Torrey et al., 2021). The use of genetically improved poultry breeds by sub-Saharan Africa (SSA) poultry producers has made the regional poultry industry a major contributor to animal-derived sources of protein for human consumption (Nkukwana, 2018). Further to being a major source of animal-derived protein for human consumption and helping improve household food security, the SSA poultry industry also supports livelihoods by employing a significant number of people in the region (Ayayee et al., 2020). In SSA, the demand for poultry, especially broiler chicken meat is increasing and is expected to continue increasing annually up to 2050 (Yuan & Chamber, 2020). The regional

increase in the demand for chicken meat and hence the increase in its per capita consumption (Bruinsma, 2017) is driven by the sustained growth in the human population, improvement of the socio-economic status, and expansion of urban settlements (Godfray et al., 2018). Despite the observable growth in the SSA poultry industry, especially broiler chicken meat production, the poultry industry fails to meet the demand for chicken meat, and the shortfall is met by imports (Mensah & Enahoro, 2022) from Brazil and European Union countries.

Poultry producers, in addition to using genetically improved poultry breeds, also deploy technologies that help optimize growth performance and feed utilisation efficiency and thus shorten the production cycle (Yadav & Jha, 2019). One such strategy is the supplementation of broiler and pullet chicken feeds with sub-therapeutic doses of antibiotics that act as growth promoters which enhance feed intake and utilisation efficiency (Mehdi et al., 2018). This use of antibiotics as growth promoters contribute significantly to improvement in growth performance, and meat and egg yield (Callaway et al., 2021). Zinc bacitracin, a polypeptide antibiotic, is routinely added to broiler and pullet chicken feed as a growth promoter (Attia et al., 2016). Dietary ZnBcn was reported to improve feed use economy, slaughter mass and meat yield of broiler chicken (Crisol-Martínez et al., 2017). Although the use of sub-therapeutic doses of antibiotics in poultry feeds boosts productivity, prolonged use of these AGPs has been shown to be the reason behind the development and spread of antibiotic-resistant bacteria strains that have caused antibiotic resistance, a global public health challenge (Suresh et al., 2018). Antibiotic resistance is in the top five threats to global human health and annually it causes the death of approximately 700 000 people in European countries (World Health Organization, 2019). Due to the negative effects of antibiotics used as growth promoters on the environment (Bougnom et al., 2019) as well as human and animal health, some developed countries have enacted legislation to minimise, curb and prohibit antibiotic use poultry feed supplements (Laxminarayan et al., 2015). The withdrawal of antibiotic from poultry feeds causes significant reduction in productive, product quality and enterprise profitability (Cardinal et al., 2019) which if not mitigated compromises the supply of poultry meat and eggs for human consumption. In order to prevent antibiotic-induced resistance and its associated environmental and health challenges, research is evaluating potential plant-derived alternatives that can promote and boost poultry productivity and profitability (Aitfella Lahlou et al., 2021).

Ginger (*Zingiber officinale* Roscoe), family *Zingiberaceae*, a perennial plant (Alsherbiny et al., 2019) is globally used as a medicinal plant (Mehrzadi et al., 2021). Phytochemicals such as gingerols, shogaols and zingerone impart ginger its medicinal properties (Hosseinzadeh et al., 2017). When used as a feed supplement in broiler chicken feeds, ginger stimulated improved growth performance and enhanced feed utilisation efficiency (Zhang et al., 2009). Substantial evidence demonstrates that the use of gingerol, shogaol, and paradol as feed supplements enhances chicken growth performance (Ali et al., 2019; Wen et al., 2020). However, the potential of zingerone, one of the major active compounds in ginger (Gungor et al., 2020), to fortify broiler chicken feeds as a growth promoter, has not been evaluated. In rodent models zingerone has been shown to possess anti-inflammatory, antioxidant (Mehrzadi et al., 2021), hepato-protective (Narayanan & Jesudoss, 2016), nephro-protective (Firoz et al., 2020), gastro-protective (Karampour et al., 2019), appetite and growth stimulating and immune-enhancing properties (Poornamathy & Parameswari, 2019). These health-beneficial biological properties suggest that zingerone can possibly substitute antibiotics as a growth promoter. Hence this study evaluated the potential of zingerone to substitute zinc bacitracin in broiler chicken feeds.

Hypothesis

H_0: Supplemental zingerone does not affect the growth, feed use economy, gastrointestinal tract (GIT) and accessory GIT viscera macromorphometry of Cobb 500 broiler chickens.

H_1: Supplementary zingerone affects affect the growth, feed use economy, gastrointestinal tract (GIT) and accessory GIT viscera macromorphometry of Cobb 500 broiler chickens

3.1 Materials and methods

3.1.1 Study site and ethical clearance

The feeding trial and assays of collected samples were done at the Wits Research Animal Facility (WRAF) and Wits School of Physiology laboratories, respectively. Following the granting of ethical clearance by the Animal Research Ethics Committee (ethics approval number: 2020/10/02C; for a copy of the ethical clearance certificate see appendix 2) of the University of the Witwatersrand, the feeding trial proceeded with the handling of the birds during the trial based on the Helsinki protocol for use of animals in research.

3.1.2 Feed ingredients and diet formulation

Whole yellow maize grain, grounded to a meal before use in feed formulation, was obtained from Obaro [Pretoria, South Africa (SA)]. Epol (Pty Ltd) Animal Feed Manufacturers (Johannesburg, SA) supplied the soybean meal, wheat bran, salt, dicalcium phosphate, and feed-grade limestone. Corn gluten meal-60 was purchased from Ingrain Company (Germiston, SA). The vitamin-mineral premix, synthetic lysine and methionine were purchased from Trouw Nutrition (Johannesburg, SA). Zingerone was bought from Sigma-Aldrich (Pty Ltd) (Germany) and zinc bacitracin was sourced from Zeuw Raw Material (Boksburg, SA). Canola oil was obtained from Makro Wholesalers (Johannesburg, SA). The broiler brooder/starter, grower, and finisher diets were formulated to meet the nutritional requirements of broiler chicken for the three growth stages, respectively as recommended by the National Research Council (NRC, 1994). Table 3.1 show the feed ingredients, proximate, fibre, calcium and phosphorus content and the gross energy content of the starter, grower and finisher diets, respectively.

Table 3.1: The ingredient and chemical nutrient composition of the starter, grower and finisher diets

Ingredients	Starter				Grower				Finisher			
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Yellow maize meal (g kg^{-1})	447.94	447.94	447.94	447.94	494.09	494.09	494.09	494.09	520.65	520.65	520.65	520.65
Soybean meal (g kg^{-1})	383.95	383.95	383.95	383.95	264.69	264.69	264.69	264.69	225.62	225.62	225.62	225.62
Corn gluten meal (g kg^{-1})	118.84	118.84	118.84	118.84	88.23	88.23	88.23	88.23	82.44	82.44	82.44	82.44
Wheat bran (g kg^{-1})	18.28	18.28	18.28	18.28	105.88	105.88	105.88	105.88	121.49	121.49	121.49	121.49
Canola oil (g kg^{-1})	-	-	-	-	22.94	22.94	22.94	22.94	26.03	26.03	26.03	26.03
Limestone (g kg^{-1})	20.11	20.11	20.11	20.11	14.12	14.12	14.12	14.12	13.88	13.88	13.88	13.88
DL-Methionine, 99% (g kg^{-1})	1.28	1.28	1.28	1.28	1.24	1.24	1.24	1.24	1.21	1.21	1.21	1.21
Dicalcium phosphate (g kg^{-1})	2.74	2.74	2.74	2.74	2.21	2.21	2.21	2.21	2.17	2.17	2.17	2.17
Salt (NaCl; g kg^{-1})	2.29	2.29	2.29	2.29	2.21	2.21	2.21	2.21	2.17	2.17	2.17	2.17
Vitamin and min premix (g kg^{-1})	4.57	4.57	4.57	4.57	4.41	4.41	4.41	4.41	4.34	4.34	4.34	4.34
Zinc bacitracin (mg kg^{-1})	500	-	-	-	500	-	-	-	500	-	-	-
Zingerone (mg kg^{-1})	-	40.00	80.00	120.00	-	40.00	80.00	120.00	-	40.00	80.00	120.00
Chemical nutrient composition												
Dry matter (%)	89.54	88.52	90.16	89.89	90.12	89.93	88.38	89.35	90.50	89.58	90.82	90.18
Crude protein (% DM)	27.20	28.30	30.40	31.18	21.27	25.25	24.91	23.96	21.82	22.47	23.51	23.36
Ether extract (% DM)	2.21	2.17	2.34	2.09	3.27	3.32	3.35	3.41	3.52	3.53	3.55	3.60
Crude fibre (% DM)	3.58	3.54	3.86	3.81	3.72	4.57	4.50	5.46	3.92	3.69	3.71	3.74
Calcium (% DM)	0.71	0.83	0.92	0.85	0.54	0.54	1.06	0.94	0.54	0.33	0.54	0.60
Phosphate (% DM)	0.42	0.43	0.44	0.44	0.38	0.48	0.48	0.49	0.41	0.37	0.41	0.48
Gross energy (MJ/kg DM)	18.01	18.60	18.47	18.28	18.25	18.38	18.15	18.07	18.29	18.39	18.57	18.45

Vitamin-mineral premix: each kg contained vitamin A 4000 000IU, vitamin D3 600 000IU, vitamin E 8000IU, vitamin K3 0.258g, vitamin B1 0.6g, vitamin B2 1.6g, niacin 11.94g, calcium pantothenate 3.92g, vitamin B12 0.1g, vitamin B6 0.98, choline 72.73g, folic acid 0.288g, biotin 0.0008g, $MnSO_4$ 9.92g, Zn 6.3g, Cu 0.252g, KI 0.2g, Co 0.0042g, Fe 2.1g, Se 0.0036g.

3.1.3 Animals, feeding, and housing

One hundred and twenty, one-day-old, unsexed Cobb 500 broiler chicks vaccinated against Newcastle, Marek`s and infectious bursal diseases sourced from Alfa Kuikenplaas Chicks, Pretoria, South Africa, were used in the feeding trial. The chicks were allowed a 2-day habituation period during which they were fed a plain formulated starter diet before commencement of the feeding trial. During the 2-day habituation, chicks were dewormed with piperazine (Kyron Laboratories Pty Ltd, Johannesburg, South Africa) added in drinking water at 90 mg/L. The chicks were housed in a deep litter system with clean dry wood shavings providing for bedding. Ten chicks were housed in a pen [1.7 m (L) x 1.1 m (W) x 1.3 m (H)]. Room temperature where the pens were housed was controlled at 34-29°C for the starter growth phase and 28-26°C and 25-23°C for the grower and finisher phases, respectively and relative humidity was maintained at 60-80% as recommended for Cobb 500 broiler chicken (Cobb-Vantress, 2021). A 12-hour lighting cycle was maintained with lights on from 06h00 to 18h00. During the starter growth phase, infrared lighting provided additional warmth. The chicks had *ad libitum* access to feed and clean drinking water. The starter, grower, and finisher diets were each fed for two weeks.

3.1.4 Experimental design

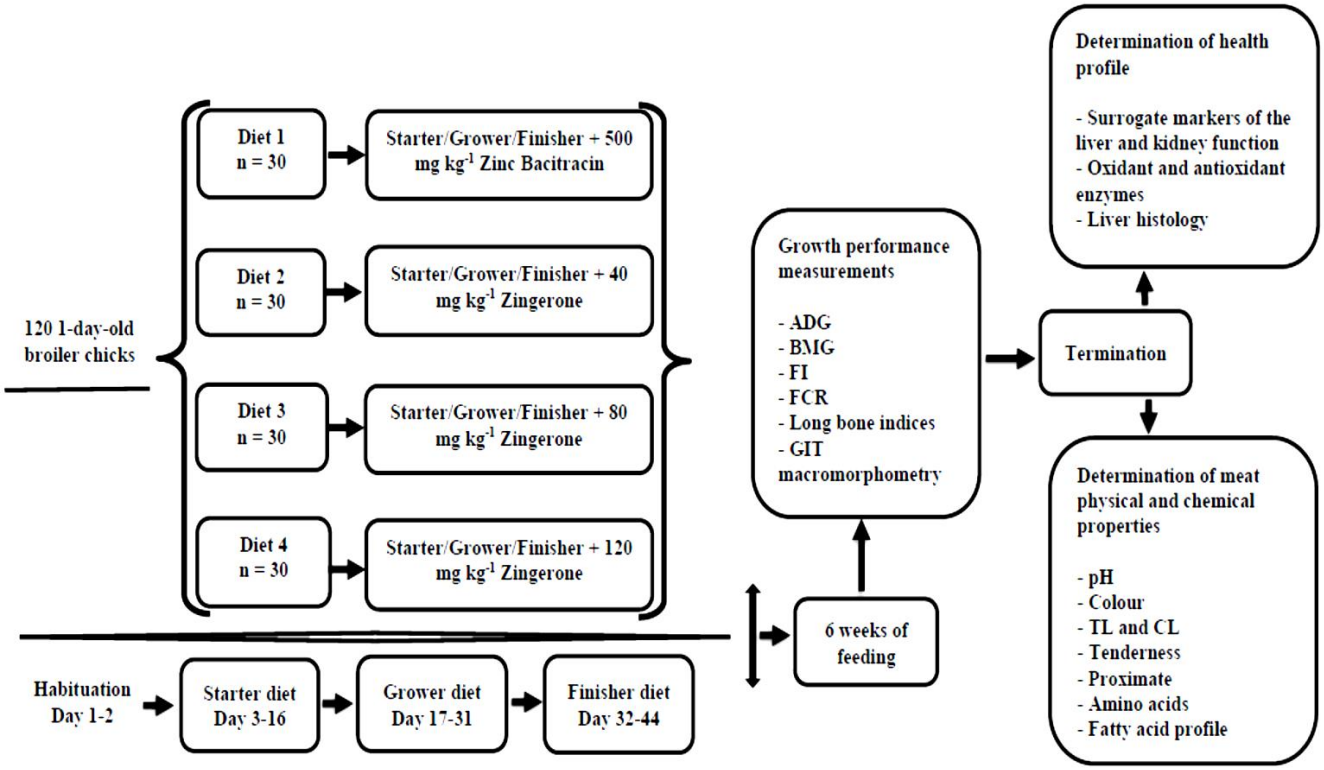

Figure 3.1: Schematic diagram of the study design

3.1.5 Measurements: body mass and feed intake

The initial body and weekly and terminal body mass and daily feed intake of the chicken were measured using an electronic scale (Waterproof Electronic Portable Scale, Clover Scales, Johannesburg, South Africa). As part of monitoring the growth and general health of the chicken, body masses were measured twice weekly. Feed intake was determined daily by subtracting refusals from the total feed given.

3.1.6 Computations

Body mass gain (BMG), average daily gain (ADG) and feed conversion ratio (FCR) were computed from the body mass and feed intake (FI) data collected in the starter, grower, and finisher phase and the overall using the following equations:

$BMG_{(starter)} = body\ mass_{(day\ 14)} - body\ mass_{(day\ 1)}$; $BMG_{(grower)} = body\ mass_{(day\ 28)} - body\ mass_{(day\ 15)}$; $BMG_{(finisher)} = body\ mass_{(day\ 42)} - body\ mass_{(day\ 29)}$ and $BMG_{(trial)} = body\ mass_{(day\ 42)} - body\ mass_{(day\ 1)}$. ADG (g) = BMG/length (in days of feeding). FI (g) = feed offered – feed refusal. FCR = feed intake (g) / mass gain (g) (Onu et al., 2004).

3.1.7 Terminal procedures, measurements and sample collection

At the end feeding trial, the chickens were fasted for 4 hours but with *ad libitum* access to clean drinking water and then killed. Each chicken was humanely decapitated with a guillotine (Harvard Apparatus, Holliston, Massachusetts, United States), blood collected into heparinised blood collection tubes (Vacuette, Greiner Bio-One, Frickenhausen, German), feathers plucked off and the carcass dissected through a midline incision using a pair of scissors. Gastrointestinal tract (GIT) organs and the pancreas masses of each chicken were measured on an electronic scale (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa). Small and large intestines lengths were measured using a ruler attached to the cooled dissection board. Digesta from each GIT organ was gently removed before weighing each GIT organ. Each dressed carcass had the hot carcass mass measured on an electronic scale (Waterproof Electronic Portable Scale, Clover Scales, Johannesburg, South Africa).

3.1.8 Determination of long bone indices

The right hind leg was removed from each carcass, defleshed and then the femur and tibia were

separated. The femur and tibiae masses were then measured on an electronic scale (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa). After determining bone masses, the length of the tibia and femur bones were measured using a digital Vernier Calliper (Major Tech, Johannesburg, South Africa). The bone mass:length ratio of each of the long bones was determined as described by Seedor et al. (1991) using the equation: bone mass:length ratio (mg/mm) = dry mass (mg) / bone length (mm).

3.1.9 Data analysis

Data are presented as mean ± SD. Data were analysed using GraphPad Prism 8 statistical software was used to analyse data. Data on the effects of zingerone on weekly feed intake, body mass gain and FCR were analysed using repeated measures of one-way ANOVA. Differences between the treatment means were determined using Tukey's *post hoc* test. Statistical significance was set at $p < 0.05$.

The statistical model used for the one-way ANOVA:

$Y_{ij} = \mu + T_i + e_{ij}$, where:

Y_{ij} = dependent variable of interest (weekly feed intake, body mass gain and feed conversion ratio).

μ = overall mean effect

T_i = effect of the dietary treatment (i = 1, 2..., 4)

e_{ij} = random residual error

Parametric data on the overall feed intake, overall body mass gain, overall feed conversion ratio, meat yield, long bone indices, and GIT macromorphometry were analysed using the one-way ANOVA. Mean comparison were determined by Tukey's *post hoc* test. Statistical significance was set at $p < 0.05$.

The linear statistical model used was as follows:

$Y_i = \mu + T_i + e_i$; where;

Y_i = dependent variable of interest (overall feed intake, overall body mass gain, overall feed conversion ratio, meat yield, long bone indices and GIT macromorphometry)

μ = overall mean to all observations

T_i = effect of dietary treatment (i = 1, 2...4)

e_i = residual random error

3.2 Results

3.2.0 Performance indices and feed use economy

3.2.1 Effects on body mass based indices, feed intake and utilisation efficiency

In the present study, there was no mortality throughout the course of the feeding trials. The effect of substituting zinc bacitracin with graded levels of zingerone on Cobb 500 broiler chicken in the starter, grower, finisher and trial performance is presented in Table 3.2. There were no differences in the induction body masses. Dietary zingerone at 40, 80 and 120 mg kg^{-1} feed had similar effects ($p > 0.05$) as ZnBcn on terminal body mass (TBM). In the starter growth phase (days 1–14) of the feeding trial, dietary zingerone had similar effects ($p > 0.05$) as a control on the BMG, ADG, FI, and FCR. Supplemental zingerone had similar effects ($p > 0.05$) as ZnBcn on the BMG, ADG, FI, and FCR in the grower growth phase (days 15–28) of the experiment. In the finisher growth phase (days 28–42), supplemental zingerone had similar effects ($p > 0.05$) as a control diet on the BMG, ADG, FI, and FCR. The trial BMG, ADG, FI and FCR of the broiler chickens were similar across the dietary treatments.

Table 3.2: Effect of substituting zinc bacitracin with zingerone on growth performance and feed utilisation efficiency of Cobb 500 broiler chickens

Parameter	Growth phases	Dietary treatments				Significance level
		Diet 1	Diet 2	Diet 3	Diet 4	
Initial mass (g)		61.13 ± 1.06	60.25 ± 2.12	60.72 ± 3.84	61.27 ± 1.78	ns
Terminal body mass (g) [42 days]		2130.57 ± 167.19	2374.38 ± 188.06	2272. 18 ± 250.22	2180.75 ± 165.71	ns
Body mass gain [BMG] (g)	Starter (days 1–14)	303.76 ± 15.73	340.94 ± 14.51	331.32 ± 38.28	336.92 ± 80.85	ns
	Grower (days 15–28)	719.09 ± 94.87	804.84 ± 134.08	778.31 ± 95.98	708.60 ± 45.03	ns
	Finisher (days 29–42)	1046.65 ± 189.69	1169.09 ± 157.89	1101.80 ± 178.30	1074.05 ± 174.00	ns
Total BMG		**2069.49 ± 167.83**	**2314.18 ± 187.90**	**2211.48 ± 246.92**	**2119.57 ± 165.92**	**ns**
Average daily gain [ADG] (g/d)	Starter (days 1–14)	21.69 ± 1.12	24.35 ± 1.04	23.68 ± 2.73	24.07 ± 5.78	ns
	Grower (days 15–28)	51.36 ± 6.78	57.49 ± 9.58	55.59 ± 6.85	50.61 ± 3.21	ns
	Finisher (days 29–42)	74.76 ± 13.55	79.94 ± 11.28	78.70 ± 12.74	76.72 ± 12.43	ns
ADG (Trial)		**49.27 ± 3.99**	**55.10 ± 4.43**	**52.65 ± 5.88**	**50.47 ± 3.95**	**ns**
Feed intake [FI] (g)	Starter (days 1–14)	830.07 ± 89.08	832.40 ± 89.88	836.79 ± 91.13	829.09 ± 79.48	ns
	Grower (days 15–28)	1577.80 ± 188.62	1698.39 ± 223.07	1621.18 ± 235.58	1585.49 ± 191.39	ns
	Finisher (days 29–42)	3027.40 ± 264.69	3058.88 ± 251.18	3050.66 ± 246.26	2979.30 ± 323.49	ns
Total FI		**5435.30 ± 542.30**	**5589.58 ± 562.80**	**5508.69 ± 569.75**	**5394.00 ± 592.70**	**ns**
Feed conversion ratio (FCR)	Starter (days 1–14)	2.74 ± 0.33	2.44 ± 0.18	2.55 ± 0.42	2.59 ± 0.87	ns
	Grower (days 15–28)	2.23 ± 0.44	2.12 ± 0.19	2.08 ± 0.22	2.24 ± 0.31	ns
	Finisher (days 29–42)	2.93 ± 0.32	2.75 ± 0.19	2.79 ± 0.25	2.79 ± 0.19	ns
FCR (Trial)		**2.63 ± 0.08**	**2.41 ± 0.07**	**2.49 ± 0.09**	**2.54 ± 0.14**	**ns**

ns = not significant, $p > 0.05$. Supplemental zingerone had a similar effect ($p > 0.05$) as the control diet on growth performance, feed intake, feed conversion ratio and meat yield in the starter, grower and finisher phases and overall trial across the dietary treatments. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

3.2.2 Effect of supplemental zingerone on meat yield

Table 3.3 below shows the effects of dietary zingerone on slaughter body mass, carcass mass and percentage dressing of Cobb 500 broiler chickens. Dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on the slaughter body mass, empty carcass mass, and dressing percentage of broiler chickens.

Table 3.3: Effect of substituting zinc bacitracin with zingerone on meat yield of Cobb 500 broiler chickens

Parameter	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Slaughter body mass (g)	2189.71 ± 199.80	2417.27 ± 178.79	2322.52 ± 245.96	2214.83 ± 156.07	ns
Empty carcass mass (g)	1636.02 ± 222.69	1867.58 ± 168.87	1813.10 ± 187.82	1708.04 ± 113.24	ns
Dressing percentage (%)	77.59 ± 1.50	78.89 ± 2.25	78.40 ± 1.43	77.03 ± 0.82	ns

ns = not significant, $p > 0.05$. Supplemental zingerone has similar effects as the control diet ($p > 0.05$) on the birds' slaughter mass gain, empty carcass mass and dressing percentage. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

3.2.3 Effect of supplemental zingerone on long bone indices

Table 3.4 below shows the effects of dietary zingerone on the tibiae and femora masses, lengths and mass/length ratio of Cobb 500 broiler chickens. Dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on the tibiae and femora masses and lengths as well as the mass/length ratio of the broiler chickens.

Table 3.4: Effect of substituting zinc bacitracin with zingerone on the tibia and femur masses and lengths as well as mass/length ratio of Cobb 500 broiler chickens

Parameter	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Tibia					
Mass (mg)	656.47 ± 24.02	709.60 ± 72.83	706.30 ± 69.11	632.33 ± 10.63	ns
Length (mm)	97.73 ± 0.31	96.67 ± 0.50	98.73 ± 0.31	96.67 ± 1.70	ns
Mass/length ratio (mg/mm)	6.72 ± 0.22	7.34 ± 0.72	7.70 ± 0.72	6.54 ± 0.18	ns
Femur					
Mass (mg)	526.13 ± 28.23	575.73 ±71.33	571.70 ± 55.05	505.27 ± 15.74	ns
Length (mm)	70.67 ± 1.47	71.06 ± 0.64	72.70 ± 0.63	72.53 ± 2.84	ns
Mass/length ratio (mg/mm)	7.44 ± 0.55	8.10 ± 0.96	7.87 ± 0.81	6.97 ± 0.26	ns

ns = not significant, $p > 0.05$. Supplemental zingerone had similar effects ($p > 0.05$) as the control diet on the tibia and femur masses, lengths and mass/length ratio of broiler chicken across the dietary treatments. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

3.2.4 Effects on GIT viscera macromorphometry

The effects of dietary zingerone on GIT organ and GIT accessory organ masses and small and large intestine lengths are shown in Table 3.5. Zingerone had similar effects ($p > 0.05$) as ZnBcn on the absolute and relative masses of the ventriculi, proventriculi, small and large intestines, caeca, liver, pancreata, visceral fat and the lengths of the small and large intestines.

Table 3.5: Effect of substituting zinc bacitracin with zingerone on gastrointestinal organ and accessory organ masses and lengths of Cobb 500 broiler chickens

	Dietary treatments				Significance
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Level**
Heart (g)	11.18 ± 1.14	11.11 ± 0.32	11.50 ± 1.03	10.29 ± 1.11	ns
(% body mass)	0.49 ± 0.03	0.45 ± 0.02	0.49 ± 0.04	0.46 ± 0.05	ns
Liver (g)	37.19 ± 2.29	40.97 ± 2.12	42.25 ± 4.42	37.65 ± 3.8	ns
(% body mass)	1.73 ± 0.15	1.67 ± 0.08	1.80 ± 0.02	1.68 ± 0.15	ns
Pancreas (g)	4.10 ± 0.52	3.96 ± 0.22	4.02 ± 0.53	3.83 ± 0.10	ns
(% body mass)	0.18 ± 0.02	0.16 ± 0.01	0.17± 0.03	0.17 ± 0.02	ns
Proventriculus (g)	8.80 ± 0.67	9.45 ± 1.47	9.07 ± 0.36	8.70 ± 0.62	ns
(% body mass)	0.39 ± 0.03	0.41 ± 0.12	0.40 ± 0.06	0.39 ± 0.01	ns
Ventriculus (g)	36.78 ± 2.45	39.20 ± 3.35	39.74 ± 0.97	37.52 ± 1.85	ns
(% body mass)	1.65 ± 0.06	1.67 ± 0.09	1.74± 0.18	1.69 ± 0.05	ns
Small intestines (g)	42.87 ± 2.71	47.89 ± 2.67	46.47 ± 2.99	42.74 ± 3.86	ns
(% body mass)	1.95 ± 0.19	1.97 ± 0.13	2.02 ± 0.23	1.93 ± 0.21	ns
Small intestines length (mm)	1585.90 ± 140.87	1688.29 ± 130.06	1674.75 ± 71.75	1601.10 ± 99.73	ns
Large intestines (g)	3.45 ± 0.53	3.78 ± 0.41	3.49 ± 0.14	3.28 ± 0.07	ns
(% body mass)	0.15 ± 0.02	0.16 ± 0.03	0.15 ± 0.02	0.15 ±0.01	ns
Large intestines length (mm)	111.13 ± 5.57	111.91 ± 4.11	113.52 ± 7.35	110.61 ± 1.15	ns
Caecum (g)	6.25 ± 0.58	7.44 ± 0.48	6.69 ± 0.58	0.58 ± 0.73	ns
(% body mass)	0.28 ± 0.01	0.32 ± 0.02	0.29 ± 0.04	0.31 ± 0.02	ns
Spleen (g)	2.19 ± 0.25	2.34 ± 0.30	2.07 ± 0.16	2.38 ± 0.36	ns
(% body mass)	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.11 ± 0.01	ns

Visceral fat (g)	35.88 ± 3.19	42.18 ± 5.22	45.73 ± 1.05	39.85 ± 5.98	ns
(% body mass)	1.62 ± 0.04	1.72 ± 0.09	1.95 ± 0.27	1.76 ± 0.15	ns

ns = not significant, $p > 0.05$. Supplemental zingerone had similar effects ($p > 0.05$) as the control diet on viscera macro-morphometry of broiler chicken across the dietary treatments. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

3.3 Discussion

3.3.1 Growth performance

Growth performance, a function of body mass gain with which feed is converted to mass as measured by the feed conversion ratio (Nogueira et al., 2019; Zampiga et al., 2021). Findings from this study show that dietary zingerone used in place of ZnBcn had similar effects on terminal body mass, BMG, ADG, FI, and FCR by growth phase and combined trial performance. It also had a similar impact on meat yield as the zinc bacitracin-based control diet. The similarities in the terminal body mass, BMG, ADG, FI, and FCR by growth phase and trial across dietary treatments indicate that zingerone neither improved nor compromised growth performance and feed utilisation efficiency. In fact, the performance of the broiler chicken, across the three growth phases and combined trial performance, was similar to that achieved with zinc bacitracin as a growth promoter demonstrating that zingerone can be used as an alternative growth-promoting supplement in Cobb 500 broiler chicken feeds. It has been shown that supplementing broiler chicken feeds with ginger, the source of zingerone, improved BMG, ADG, and FCR but had a similar effect on FI as the control (Zhang et al., 2009; Habibi et al., 2014; Qorbanpour et al., 2018). The reported improvement in BMG and ADG could have been due to the synergist effects of several (gingerol, shogaol, and paradol) phytochemicals in ginger (Ahmad et al., 2015; Raza et al., 2016) in addition to zingerone. Therefore, it is contended that when administered alone with zingerone might not stimulate enhanced growth performance. Although that could have been the case, it is important to note that zingerone in place of zinc bacitracin did not compromise growth performance and feed utilisation efficiency thus broiler chicken productivity and efficiency can be maintained with the use of zingerone as a growth promoter in place of zinc bacitracin, an antibiotic growth promoter. Based on the findings, it is contended that zingerone can be used to replace antibiotics as growth promoters in broiler chicken production and thus offers a window of opportunity to mitigate the challenges and costs of antibiotic resistance and environmental pollution when antibiotics are used at sub-therapeutic doses as growth-stimulating supplements in broiler chicken production.

3.3.2 Meat yield

Meat yield is a critical production efficiency indicator for broiler chicken as well as in other poultry species. Proposed and potential dietary interventions, while perhaps providing a solution to the challenges of antibiotic resistance and environmental pollution must not negatively impact the product yield, in this case, meat yield. Qorbanpour et al. (2018) and

Barazesh et al. (2013) showed that fortifying broiler chicken diets with graded inclusion levels of powdered ginger had a similar effect on empty carcass mass and dressing percentage. Similarly, Onu (2010) did not observe any significant effect on slaughter traits of broiler chicken from supplementing broiler chicken with ginger. Findings from the current study showed that supplemental zingerone had similar effects as ZnBcn on the slaughter and empty carcass masses and the dressing percentage of the broiler chicken. These findings suggest that in addition to its potential to be used as a growth promoter without loss in growth performance and feed utilisation efficiency by broiler chicken, zingerone can also be used to replace zinc bacitracin without risking a reduction in meat yield.

3.3.3 Long bone indices

Skeletal system integrity is vital in poultry production (Zuidhof et al., 2014). Selection of broiler chicken strains based on rapid mass gain results in skeletal and leg deformities due to quicker deposition of muscles on a poorly developed skeletal system (Shim et al., 2012). Tibiae and femora problems are major issue that affects broiler chickens` health and welfare resulting in economic loss in the poultry industry (Nakhon et al., 2019). Thus bone mineralisation is a key component to make bones harder and enables the skeleton to support and protect the body mass of the birds (Guo et al., 2020). In poultry production, body mass is a critical attribute that is used to evaluate the physical growth performance of broiler chickens (Mortensen et al., 2016). However, body mass is an inaccurate tool for assessing growth since it can be influenced by gut fill, hydration and viscera organ mass (Borga et al., 2018) and does not take into account the distribution and proportions of lean and fat weight (Shuster et al., 2012). Consequently, the growth and development of long bone indices such as tibiae and femora have been shown to be a more accurate proxy for assessing growth and health in chickens (González-Cerón et al., 2015). Long bone weight and length are important parameters of chicken bone health and have an essential impact on actual production (Li et al., 2021). In the present study, fortifying broiler chicken diets with zingerone had similar effects as ZnBcn on the tibiae and femora masses, lengths and mass-to-length ratio across dietary treatments. These findings suggest that zingerone can effectively substitute ZnBcn in poultry diets without compromising the growth and development of broiler chicken long bone indices.

3.3.4 Gastrointestinal organs macromorphometry

The GIT is the first point of contact between the animal and its diet and GIT organs and GIT accessory organs are critical to nutrient digestion and absorption (Adedokun & Olojede,

2019). Feed composition has been shown to impact the growth and development of the GIT (Ravindran & Reza Abdollahi, 2021). In birds, for instance, phytochemicals have been shown to stimulate digestion (Kiarie & Mills, 2019). Fortifying broiler chicken feeds with cinnamaldehyde has been reported to enhance the growth of GIT organs and GIT accessory organs (Ali et al., 2021). The findings of the present study show that zingerone had similar effects as ZnBcn on the masses of the proventriculi, ventriculi, small and large intestines, caeca, the lengths of the small, and intestines and the masses of the pancreata and livers of the broiler chicken. This finding is in tandem with the reported lack of effect on broiler chicken viscera reported by Onu (2010) when ginger was used to fortify broiler chicken feed. The fundamental message from the present study findings is that fortifying broiler chicken diets with zingerone did not compromise the growth and development and physiological function of GIT organs and the pancreata and livers of broiler chickens, especially in view of similarities in growth performance and feed utilization between zingerone-fed and ZnBcn-fed chickens.

3.4 Conclusion

In conclusion, findings from this study show that zingerone can be used to replace the antibiotic as a growth promoting feed supplement in Cobb 500 broiler chicken feeds without altering growth performance, feed utilisation efficiency, meat yield, tibia and femora indices and GIT viscera organs. Importantly and interestingly, even the lowest dietary inclusion level of zingerone at 40 mg kg^{-1} of feed, is equally effective as a substitute to zinc bacitracin which can be possibly deployed in broiler chicken feeds and contribute to reduced costs of fortifying broiler chicken feeds.

The following experimental chapter gives a narrative on the effects of supplemental zingerone on meat quality traits of Cobb 500 broiler chickens.

CHAPTER FOUR: EFFECT OF SUPPLEMENTAL ZINGERONE ON MEAT QUALITY ATTRIBUTES OF COBB 500 BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*)

Abstract

Zingerone`s potential to substitute ZnBcn as a feed supplement in Cobb 500 chicken diets was evaluated by determining its effects on broiler chicken breast and thigh meat`s physical quality traits and breast meat chemical nutrient content. One hundred and twenty 1-day-old Cobb 500 chicks were randomly assigned into 4 diets, each having 3 replicates of 10 chicks where zingerone replaced zinc bacitracin (ZnBcn) at 0 (control: 500 mg kg^{-1} of ZnBcn); 40; 80 and 120 mg kg^{-1} in the starter, grower and finisher diets. The broiler chicks were fed *ad libitum* for 6 weeks: 2 weeks for each of the starter, grower and finisher growth phases. On day 42, the chickens were humanely slaughtered and dressed. The chicken thigh meat`s initial and ultimate pH (pH_i and pHu) and colour were determined and its breast meat's pH_i and pH_u, colour, thawing loss (TL), cooking loss (CL), tenderness, proximate content and amino acid and fatty acid profiles were determined. Supplemental zingerone had similar effects as the control diet on the broiler chicken thigh meat`s pH_i, pH_u and colourimetric coordinates. Dietary zingerone had similar ($p > 0.05$) effects as ZnBcn on the broiler chicken breast meat's pH_i, pH_u, CL, TL and tenderness but at 40 mg kg^{-1} feed (diet 2) it increased the meat's redness (a^*). Dietary zingerone had a similar effect as ZnBcn on the meat's crude protein content ($p > 0.05$) but significantly increased its ash and fat contents ($p < 0.01$; $p < 0.0001$). Meat from chickens fed diet 2 (40 mg kg^{-1} feed zingerone) had the highest concentration of essential amino acids ($p < 0.05$) while that from chickens fed diet 3 (80 mg kg^{-1} feed zingerone) had the lowest ($p > 0.001$) total amino acid content. Dietary zingerone had a similar ($p > 0.05$) effect as ZnBcn on the meat's total saturated fatty acid content which ranged from 29.02 to 29.37%. Meat from chickens fed diets 3 and 4 (80 mg kg^{-1} feed and 120 mg kg^{-1} feed zingerone, respectively) had significantly increased ($p < 0.0001$) total monounsaturated fatty acid, oleic acid, total polyunsaturated fatty acid and linoleic acid contents and PUFA/SFA ratio. Dietary zingerone at 40 mg kg^{-1} feed can be used to enhance consumer acceptability of broiler chicken breast meat since it increased its redness and at 80 and 120 mg kg^{-1} feed it enhanced the nutritional value of the meat since it mediated an increase in the desirable fatty acids.

4.0 Introduction

Nowadays consumers demand high-quality and antibiotic-free poultry meat and eggs (Van Niekerk et al., 2020). Globally, chicken meat is the most consumed type of meat when compared to red meats due to its relative affordability, high nutrient content and lower caloric value (Gou et al., 2020). However, the accrual of antibiotic residues in chicken meat portions has raised public concerns and triggered many countries to prohibit the use of antibiotics in

poultry production (Thi Huong-Anh et al., 2020). The consumer-driven demand for use of environmentally friendly technologies in the food production chain has seen an increase in the evaluation of the potential of phytochemicals to replace antibiotics as feed supplements in poultry production (Ali et al., 2021). Phytochemicals are natural compounds that are deemed less toxic when used in small proportions or doses and can potentially lower production costs (Akbarian et al., 2016). Ginger, a rhizome containing a high proportion of bioactive compounds (Mao et al., 2019) such as gingerol, shogoal and zingerone (Wen et al., 2020) exerts strong health-beneficial biological properties (Alsherbiny et al., 2019). Supplementation of broiler chicken diets with ginger powder has been reported to enhance the growth performance as well as the physical and chemical quality traits of chicken meat (Zhang et al., 2009). Gingerol, one of the phytochemical constituents of ginger has also been shown to enhance chicken meat quality traits (Wen et al., 2020).

Zingerone, a less pungent ginger-derived phytochemical (Sahoo et al., 2022) exhibits antioxidant, antibacterial and anti-inflammatory properties (Alibakhshi et al., 2018). Plant-derived dietary supplements have been shown to impact meat quality (Tashla et al., 2020; Goliomytis et al., 2015). Phytochemical-based feed additives have been shown to improve broiler chicken meat colour (Goliomytis et al., 2015), enhance the meat's favourable fatty acid content (Kamboh & Zhu, 2013) and the tenderness (Qaid et al., 2022). However, despite its health-beneficial biological activities, the effects of zingerone on broiler chicken meat quality have not been interrogated. Thus, in this part of the study, the effect of dietary supplemental zingerone on the physical quality traits and nutrient content of Cobb 500 broiler chicken breast and thigh meat was evaluated.

Hypothesis

H_0: Supplemental zingerone does not affect the physico-chemical properties (pH, colour, TL, CL, tenderness, proximate and amino acid content and fatty acid profile) of the breast meat and physical properties (pH, colour, TL, CL and tenderness) of the thigh meat from Cobb 500 broiler chicken.

H_1: Supplementary zingerone affects the physico-chemical properties (pH, colour, TL, CL, tenderness, proximate and amino acid content and fatty acid profile) of the breast meat and physical properties (pH, colour, TL, CL, tenderness) of the thigh meat from Cobb 500 broiler chicken.

4.1 Material and methods

4.1.1 Study site and ethical clearance

The study site and ethical clearance are as previously stated in Chapter Three, subheading 3.1.1.

4.1.2 Feed ingredients and diet formulation

The sourcing of dietary ingredients and diet formulation are as previously stated in Chapter Three, subheading 3.1.2.

4.1.3 Animals, feeding, and housing

The sourcing of feed ingredients and diet formulation are as previously stated in Chapter Three, subheading 3.1.2.

4.1.4 Experimental design

The experimental design is as previously stated in Chapter Three, subheading 3.1.4.

4.1.5 Terminal procedures, measurements and sample collection

In addition to the terminal procedure described in Chapter three (3.1.7), the left breast and thigh meat of each carcass was collected and used for determining the physical properties of meat at 30 minutes and 24 hours post-slaughter. The right breast muscle was used to determine the chemical nutrient (proximate, amino acid and fatty acid) content of the meat.

4.1.6 Determination of the physical traits of meat

Thirty (30) minutes post-slaughter the initial pH (pH_i) of the breast and thigh meat was measured using a digital pH meter (Crison pH25, Allena, Spain) fitted with a piercing electrode. Prior to the measurements, the digital pH meter was subjected to a three-point calibration at pH 4.01, pH 7.00 and pH 9.21 using standard solutions provided by the pH meter supplier as per the manufacturer`s instruction. Immediately after the measurement of pH_i, the colour parameters [lightness (L*), redness (a*) and yellowness (b*)], Chroma and Hue of the breast and thigh meat were measured using a Lovibond colour meter (LC 100 Spectrophotometer, LASEC, South Africa) as recommended by the Commission International De I' Eclairage Colorimetry (1976). Following storage of the breast and thigh meat sample at 4°C for 24 hours, the ultimate pH (pH_u) and meat colour were measured.

A total of fifteen breast meat samples were randomly selected per dietary treatment group to determine thawing loss (TL) and cooking loss (CL) as described by De Marchi et al. (2011). Briefly, the whole breast samples stored at -20°C were weighed, defrosted at room temperature for approximately 8 hours, then blotted dry and weighed again using an electronic balance. The TL was computed using the equation:

$$\text{TL}\ (\%) = \frac{\text{mass of frozen meat sample (g)} - \text{mass of thawed meat sample (g)}}{\text{mass of frozen meat sample (g)}} \text{ x } 100$$

Thereafter the thawed meat samples were cooked in self-seal plastic bags in a water bath (Julabo PURA a30, Gerhard–Juchheim-Strasse, Seelbach, Germany) until an internal temperature of 75°C for 60-min and the CL was determined using the equation:

$$\text{CL}\ (\%) = \frac{\text{mass of thawed meat sample (g)} - \text{mass of cooked meat sample (g)}}{\text{mass of thawed meat sample (g)}} \text{ x } 100$$

Following the determination of CL, the breast meat samples were cooled at room temperature and meat tenderness was determined using Warner-Bratzler Shear Force (WBSF) at the Meat Science Laboratory at the University of Fort Hare, Alice, South Africa. The measurements were done according to the guidelines of the American Meat Science Association (1995), where three sub-cuts of approximately 12.5 mm core diameter were extracted from each breast sample parallel to the long axis of the muscle fibre. The cored sub-cuts of the cooked breast meat were sheared once through the midpoint at a perpendicular angle to the fibre direction using WBSF mounted on a Universal Instron machine (Model 4301, Instron Corporation, Massachusetts, United States) at a crosshead speed of 200 mm/min with a 1 kN load cell. The results of the shear force cut were recorded on the computer screen mounted to the Universal Instron machine.

4.1.7 Determination of the nutrient content of the meat

The breast meat proximate, amino acid, mineral and fatty acid content were determined at the Analysis Laboratory at ARC-Irene Analytical Service, Pretoria and Nutrilab at the University of Pretoria.

4.1.7.1 Proximate content

The proximate components: crude protein (CP), ether extracts (EE), ash and fatty acid profile of the breast meat were determined from freeze-dried breast meat samples. Freeze-dried meat samples from each dietary treatment group were pooled, milled and then sub-sampled three times for each chemical nutrient test thus allowing for each test to be done in triplicate. The breast meat crude protein, ether extract and ash contents were determined as described by the Association of Analytical Chemistry (AOAC, 2006; method numbers: 940.05. 988.05 and 920.39, respectively).

4.1.7.2 Amino acid content

The amino acid content of the breast meat was determined as described by Einarsson et al. (1983). Briefly, the meat samples were hydrolysed in 6M HCl at 110°C for 24 hours. This was followed by pre-column fluorescence derivatisation of amino acids with 9-flourenylmethyl chloroformate. The amino acids were then extracted with pentane and separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary high-performance liquid chromatograph system (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a SpectraSystem FL3000 fluorescence detector (Thermo Fisher Scientific Inc.) and a Rheodyne 7125 valve (IDEXX Corp., Rohnert Park, CA, USA) with a 20 μL injection loop. A concave curve from sodium citrate buffer (pH 2.95) acetonitrile (70:30) to sodium citrate buffer (pH 4.5) methanol-acetonitrile (14:6:70) and a flow rate of 1.4 ml min^{-1} was used to mix the eluents. The amino acids were separated using an OmniSper 5 C18 150 × 4.6 analytical column and guard column (Varian Australia Pty Ltd, Perth, Australia). Identification of the amino acids was done at an excitation wavelength of 264 nm and an emission wavelength of 340 nm. A PC equipped with TSP software was used for quantification.

4.1.7.3 Fatty acid profile

The Soxhlet method was used to extract the oil from the breast meat samples as described by the Association of Official Analytical Chemistry (AOAC, 2005; method number 920.39) and the meat's fatty acid profile was determined as described by Christopherson and Glass (1969). Briefly, the fat extracts were transmethylated with 2 M methanol sodium hydroxide. The resulting fatty acid methyl esters were extracted using heptane and then filtered using Nylon syringe filters and dried under nitrogen after which they were separated by a temperature gradient over 45 minutes on a gas chromatograph with nitrogen as carrier gas on a DB-23 capillary column (90 cm × 250 μm × 0.25 μm; Supelco, Sigma-Aldrich). The gas

chromatograph consisted of an HP6890 GC (Hewlett Packard, Bristol, UK) with a flame ionisation detector. Both the detector and injector temperatures were set at 300°C. A personal computer equipped with Chemstation software (Agilent Technologies Inc., Santa Clara, CA, USA) was used for quantification.

4.1.8 Data analysis

The data was analysed using GraphPad Prism 8 software (Graph-Pad Software Inc., San Diego, CA, USA). Multiple-group parametric data on the breast and thigh meat physical traits and breast meat chemical traits were analysed using the one-way ANOVA. The differences between the treatment means were determined using Tukey's *posthoc* test. Statistical significance was set at $p < 0.05$.

The statistical model used for the one-way ANOVA:

$Y_{ij} = \mu + T_i + e_{ij;}$ where,

Y_{ij} = dependent variable of interest (meat pH, colour, TL, CL, tenderness, proximate, amino acid content and fatty acid content)

μ = overall mean effect

T_i = is the fixed effect of the i^{th} dietary treatments (i = 1, 2..., 4)

e_{ij} = random residual error

4.2 Results

4.2.0 Meat quality

4.2.1 Effect on physical attributes of breast and thigh meat

Tables 4.1 and 4.2 below show the effect of substituting zinc bacitracin with zingerone on the initial and ultimate pH and colour coordinates of the breast and thigh meat, respectively from Cobb 500 broiler chickens. Dietary zingerone had similar ($p > 0.05$) effects as ZnBcn on the pH_i and pH_u of the breast meat from broiler chickens and at 30 minutes post-slaughter. It also had similar ($p > 0.05$) effects as the ZnBcn-based control diet on the colour coordinates of the breast meat from carcasses of broiler chicken. However, at 24 hours post-slaughter supplementing broiler chicken diets with zingerone at 40 mg kg^{-1} of feed increased the redness (a*, $p < 0.05$) of the breast meat compared to that from control counterparts fed the ZnBcn-fortified diet. Dietary zingerone had similar effects as ZnBcn on the initial and ultimate pH and colour coordinates of thigh meat from broiler chickens.

Table 4.1: Effect of substituting zinc bacitracin with zingerone on the pH and colour of breast meat from Cobb 500 broiler chickens

Parameter	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
30 mins post-slaughter					
Initial pH (pH_i)	6.74 ± 0.24	6.63 ± 0.29	6.67 ± 0.14	6.78 ± 0.24[a]	ns
Lightness (L*)	45.71 ± 1.29	45.89 ± 2.15	50.64 ± 8.08	48.72 ± 4.21[a]	ns
Redness (a*)	1.23 ± 0.45	1.63 ± 0.33	1.54 ± 0.32	1.36 ± 0.33[a]	ns
Yellowness (b*)	13.26 ± 1.22	12.45 ± 0.42	12.58 ± 1.01	13.83 ± 0.83[a]	ns
Chroma (C*)	13.48 ± 1.04	12.62 ± 0.43	12.72 ± 0.95	13.39 ± 0.82[a]	ns
Hue (H*)	94.39 ± 2.21	96.67 ± 3.25	96.48 ± 2.47	94.71 ± 2.92[a]	ns
24-hrs post-slaughter					
Ultimate pH (pH_u)	5.74 ± 0.12	5.79 ± 0.15	5.73 ± 0.05	5.77 ± 0.11	ns
Lightness (L*)	55.39 ± 3.61	54.15 ± 2.13	55.08 ± 3.09	54.27 ± 2.76	ns
Redness (a*)	1.11 ± 0.34[a]	1.67 ± 0.12[b]	1.36 ± 0.07[ab]	1.44 ± 0.13[ab]	*
Yellowness (b*)	19.15 ± 0.61	18.34 ± 1.13	18.33 ± 0.75	19.07 ± 0.64	ns
Chroma (C*)	19.18 ± 0.62	18.41 ± 1.14	18.42 ± 0.76	19.24 ± 0.77	ns
Hue (H*)	89.53 ± 1.13	90.95 ± 0.95	90.66± 2.86	89.09 ± 1.22	ns

ns = not significant, $p > 0.05$, *$p < 0.05$. [ab] Within row means with different superscripts are significantly different at $p < 0.05$. Dietary zingerone had similar effects as ZnBcn on the birds' breast meat pH_i, L*, a*, b*, C* and H^*. However, at 24 hours post slaughter, chickens fed diet 2 had significantly increased ($p > 0.05$) meat redness (a*) compared to counterparts fed diet 1. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

Table 4.2: Effect of substituting zinc bacitracin with zingerone on the pH and colour of thigh meat from Cobb 500 broiler chickens

Parameters	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
30-mins post-slaughter					
Meat pH_i	6.16 ± 0.04	6.06 ± 0.08	6.09 ± 0.03	6.07 ± 0.09	ns
Lightness (L*)	49.90 ± 0.66	49.88 ± 1.50	50.78 ± 1.53	49.57 ± 1.76	ns
Redness (a*)	2.43 ± 0.36	1.35 ± 0.26	1.84 ± 0.06	2.20 ± 1.02	ns
Yellowness (b*)	14.84 ± 0.75	13.98 ± 0.59	13.96 ± 1.71	14.14 ± 0.59	ns
Chroma (C*)	14.92 ± 0.45	14.10 ± 0.57	14.16 ± 1.70	15.03 ± 1.00	ns
Hue (H*)	82.85 ± 1.28	86.99 ± 2.51	86.55 ± 3.35	88.51 ± 3.21	ns
24-hrs post-slaughter					
Meat pH_u	6.04 ± 0.20	6.03 ± 0.06	6.01 ± 0.14	6.02 ± 0.06	ns
Lightness (L*)	54.64 ± 4.81	56.98 ± 3.24	56.26 ± 4.99	58.45 ± 6.54	ns
Redness (a*)	4.35 ± 0.53	3.59 ± 0.25	3.45 ± 0.69	3.92 ± 0.31	ns
Yellowness (b*)	23.17 ± 4.56	21.42 ± 3.83	21.69 ± 4.10	21.80 ± 5.87	ns
Chroma (C*)	23.68 ± 4.52	22.36 ± 4.56	22.08 ± 3.92	22.25 ± 5.75	ns
Hue (H*)	79.35 ± 1.24	81.63 ± 1.07	80.45± 3.97	81.57 ± 3.57	ns

ns = not significant, $p > 0.05$. At 30 minutes and 24 hours post-slaughter, dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on the thigh meat pH, L*, a*, b*, C* and H* across the dietary treatments. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

Table 4.3 shows the effect of substituting zinc bacitracin with zingerone on the moisture characteristics and tenderness of breast meat from Cobb 500 broiler chickens. Fortifying broiler chicken diets with dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on the breast meat moisture characteristics and tenderness.

Table 4.3: Effect of substituting zinc bacitracin with zingerone on thawing loss, cooking loss and tenderness of breast meat from Cobb 500 broiler chickens

Parameters	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Thawing loss (%)	5.12 ± 3.19	6.20 ± 3.21	7.22 ± 3.43	7.13 ± 4.42	ns
Cooking loss (%)	26.08 ± 4.35	29.51 ± 3.65	29.08 ± 3.63	29.77 ± 2.87	ns
Shear force (N)	15.77 ± 6.67	14.68 ± 5.65	13.04 ± 6.27	10.63 ± 3.36	ns

ns = not significant, $p > 0.05$. Dietary zingerone had similar effects ($p > 0.05$) as ZnBcn on TL, CL and tenderness across the dietary treatments. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

4.2.3 Effect on the breast meat chemical nutrient profile

The effects of dietary zingerone on the proximate content of the broiler chicken breast meat are presented in Table 4.4. Dietary zingerone had a similar ($p > 0.05$) effect as ZnBcn on the CP content of the breast meat from broiler chickens across the dietary treatments. The breast meat from broiler chickens fed diet 4 had higher ($p < 0.01$) ash content compared to counterparts fed diets 1 and 3. Fortifying the broiler chicken diets with zingerone significantly increased ($p < 0.0001$) the fat content of the chicken breast meat compared to that of counterparts fed the ZnBcn-fortified control diet. Breast meat from chicken-fed diet 3 (zingerone at 80 mg kg^{-1} of feed) had the highest fat (ether extract) content.

Table 4.4: Effect of substituting zinc bacitracin with zingerone on the proximate composition of breast meat from Cobb 500 broiler chickens

Proximate (% DM)	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Crude Protein (CP)	78.06 ± 0.83	77.39 ± 2.96	74.02 ± 0.93	78.12 ± 1.41	ns
Ash	4.85 ± 0.14^{a}	5.03 ± 0.04^{ab}	4.68 ± 0.11^{a}	5.35 ± 0.02^{b}	**
Dry matter (DM)	94.50 ± 0.07^{b}	95.08 ± 0.04^{c}	93.48 ± 0.04^{a}	94.38 ± 0.04^{b}	****
Ether extract (EE)	3.31 ± 0.09^{a}	4.56 ± 0.05^{c}	5.79 ± 0.10^{d}	3.95 ± 0.05^{b}	****

ns = not significant, $p > 0.05$, ** $p < 0.01$, **** $p < 0.0001$. ^{abcd} Within row means with different superscripts are significantly different at $p < 0.05$. The CF content of the breast meat from birds was similar ($p > 0.05$) across the dietary treatments. The ash content of the breast meat from birds fed diet 4 was higher ($p < 0.01$) compared to those counterparts fed diets 1 and diet 3. The DM and EE content of the breast meat from birds fed diet 2 was significantly higher ($p < 0.0001$) than birds fed diets 1, 2, and 4. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

The amino acid content of the breast meat from carcasses of broiler chickens is shown in Table 4.5. Dietary zingerone at 40 mg kg^{-1} of feed significantly increased ($p < 0.0001$) the arginine, leucine and lysine contents of the broiler chicken breast meat compared to those from chicken-fed diets 1, 3 and 4. The breast meat from broiler chicken fed diet 2 fortified with zingerone at 40 mg kg^{-1} of feed had the highest concentration of essential amino acids ($p < 0.05$). The chicken breast meat from birds fed diet 3 had the lowest ($p < 0.0001$) total amino acid content when compared to that from counterparts fed diets 1, 2 and 4.

Table 4.5: Effect of substituting zinc bacitracin with zingerone on amino acids content of breast meat from Cobb 500 broiler chickens

Amino acids	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Essential amino acids (g/100g DM)					
Arginine	6.39 ± 0.10^{a}	8.91 ± 0.07^{b}	6.23 ± 0.11^{a}	6.47 ± 0.22^{a}	***
Histidine	4.32 ± 0.21^{a}	4.61 ± 0.08^{b}	4.74 ± 0.10^{b}	4.15 ± 0.17^{a}	*
Isoleucine	4.28 ± 0.12^{b}	4.53 ± 0.18^{c}	3.94 ± 0.10^{a}	4.06 ± 0.31^{ab}	**
Leucine	6.40 ± 0.15^{a}	6.78 ± 0.31^{b}	6.19 ± 0.14^{a}	6.43 ± 0.28^{ab}	*
Lysine	8.11 ± 0.08^{b}	9.00 ± 0.05^{c}	7.78 ± 0.23^{a}	8.16 ± 0.10^{b}	***
Methionine	2.19 ± 0.17^{a}	2.81 ± 0.07^{b}	2.24 ± 0.16^{a}	2.33 ± 0.10^{a}	**
Phenylalanine	3.32 ± 0.10^{a}	4.15 ± 0.20^{b}	3.15 ± 0.18^{a}	3.30 ± 0.04^{a}	**
Threonine	3.93 ± 0.11^{a}	4.24 ± 0.08^{b}	3.85 ± 0.13^{a}	3.85 ± 0.10^{a}	**
Tyrosine	2.15 ± 0.15^{ab}	1.88 ± 0.21^{a}	2.02 ± 0.10^{a}	2.26 ± 0.15^{b}	*
Valine	4.42 ± 0.23^{b}	4.59 ± 0.16^{b}	3.94 ± 0.31^{a}	4.08 ± 0.12^{a}	**
Non-essential amino acid (g/100g DM)					
Alanine	4.37 ± 0.15^{b}	3.98 ± 0.19^{a}	4.24 ± 0.14^{b}	4.40 ± 0.32^{a}	**
Aspartic acid	7.75 ± 0.06^{b}	7.39 ± 0.18^{a}	7.57 ± 0.17^{a}	7.83 ± 0.08^{b}	***
Glutamic acid	12.12 ± 0.08^{b}	6.03 ± 0.10^{a}	11.87 ± 0.11^{b}	12.25 ± 0.07^{b}	***
Glycine	4.16 ± 0.23^{a}	6.58 ± 0.05^{b}	3.87 ± 0.26^{a}	4.12 ± 0.20^{a}	***
Hydroxyproline	0.31 ± 0.12^{a}	1.02 ± 0.10^{b}	0.15 ± 0.09^{a}	0.25 ± 0.08^{a}	***
Proline	2.99 ± 0.08^{a}	4.48 ± 0.07^{b}	2.88 ± 0.20^{a}	3.01 ± 0.14^{a}	***
Serine	3.72 ± 0.13^{a}	4.61 ± 0.10^{b}	3.74 ± 0.10^{a}	3.67 ± 0.15^{a}	***
Total	**80.93 ± 0.23^{b}**	**80.59 ± 0.17^{b}**	**78.40 ± 0.08^{a}**	**80.62 ± 0.10^{b}**	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. [abc] Within row means with different superscripts are significantly different at $p < 0.05$. Broiler chickens fed diet 3 had decreased breast meat amino acids content compared to counterparts fed diets 1, 2 and 4. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

Table 4.6 shows the effect of dietary zingerone on the chicken breast meat's fatty acid profile. Dietary zingerone had a similar ($p > 0.05$) effect as ZnBcn on the meat's total saturated fatty acid content but the breast meat from chickens fed diet 3 (80 mg kg^{-1} of feed zingerone) had significantly increased ($p < 0.0001$) total monounsaturated fatty acid and oleic acid content. Meat from chicken-fed diet 4 (120 mg kg^{-1} of feed zingerone) had the highest total polyunsaturated fatty acid and linoleic acid content and a higher PUFA/SFA ratio compared to that from counterparts fed diets 1, 2 and 3. Breast meat from chicken-fed diets 2 and 3 had a higher n6PUFA/n3PUFA ratio compared to breast meat from chicken-fed diets 1 and 4.

Table 4.6: Effect of substituting zinc bacitracin with zingerone on the fatty acid profile of breast meat from Cobb 500 broiler chickens

Fatty acids (%)	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Saturated fatty acids					
C16:0 (palmitic acid)	20.08 ± 0.24[a]	20.94 ± 0.21[b]	20.63 ± 0.31[b]	20.30 ± 0.18[a]	*
C18:0 (stearic acid)	8.94 ± 0.11[b]	8.38 ± 0.36[a]	8.43 ± 0.22[a]	9.07 ± 0.08[b]	***
TSFAs	**29.02 ± 0.22[a]**	**29.32 ± 0.27[a]**	**29.06 ± 0.31[a]**	**29.37 ± 0.33[a]**	**ns**
Mono-unsaturated fatty acids					
C16:1 (palmitoleic acid)	4.68 ± 0.10[b]	4.52 ± 0.14[b]	4.98 ± 0.11[c]	3.47 ± 0.15[a]	***
C18:1n9t (elaidic acid)	1.30 ± 0.21[b]	1.31 ± 0.20[b]	1.52 ± 0.18[c]	1.08 ± 0.11[a]	***
C18:1n9c (oleic acid)	39.54 ± 0.11[a]	39.67 ± 0.13[a]	41.66 ± 0.17[b]	39.76 ± 0.10[a]	**
TMUFAs	**45.50 ± 0.33[a]**	**45.50 ± 0.37[a]**	**48.16 ± 0.10[b]**	**44.31 ± 0.12[a]**	*******
Poly-unsaturated fatty acids					
C18:2n6c (linoleic acid)	18.39 ± 0.21[a]	18.29 ± 0.11[a]	18.42 ± 0.08[a]	21.17 ± 0.01[b]	***
C18:3n3 (α-linolenic acid)	1.97 ± 0.08[b]	1.72 ± 0.12[a]	2.07 ± 0.05[c]	1.72 ± 0.13[a]	***
C20:3n3 (eicosatrienoic acid)	0.62 ± 0.22[a]	0.64 ± 0.11[a]	0.54 ± 0.10[a]	0.81 ± 0.08[b]	***
C20:5n3 (eicosapentaenoic acid)	4.02 ± 0.11[a]	4.13 ± 0.21[c]	3.15 ± 0.34[a]	5.13 ± 0.1[c]	***
TPUFAs	**25.00 ± 1.09[a]**	**24.78 ± 1.21[a]**	**24.18 ± 0.98[a]**	**28.82 ± 2.08[b]**	*******
PUFA/SFA ratio	0.81:1[a]	0.83:1[a]	0.84:1[a]	0.94:1[b]	***
n6PUFA/n3PUFA ratio	2.78:1[a]	2.82:1[b]	3.19:1[c]	2.76:1[a]	***

ns = no significant, $p > 0.05$; **$p < 0.01$; *** $p < 0.001$. [abc] Within row means with different superscripts are significantly different at $p < 0.05$. Dietary zingerone had similar effects as a control diet on the meat`s TSFA across the dietary treatments. The total MUFA of chickens fed diet 3 was significantly higher ($p < 0.0001$) than that of chickens fed diet 1, 2 and 4. Breast meat from chicken-fed diets 2 and 3 had a higher n6PUFA/n3PUFA ratio compared to breast meat from chicken-fed diets 1 and 4. The chickens fed diet 4 had significantly increased ($p < 0.0001$) breast meat TPUFA content and PUFA/SFA ratio compared to chickens fed diets 1, 2 and 3. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2:

Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

4.3 Discussion

Nowadays, consumers prefer meat and meat products that are grown on natural feed additives rather than antibiotics due to the health concerns that arise from the deposition of antibiotic residues in the meat. Due to both the negative public health and environmental effects of routine use of antibiotics in poultry and production, efforts to determine the potential of phytochemicals which are deemed non-toxicity, are gaining momentum in research and the feed additive market (Dhama et al., 2014). This study evaluated the effects of substituting zinc bacitracin with zingerone in Cobb 500 broiler chickens' diets on the breast and thigh meat quality.

4.3.1 Physical attributes of the broiler chickens` breast and thigh meat

The meat quality traits are influenced by physical and biochemical changes in muscle metabolism during post-mortem (Ismail & Joo, 2017). Pre-slaughter stress and anaerobic glycolysis affect muscle metabolism causing a depletion of muscle glycogen stores and a breakdown of adenosine triphosphate that mediate a decline in muscle pH after slaughter (Adzitey & Nurul, 2011). The pH of broiler chicken meat substantially impacts meat colour and visual acceptance by consumers, its water-holding capacity and tenderness (Ristic & Damme, 2010) and it (pH) is a reliable indicator of meat quality (Mir et al., 2017). Kralik et al. (2018) reported that a low pH in chicken meat stimulates the oxidation of myoglobin (pink colour) and oxyhemoglobin (red colour) to metmyoglobin (brown colour). The normal pH values of broiler chicken breast meat range from 5.72 to 6.21 while that of thigh meat ranges from 6.42 to 6.54 (Barbut, 1997; Liu & Niu, 2008). A pH value of chicken meat that is below 5.70 represents pale, soft and exudative (PSE) meat while a pH value greater than 7.21 represents dark, firm and dry (DFD) meat (Adzitey & Nurul, 2011). Fortification of broiler chicken diets with phytochemicals has been shown to enhance physico-chemical quality traits of meat (Martínez Aispuro et al., 2020). Partovi et al. (2019) and Qaid et al. (2021) reported that fortifying broiler chicken diets with phytochemicals enhanced meat pH, colour, water-holding capacity and shear force. Findings from the current study show that the pH_i and pH_u values of broiler chicken breast meat ranged between 5.73 to 6.78 while the thigh meat`s pH_i and pH_u values ranged from 6.01 to 6.16, respectively. Feeding zingerone-fortified diets resulted in broiler chicken breast and thigh meat with a pH that falls within the normal pH (pH_i and pH_u) range. Findings from the current study showed a decrease in the pH of broiler chicken meat 24 hours post-slaughter which is attributable to post-slaughter anaerobic conversion of muscle glycogen to lactate (Adzitey & Nurul, 2011). Similarities in the breast

and thigh meat pH_i and pH_u from zingerone and ZnBcn-supplemented chicken suggest that graded levels of dietary zingerone did not have a negative impact on the process of converting muscle to meat. This suggests that zingerone neither increased nor decreased acid production from muscle glycogen during the process of converting muscle to meat.

Meat colour, especially its redness, is a critical determinant of acceptability by consumers since it (redness) is indicative of both the freshness and tenderness of the meat (Testa et al., 2021). It is also a major determinant of satisfaction and willingness by consumers to pay premium prices (Mir et al., 2017). An increase in the redness of meat is associated with higher muscular myoglobin content (Keeton & Dikeman, 2017; Hwang & Claus, 2021). Findings from the current study show that at 30 minutes post-slaughter, dietary zingerone had similar effects as ZnBcn on the colour colorimetric values of the chicken breast and thigh meat across diets. Dietary zingerone also had similar effects as the ZnBcn-based control diet on the colourimetric values of the thigh meat at 24 hours post-slaughter. However, the breast meat from broiler chickens fed diet 2 fortified with zingerone at 40 mg kg^{-1} was the most reddest. The increased redness shows an increase in muscle myoglobin content suggesting that zingerone when used to fortify broiler chicken diets at 40 mg kg^{-1} of feed can potentially be harnessed to increase redness and hence enhance the acceptability of the meat by consumers. Zingerone is a yellow to yellowish-brown pigmented crystalline substance". It has been shown that phytochemicals with "colour" when used as feed supplements can affect meat colour (Cabrol et al., 2022). In the current study, it is therefore hypothesized that the "Zingerone colour" could have been "mirrored" in the meat hence the observation that meat from chicken fed a diet with Zingerone at 40 mg kg^{-1} of feed was more red compared to that of counterparts fed diets 1, 3 and 4. The increased redness of the meat at 40 mg kg-1 of feed zingerone inclusion could have been due to positive associative effects at that level of dietary supplementation. Similarities in the colour colorimetric values of the thigh meat at 30 minutes and 24 hours post-slaughter and breast meat at 30 minutes post-slaughter indicate that replacing zinc bacitracin with zingerone in broiler chicken diets did not adversely impact the meat`s physical appearance.

The ability of meat to retain moisture is one of the vital physicochemical properties which significantly impact the palatability of meat (Mir et al., 2017). Approximately 88 to 95% of the water is held intracellularly between actin and myosin filaments and myofibrils (Offer et al., 1989). An increase in muscle moisture content has been shown to improve the appearance, firmness, juiciness and tenderness of the meat and its economic value (Mir et al., 2017).

However, a decrease in muscle moisture content results in protein denaturation and loss of protein solubility (Offer et al., 1989). Muscle fibre characteristics are key determinants of both appearance and eating quality traits in poultry (Ismail & Joo, 2017). The thigh muscle of broiler chicken is a red muscle which contains more red type I fibre (slow-twitch) and the breast muscle is a white muscle which contains more white type IIB fibre (fast-twitch) (Verdiglione & Cassandro, 2013). Red muscle has more muscle contraction than white muscle, hence thigh muscles are tougher compared to breast muscles (Huo et al., 2021). Matshogo et al. (2020) reported that TL, CL and tenderness of the broiler chicken breast meat that ranges between 6.45 to 8.55%, 23.54 to 25.40% and 7.55 to 11.63N. Findings from the present study show that the chicken breast meat's TL, CL and tenderness ranged from 5.12 to 7.22%, 26.08 to 29.77% and 10.63 to 15.77N, respectively. The observed TL, CL and tenderness values are within the range reported by Matshogo et al. (2020) which suggests that fortifying broiler chickens` feed with zingerone in place of ZnBcn did not negatively impact the meat's moisture-holding characteristics and tenderness. Furthermore, similarities in the TL and CL of the meat from chicken fed the zinc bacitracin-based control diet and the zingerone-based test diets suggest that zingerone can be used to fortify broiler chicken diets without risking muscle (meat) denaturation and compromising protein solubility.

4.3.2 Chemical nutrient profile of the broiler chicken breast meat

The meat's chemical nutrient profile is an important parameter in evaluating meat quality as it points to the nutritive value of the meat and its potential effects on consumer health (Gheorghe et al., 2021). Singh et al. (2014) reported that supplementing broiler chicken diets with cinnamon powder, a source of phytochemicals that exhibit health-beneficial biological activities enhanced the meat's proximate nutrient composition. A study by Marcinčk et al. (2011) showed that fortifying broiler chicken feeds with a combination of clover powder and lemon balm extract (0.2%) increased the crude protein content of chicken meat but decreased its fat content. The breast chicken meat from birds-fed diets fortified with genistein and hesperidin had increased polyunsaturated fatty acids, n-3 to n-6 and polyunsaturated to saturated fatty acid ratios (Kamboh & Zhu, 2013). Fresh broiler chicken breast meat contains on average 15 to 35% protein and 1.5 to 5.3% fat (De Oliveira et al., 2016). In the current study, fresh chicken meat was freeze-dried prior to chemically quantifying its various chemical nutrient constituents. Findings from the present study reported 4.85 to 5.35% ash, 74.02 to 78.12% crude protein and 3.31 to 5.79% ether extract in the chicken breast meat. Fresh broiler chicken breast meat has on average 74.08 to 77.13% moisture (Domínguez-Niño et al., 2020) thus when dried, the moisture loss results in a concentration of the nutrients,

especially protein. The meat samples assayed for nutrient content were freeze-dried, hence had very little moisture remaining, and this explains the seemingly very high CP content of the meat. However, the similarity in the meat's CP content shows that dietary zingerone neither decreased nor increased the meat's protein content. This observation is at variance with the findings of Marcinčk et al. (2011), who observed that a combination of clover powder and lemon balm extract when used to fortify broiler chicken diets resulted in increased crude protein in chicken breast meat. The lipid content of the breast meat from broiler chickens fed diets fortified with zingerone at 40, 80 and 120 mg kg^{-1} of feed was substantially increased compared to that of birds fed the control ZnBcn-based diet. Despite, dietary zingerone being shown to increase the meat's fat content, its fat content was within the reported range (De Oliveira et al., 2016). Thus, taken together findings on the proximate content of the meat suggest zingerone can be used to replace zinc bacitracin without negatively impacting the meat's protein content, however, caution must be taken regarding its use as it might cause an increase in the fat content of the meat which (fat) might negatively affect consumer health.

Amino acids, especially essential amino acids play a critical role in promoting in-*utero* foetal and neonatal growth and development. Broiler chicken meat is characterised by abundant content of arginine, leucine, lysine, glutamic acid and aspartic acid (Kim et al., 2017) which are essential amino acids. Plant-derived phytochemicals are rich in bioactive compounds with potent antioxidant properties that can potentially improve meat shelf life and attenuate lipid peroxidation in chicken meat (Mehdi et al., 2018). Waheed et al. (2018) reported that fortifying broiler chicken diets with plant-derived phytochemicals improve the essential amino acids content in the meat. Amino acids play specific roles in modulating metabolic pathways, antioxidant systems, and enzymatic processes, which can potentially enhance meat production and meat quality (Estévez et al., 2020). In their study, Waheeda et al. (2018) reported that the arginine, leucine, lysine, glutamic acid and aspartic acid content of broiler chicken ranged from 5.5 – 8.5%, 5.4 – 12.2%, 5.3 – 8.1%, 8.6 – 13% and 7.5 – 14.5%, respectively. The findings of the present study reported 6.23 to 8.91% arginine, 6.19 to 6.78% leucine, 7.78 to 9.00% lysine, 6.03 to 12.25% glutamic acid and 7.39 to 7.83% aspartic acid which are within the ranged reported by Waheeda et al. (2018). Importantly and interestingly, compared to the control, the concentration of these key amino acids was higher in the meat from chicken-fed zingerone-fortified diets which suggests that zingerone positively impacted the amino acid content, hence the nutritional value of the meat. These results might be attributed to the presence of antioxidant properties in zingerone.

The World Health Organisation recommends a daily dietary fat intake of not greater than 35% of the total energy requirement of which saturated fatty acids and polyunsaturated fatty acids should not exceed 10% and 11% of that designated energy intake (WHO, 2018). Due to its relatively lower saturated fatty acids and higher monounsaturated and polyunsaturated fatty acid content, broiler chicken meat is a vital constituent of a healthy diet (Wood & Enser, 2017; FAO and WHO, 2020). Dietary mono-and poly-unsaturated fats acids have been shown to mitigate the risk of the development of metabolic diseases, among many, coronary heart diseases (Gecgel et al., 2015; Kwon, 2016). The incorporation of plant-derived phytochemicals in broiler chicken diets has been shown to enhance oxygen-scavenging responses that potentially inhibit saturated fatty acid levels by modulating the activity of 9-desaturase enzyme complex, which converts SFA into UFA (Ahmed et al., 2015; Waheed et al., 2018). Broiler chicken breast meat is reported to contain 30.42 to 33.47% total saturated fatty acids (TSFAs), 43.37 to 47.04% total monounsaturated fatty acids (TMUFAs) and 22.40 to 23.15% total polyunsaturated (TPUFAs) (Moyo et al., 2021). In the current study, the TSFAs, TMUFAs and TPUFAs contents of broiler chicken breast meat ranged from 29.02 to 29.62%, 44.31 to 48.16% and 24.18 to 28.02%, respectively. Findings from the current study showed that dietary zingerone had a similar ($p > 0.05$) effect as ZnBcn on the meat's TSFAs but breast meat from chickens fed diets 3 (80 mg kg^{-1} feed zingerone) had significantly increased ($p < 0.0001$) TMUFAs and oleic acid (OA) content and meat from chicken fed diet 4 (120 mg kg^{-1} feed zingerone) had the highest TPUFAs and linoleic acid (LA) content and a higher PUFA/SFA ratio compared to that from counterparts fed diets 1, 2 and 3. Monounsaturated fatty acids, especially OA have been shown to positively impact consumer health by mitigating oxidative stress (Gillingham et al., 2011) while LA, an essential fatty acid, is metabolized to dihomo-γ-linolenic acid. The latter serves as an important constituent of neuronal membrane phospholipids and as a precursor of prostaglandin E (Nagy & Tiuca, 2017) thus is vital in the preservation of blood flow in the nerves and cellular communication and metabolic regulation.

Unsaturated fatty acids and essential fatty acids play several beneficial roles in human health (Peña-Saldarriaga et al., 2020). Higher dietary levels of monounsaturated fatty acids and OA decrease the concentration of circulating LDL-cholesterol and protect against coronary heart disease (Wood & Enser, 2017; Martins et al., 2018). Phytochemical feed additives have been shown to have beneficial biological effects including antioxidant and antimicrobial properties that improve poultry meat quality characteristics. Dietary zingerone, therefore, can potentially

be harnessed to increase chicken breast meat's monounsaturated fatty acid, OA and LA content with concomitant benefits to consumer health. Dietary PUFA/SFA ratio is one of the key metrics considered in determining both the nutritional value and the impacts of the diet on consumer health (Gouaref et al., 2020) and the recommended dietary PUFA/SFA ratio should be above 0.4 (Wood et al., 2004). In addition, the recommended n6PUFA/n3PUFA ratio in human diets ranges from 1 to 4.1. Findings from the current study reported a PUFA/SFA ratio range in the chicken breast meat of 0.83 to 0.94 with meat from chicken fed a diet fortified with zingerone at 120 mg kg^{-1} of feed having the highest PUFA/SFA ratio. These findings also show that the n6PUFA/n3PUFA ratio in chicken breast meat range from 2.76 to 3.19 with meat from chickens fortified with zingerone at 80 mg kg^{-1} of feed having the highest n6PUFA/n3PUFA ratio. Taken together our findings demonstrate that fortifying broiler chicken diets with zingerone increased beneficial fatty acids in the chicken breast meat which would positively impact consumer health.

4.4 Conclusion

Based on findings from the current study, it is concluded that zingerone can replace zinc bacitracin as a dietary supplement in Cobb 500 chicken diets without altering the meat's pH, TL, CL and tenderness. Importantly, fortifying broiler chicken diets with zingerone can be used to enhance the meat's redness translating to increased acceptability and can also be used to improve the meat's nutritional value by increasing its TMUFA, TPUFA, OA and LA content.

The studies that evaluate the feed additives and cone-conventional feed resources have been shown to predominantly interrogate their effects on production performance and product quality but with minimal attention to effects on poultry and or livestock welfare. Thus, the next chapter focuses on a narrative that reports on the effects of supplemental zingerone on the chickens' health.

CHAPTER FIVE: EFFECT OF SUPPLEMENTAL ZINGERONE ON THE OXIDANT AND ANTIOXIDANT STATUS AND KIDNEY AND LIVER HEALTH OF COBB 500 BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*)

Abstract

The study evaluated the effects of dietary fortification with zingerone in place of zinc bacitracin (ZnBcn) on the oxidant and antioxidant status, hepatic and kidney health of broiler chicken. One hundred and twenty 1-day-old unsexed Cobb 500 broiler chicks were, in a completely randomized design, allocated to four diets where zingerone replaced ZnBcn at 0 (control diet; 500 mg kg^{-1} of feed ZnBcn); 40; 80 and 120 mg kg^{-1} of feed as a growth-promoting supplement. Zingerone doses were the same for the starter, grower and finisher diets. Each diet was replicated 3 times with 10 chicks per replicate. The chickens were fed for 6 weeks: 2 weeks for each of the starter, grower and finisher growth phases. Terminally, plasma was harvested from the collected blood. Plasma malonaldehyde (MDA) concentration, glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activities and surrogate markers of liver and kidney function, liver fat and histology were determined. Supplemental zingerone had similar effects as ZnBcn on the chickens' liver fat content, plasma MDA concentration, GSH-Px, GST, SOD, CAT, alkaline phosphatase (ALP) and alanine transaminase (ALT) activities. Chickens' hepatic inflammation and steatosis scores were similar across diets ($p > 0.05$). At 120 mg kg^{-1} of feed zingerone, though similar to the control, decreased the chickens' plasma globulin and total protein concentration ($p < 0.01$; $p < 0.05$) compared to counterparts supplemented at 40 and 80 mg kg^{-1} of feed. Dietary zingerone had similar ($p > 0.05$) effects as a control diet on the plasma urea, total bilirubin and creatinine concentrations. Zingerone can replace ZnBcn in Cobb 500 broiler chicken diets without altering liver fat accretion and kidney function, eliciting oxidative stress, perturbing the systematic antioxidant pool activity, compromising liver synthetic function, damaging hepatocytes, bile canaliculi and bile duct cells, and without causing hepatic inflammation and steatosis.

5.0 Introduction

Several strategies, among them the use of antibiotics as growth promoters, have been and continue to be employed to enhance broiler chicken productive efficiency (Salim et al., 2018). However, due to the environmental pollution and public health concern of antibiotic resistance caused by fortifying broiler chicken diets with antibiotics at sub-therapeutic doses, there is a growing preference towards the use of natural products, especially plant-derived extracts and phytochemicals in the poultry production chain (Krauze, 2021). Plant-derived additives used in poultry diets to enhance the antioxidant status and immune response of chickens are known as phytogenic feed additives, and ginger is one such additive (Attia et al., 2022). Ginger, a known spice, in addition to carbohydrates and lipids contains terpenes and

phenolics with shogaols, gingerols and zingerone as its major phytochemicals (Mao et al., 2019). Ginger, due to the health-beneficial biological activities exhibited by its phytochemicals is used in ethnomedicine (Adebayo et al., 2021). Fortifying broiler chicken diets with ginger powder, in addition to enhancing feed intake and utilisation efficiency and growth performance (Shewita & Taha, 2018) has been shown to significantly improve the plasma and hepatic antioxidation indices of broiler chicken (Al-Khalaifah et al., 2022). Dietary fortification of broiler chicken diets with ginger was shown to increase white blood cell counts and the percentage of heterophils in the blood of the birds and to decrease the plasma cholesterol, triglyceride, and very low-density lipoprotein concentration while mediating and increase in plasma lipoprotein concentration (Al-Khalaifah et al., 2022). Furthermore, fortifying broiler chicken feed with ginger powder has been shown to enhance the chickens' immune status and health (Abd El-Hack et al., 2020). These positive effects of ginger on broiler chicken health can be ascribed to the presence of phytochemicals which exhibit health-beneficial biological properties

Zingerone, one of the phytochemicals found in high concentration in ginger has been shown to possess antioxidant, anti-inflammatory, lipolytic and hepatoprotective properties (Ahmad et al., 2015). In rodents, it inhibited the genesis of free radicals and downregulated the synthesis and secretion of inflammatory cytokines resulting in hepatic protection (Türk et al., 2020; Wali et al., 2020). Environmental stressors and high-fat diets negatively impact broiler chicken health. While the previous two experimental chapters evaluated the effects of dietary zingerone on broiler chicken productive performance and meat quality, it became necessary to evaluate its effects on bird welfare and health as these are pertinent issues in poultry production. The liver and kidneys are critical organs whose functional efficiency has a great bearing on the health status of any poultry and/or livestock. This study, therefore, evaluated the effects of supplemental zingerone on the oxidant and antioxidant status and kidney and liver health of Cobb 500 broiler chicken.

Hypothesis

H_0: Supplemental zingerone does not affect the oxidant and antioxidant status, kidney and liver function and general health of Cobb 500 broiler chicken.

H_1: Supplementary zingerone affects the oxidant and antioxidant status, kidney and liver function and general health of Cobb 500 broiler chicken.

5.1 Material and methods

5.1.1 Study site and ethical clearance

The study site and ethical clearance are as previously stated in Chapter Three, subheading 3.1.1.

5.1.2 Feed ingredients and diet formulation

The sourcing of dietary ingredients and diet formulation are as previously stated in Chapter Three, subheading 3.1.2.

5.1.3 Animals, feeding and housing

Animal feeding and housing are as previously stated in Chapter Three, subheading 3.1.3.

5.1.4 Experimental design

The experimental design is as previously stated in Chapter Three, subheading 3.1.4.

5.1.5 Terminal procedures and blood sample collection

Following the humane slaughter of each chicken as described under "terminal procedures" in chapter three (subheading 3.1.7), blood from each chicken collected into heparinised blood collection tubes (Vacuette, Greiner Bio-One, Frickenhausen, German) was centrifuged for 15 minutes at a force of 5500 × g. Plasma was decanted into microtubes and stored at -20°C pending laboratory assays to determine oxidant (stress) and antioxidant status, kidney and liver health of the birds. The liver was excised from each carcass and weighed. A sample of the liver was preserved in 10% phosphate-buffered formalin pending histological assays and the remainder was frozen-stored at -20°C in a freezer (Bosch, Stuttgart, Germany) pending determination of liver fat content.

5.1.6 Determination of plasma markers of health

5.1.6.1 Oxidant and antioxidant status

The plasma concentration of malondialdehyde (MDA), a marker of oxidative stress, was determined as described by Habibi et al. (2014) using the thiobarbituric acid reactive substance (TBARS) assay kit (Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's instructions. Briefly, following thawing at room temperature (24˚C) for 1 hour, the thawed samples were then acid treated with TBARS acid reagent to precipitate

interfering proteins and other substances. The TBARS acid reagent-treated samples were incubated at room temperature for 15 minutes and then centrifuged in a microtube centrifuge (Eppendorf, Hamburg, Germany) at 12 000 × g for 4 minutes. The supernatant was collected and analysed wherein its optical density was pre-read at 540 nm using a microplate reader (Elabscience Biotechnology®, Wuhan, Hubei, China). The samples were then incubated at 48°C in an oven (Labcon, South Africa) for 2:30 hours, and the optical densities were read again. The initial optical densities were subtracted from the final readings. A standard curve was generated from the test standards and used to determine the TBARS concentration of the test samples.

The plasma superoxide glutathione-peroxidase (GSH-Px), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities were measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China) specific for chickens as per manufacturer's instructions as described by Cowell et al. (1994) and Sun et al. (1988), respectively. The total SOD activity was determined using the xanthine oxidase method, which monitors the inhibition of nitro blue tetrazolium reduction by the sample as described by Sun et al. (1988). Plasma CAT activity was determined by measuring the catalase degradation of hydrogen peroxide (H_2O_2) using a redox dye as described by Cowell et al. (1994). Plasma GSH-Px activity was determined using H_2O_2 and an electronic donor dye that forms a pink colour during peroxide reaction as described by Kokkinakis & Brooks (1979). Plasma GSH-ST activity was determined by measuring the conjugation of 1-chloro-2.4-dinitrobenzene with reduced glutathione as described by Habig et al. (1974).

5.1.6.2 Surrogate markers of kidney and liver function

The plasma concentrations of uric acid, total bilirubin, blood urea nitrogen and creatinine (surrogate markers of kidney function) and plasma activities of alanine transaminase (ALT), alkaline phosphatase (ALP), plasma albumin, globulin and total protein (surrogate markers of liver function) of the broiler chicken were measured using a colourimetric-based clinical chemistry analyser (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. Briefly, the frozen-stored plasma samples were thawed at room temperature; gently mixed by gently inverting each plasma-containing microtube to create a homogeneous sample. Plasma from each sample was drawn using a pipette and transferred into a catalyst sample cup which was inserted in a colorimeter along with pre-loaded disks for analyses of the surrogate markers of kidney and liver function,

respectively. The IDEXX Clinical Chemistry Analyser automatically sampled out 200 µL of a plasma sample from the catalyst sample cup and dispensed 10µL onto each of the pre-loaded discs, analysed the sample and provided an off-print of the results.

5.1.7 Determination of hepatic lipid content

The hepatic lipid content was determined using the Soxhlet apparatus (Gebr. Rettberg GmbH, 37079 Göttingen, Germany) with petroleum ether as a solvent as described by the Association of Analytical Chemists (AOAC, 2005: method number 920.39). Briefly, the liver samples frozen-stored at -20ºC were thawed overnight at room temperature and thereafter they were cut into smaller pieces and weighed. The liver pieces (0.5g) were placed inside a petroleum ether-soaked extraction thimble containing fat-free cotton wool and then loaded into the Soxhlet extraction chamber. The weight of an empty round-bottomed distillation flask was determined prior to the extraction of fat from the liver sample using a digital scale (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). Two hundred millilitres (200 ml) of petroleum ether was poured into the distillation flask and placed onto the heating pad and then connected to the Soxhlet extractor. The reflux condenser was placed on top of the Soxhlet extractor. The heating mantel was then set at 50ºC following the turning on of the cooling water supply to the condenser. Each sample extraction cycle duration lasted 2 hours. After extraction, the petroleum ether was removed from the flask using a rotatory evaporator (Buchi Rotavapor-R, Buchi Laboratoriums, Technik AG, CH.9230 Flawl/Schweiz) leaving the extracted lipid in the flask. The flask containing the fat/oil was weighed. The percentage of fat extracted from each liver sample was computed using the equation:

$$\text{Liver fat content (\%)} = \frac{\text{mass of flask with fat (g)} - \text{mass of empty flask (g)}}{\text{mass of liver sample (g)}} \text{ x } 100$$

5.1.8 Determination of liver histology

Liver samples preserved in 10% phosphate-buffered formalin were routinely processed using the automatic tissue processor (Microm STP 120 Thermo Fisher Scientific, Inc, Massachusetts, USA), embedded in paraffin wax blocks and then sectioned at 5µm. The sections were then stained with haematoxylin and eosin (H and E) on a glass slide and covered with a glass coverslip as described by Reyes-Gordillo et al. (2007). The changes in hepatocellular microarchitecture were randomly assessed in three fields per slide under a light microscope at a high-power magnification of 40X using an eyepiece micrometre (Reichert®, Austria). Photographs of the sections were captured using a Leica ICC50 HD video camera

mounted onto the Leica DM 500 microscope (Leica Biosystems, USA). The stained liver sections were semi-quantitatively scored for steatosis and inflammation according to the criteria described by Kleiner et al. (2005). Macro- and -micro-vesicular steatosis and hepatocellular hypertrophy were semi-quantitatively analysed by determining the average percentage of a fat accumulation from the three fields per slide. A detailed description of semi-quantitative analysis criteria is attached in appendix 8.

5.1.9 Data analysis

Parametric data are expressed as mean and standard deviation and non-parametric data as median and range (mean, max). GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, USA) was used to analyse data. A one-way ANOVA, followed by Tukey *posthoc* test was used to analyse parametric multiple-group data. Multiple groups of non-parametric hepatic inflammation and steatosis data were analysed using the Kruskal-Wallis test followed by a multiple-comparisons Dunn's *posthoc* test. Statistical significance was set at $p < 0.05$.

The statistical model used for the one-way ANOVA:

$Y_{ij} = \mu + T_i + e_{ij;}$ where,

Y_{ij} = dependent variable of interest (oxidant and antioxidative parameters, plasma markers of kidney and liver function, hepatic fat content and histology).

μ = overall mean effect

T_i = is the fixed effect of the i^{th} dietary treatments (i = 1, 2..., 4)

e_{ij} = random residual error

5.2 Results

5.2.0 General health profile

5.2.1 Effect on surrogate markers of kidney and liver function

The effect of supplemental zingerone on the plasma concentration of surrogate markers of the kidney and liver function of Cobb 500 broiler chickens is shown in Table 5.1. Supplemental zingerone had similar effects ($p > 0.05$) as zinc bacitracin on chickens' plasma BUN, uric acid, creatinine and total bilirubin concentrations and plasma ALT and ALP activities, albumin concentration and the albumin/globulin ratio. The plasma globulin and total protein concentrations of chickens supplemented with zingerone at 40 and 80 mg kg^{-1} of feed were higher ($p < 0.05$) compared to that of counterparts supplemented at 120 mg kg^{-1} of feed.

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Table 5.1: Effect of substituting zinc bacitracin with zingerone on plasma surrogate markers of the kidney and liver function of Cobb 500 broiler chickens

Parameter	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Kidney surrogate markers					
Blood urea nitrogen (mg/dL)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	ns
Uric acid (mg/dL)	3.27 ± 1.45	3.49 ± 0.83	3.75 ± 0.52	3.78 ± 0.63	ns
Creatinine (mg/dL)	0.10 ± 0.00	0.11 ± 0.01	0.10 ± 0.00	0.11 ± 0.00	ns
Total bilirubin (mg/dL)	0.10 ± 0.00	0.10 ± 0.01	0.12 ± 0.03	0.11 ± 0.02	ns
Liver surrogate markers					
ALT (U/L)	11.04 ± 1.22	10.67 ± 0.58	11.73 ± 0.42	10.17 ± 0.29	ns
ALP (U/L)	194.37 ± 0.79	196.40 ± 25.72	200.52 ± 9.69	184.10 ± 36.99	ns
Albumin (g/dL)	1.17 ± 0.05	1.37 ± 0.32	1.23 ± 0.03	1.11 ± 0.08	ns
Globulin (g/dL)	1.45 ± 0.08^{ab}	1.59 ± 0.10^{a}	1.63 ± 0.08^{a}	1.28 ± 0.10^{b}	**
Albumin/globulin ratio	0.83 ± 0.13	0.90 ± 0.63	0.77 ± 0.08	0.90 ± 0.18	ns
Total protein (g/dL)	2.62 ± 0.12^{ab}	2.82 ± 0.17^{a}	2.86 ± 0.11^{a}	2.39 ± 0.18^{b}	*

ns = not significant, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, [ab] Within row means with different superscripts are significantly different at $p < 0.05$. No statistically significant difference ($p > 0.05$) in the plasma ALT and ALP activities, albumin concentration and albumin/globulin ratio and the plasma BUN, uric acid, creatinine and total bilirubin concentration across the dietary treatments. The plasma globulin concentration of broiler chickens fed diet 4 was lower ($p < 0.01$) compared to that of chickens fed diets 2 and 3. The plasma total protein concentration of broiler chickens fed diet 4 was significantly lower ($p < 0.05$) compared to that of chickens fed diets 2 and 3. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

5.2.2 Effect on oxidative stress and systemic antioxidant pool status

Table 5.2 shows the effect of substituting zinc bacitracin with dietary zingerone on broiler chickens' plasma MDA concentration and GSH-Px, GST, SOD and CAT activities. Supplemental zingerone had similar effects ($p > 0.05$) as dietary zinc bacitracin on the chickens' plasma markers of oxidative stress and the plasma activities of enzymes that constitute the systemic antioxidant pool.

Table 5.2: Effect of substituting zinc bacitracin with zingerone on plasma oxidative stress (malondialdehyde concentration) and systemic antioxidant enzymes activities of Cobb 500 broiler chickens

	Dietary treatments				Significance
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**level**
Malondialdehyde (nmol/ml)	0.64 ± 0.11	0.72 ± 0.31	0.73 ± 0.16	0.85 ± 0.18	ns
Glutathione peroxidase (ng/ml)	0.69 ± 0.07	0.80 ± 0.19	0.84 ± 0.16	0.89 ± 0.21	ns
Glutathione S-transferase (ng/ml)	0.56 ± 0.03	0.67 ± 0.20	0.66 ± 0.13	0.71 ± 0.20	ns
Superoxide dismutase (ng/ml)	0.54 ± 0.06	0.70 ± 0.22	0.72 ± 0.21	0.74 ± 0.27	ns
Catalase (ng/ml)	0.58 ± 0.07	0.71 ± 0.23	0.70 ± 0.11	0.77 ± 0.20	ns

ns = not significant, $p > 0.05$. Supplemental zingerone had similar effects ($p > 0.05$) as zinc bacitracin on plasma malondialdehyde, GSH-Px, GST, SOD and CAT concentrations of broiler chicken. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (positive control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds.

5.2.3 Effect on liver fat content

Dietary zingerone on liver fat content of Cobb 500 broiler chickens is shown in Figure 5.1 below. Substituting zinc bacitracin with zingerone had similar ($p > 0.05$) effects on the liver fat content of the chickens.

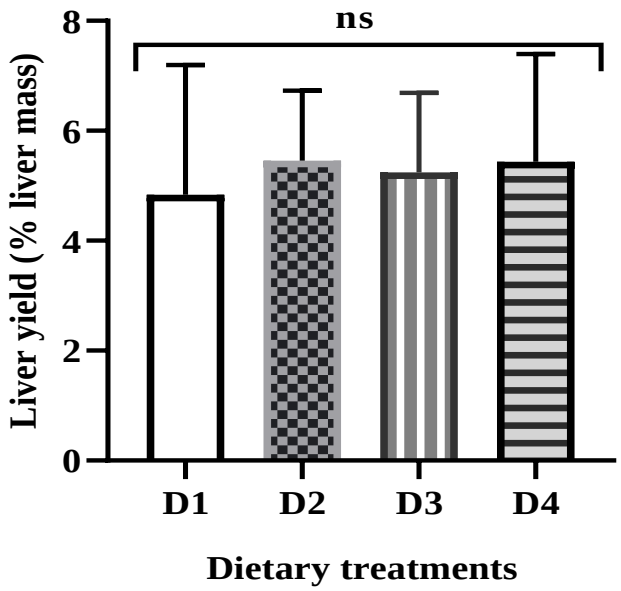

Figure 5.1: Effect of substituting zinc bacitracin with zingerone on the liver fat content of Cobb 500 broiler chickens

ns = not significant, $p > 0.05$. Supplemental zingerone had similar effects ($p > 0.05$) as dietary zinc bacitracin on the hepatic fat content of broiler chickens. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Results expressed as mean ± SD, n = 3 replicates per dietary treatment with each replicate having 10 birds. D1 – Diet 1, D2 – Diet 2, D3 – Diet 3 and D4 – Diet 4.

5.2.4 Effect on liver microarchitecture

Dietary zingerone on liver microarchitecture of Cobb 500 broiler chickens is shown in Figure 5.2 below and Table 5.3 shows the effects of substituting zinc bacitracin with zingerone on the broiler chickens' hepatocyte macro- and micro-vesicular steatosis and inflammation scores. Supplementing broiler chicken diets with zingerone in place of zinc bacitracin did not alter hepatic cell microarchitecture and fat droplet deposition. Substituting zinc bacitracin with zingerone had similar ($p > 0.05$) effects on the hepatocyte macro-and micro-vesicular steatosis and inflammation scores of the broiler chickens (Table 5.3)**.**

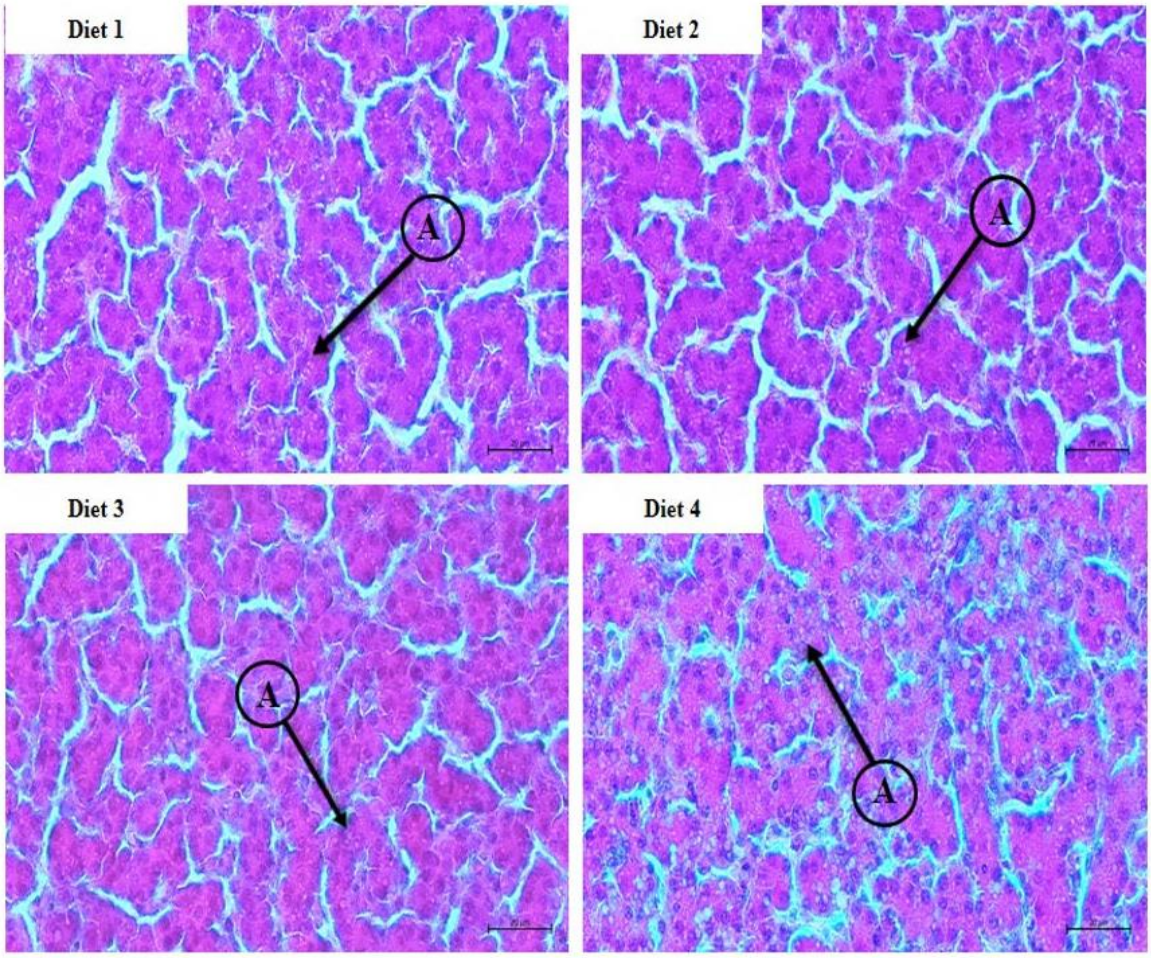

Figure 5.2: Photomicrographs of representative liver sections (H and E staining, 40X magnification) across dietary treatments

Arrows A: small fat droplets. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed.

Table 5.3: Effect of substituting zinc bacitracin with zingerone on hepatic steatosis status and inflammation of Cobb 500 broiler chickens

	Dietary treatments				Significant Level
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	
Macrosteatosis score	0(0, 0)	0(0, 0)	0(0, 0)	0(0, 0)	ns
Microsteatosis score	0(0, 2)	0(0, 2)	0.5(0, 2)	0(0, 2)	ns
Hepatic inflammation	0(0, 0)	0(0, 0)	0(0, 0)	0(0, 0)	ns

ns = not significant, $p > 0.05$. Substituting zinc bacitracin with dietary zingerone had similar effects on hepatic steatosis scores and inflammation of Cobb 500 broiler chickens. Diet 1: Starter/Grower/Finisher + zinc bacitracin at 500 mg kg^{-1} of feed (control), Diet 2: Starter/Grower/Finisher + zingerone at 40 mg kg^{-1} of feed, Diet 3: Starter/Grower/Finisher + zingerone at 80 mg kg^{-1} of feed and Diet 4: Starter/ Grower/Finisher + zingerone at 120 mg kg^{-1} of feed. Data presented as median and range (min, max), n = 3 replicates per dietary treatment with each replicate having 10 birds.

5.3 Discussion

The present study evaluated the effects of substituting zinc bacitracin with zingerone as a dietary supplement on Cobb 500 broiler chicken diets on the oxidant and antioxidant status and kidney and liver health of the birds. The present study findings show that dietary fortification of Cobb 500 broiler chickens' diets with zingerone neither altered liver fat deposition and or microarchitecture nor caused damage to hepatocytes and biliary system cells. Additionally, dietary zingerone did not alter the systemic oxidant and antioxidant status.

5.3.1 Kidney and liver functions

The kidneys are vital to the maintenance of homeostasis in birds (chickens) which is of essence to health (Sultana et al., 2021). They synthesise calcitriol, erythropoietin and renin in addition to coordinating the renin-angiotensin system (Podkowińska & Formanowicz, 2020). Kidneys also excrete metabolites from the metabolism and detoxification of xenobiotics including among many uric acid, urea, creatinine and residues of deactivated pharmacological agents (Breshears & Confer, 2017). Impaired kidney function, which manifests with higher than normal plasma concentrations of the metabolites excreted via urine, causes nephrotoxicity in poultry (Imtiaz et al., 2020) and other livestock. Supplementing broiler chicken diets with ginger powder has been shown to result in improved functional attributes of kidneys (Amaduruonye et al., 2018). These observed benefits of ginger powder to chicken kidney health are thought to be due to the presence of shogaols, gingerols and zingerone; phytochemicals with health-beneficial biological activities. Since the accumulation of waste products of metabolism excreted via urine indicates compromised kidney health, the findings of the current study, where the plasma total bilirubin, blood urea nitrogen and creatinine concentrations of broiler chickens fed zingerone-supplemented diets were similar to ZnBcn-control counterparts dietary zingerone at 40; 80 and 120 mg kg^{-1} of feed did not elicit kidney malfunction. In the current study, plasma total bilirubin, blood urea nitrogen and creatinine concentrations range from 0.10 to 0.12 mg/dL, 1.89 to 2.00 mg/dL and 0.10 to 0.11 mg/dL, respectively. Various authors reported plasma total bilirubin ranges from 0.12 to 0.18 mg/dL, blood urea nitrogen ranges from 2.00 to 3.20 mg/dL and creatinine ranges from 0.42 to 0.53 mg/dL (Ciurescu et al., 2019; Aikpitanyi & Egweh, 2020) in chicken. In the current study, plasma total bilirubin and blood urea nitrogen concentrations of broiler chickens fall within the range reported by other authors but creatinine concentration was lower compared to creatinine reported by other studies (Ciurescu et al., 2019; Aikpitanyi & Egweh, 2020). Furthermore, Amaduruonye et al. (2018) also observed similar plasma concentrations of these metabolites (bilirubin, blood urea nitrogen and creatinine) as in the current study findings, in

broiler chickens fed diets supplemented with ginger roots meal. These findings indicate that dietary zingerone at 40; 80 and 120 mg kg^{-1} of feed did not elicit kidney malfunction in Cobb 500 broiler chicken.

The liver is a critical accessory organ to avian digestive physiology: it synthesises bile and bile salts that are critical to fat digestion and absorption (Alshamy et al., 2019). Some dietary constituents, including supplements used to fortify broiler chicken diets, compromise liver function and microarchitectural manifests through altered physiological function (Massart et al., 2022). Elevated plasma ALT and ALP activities are associated with hepatocellular damage and hepatic inflammation (Kalas et al., 2021) while plasma elevated ALP activity could be associated with damaged bile duct and biliary canaliculi cells. The present study shows similarities in plasma ALT and ALP activities in broiler chicken fed zingerone-fortified diets and those of counterparts fed the control ZcBcn-fortified diet. Current study findings showed plasma ALT and ALP activities ranges of 10.17 to 11.73 U/L and 184.10 to 200.52 U/L, respectively which were similar across dietary treatments. Aikpitanyi and Egweh (2020) and Ciurescu et al. (2019) reported ALT and ALP activities of broiler chickens that range from 7.00 to 10.40 U/L and 137.75 to 309.00 U/L thus the observed activities fall within the range reported in other studies. Current study findings also showed normal hepatic microarchitecture without inflammation, ballooning and steatosis and similar liver fat content of the chickens across diets. Furthermore, the findings showed similarities in the plasma albumin (1.11 to 1.37 g/dL) and albumin/globulin ratio (0.77 to 0.90) concentration of the chickens across diets. However, broiler chickens that were fed zingerone at 120 mg kg^{-1} had decreased total protein (2.86 to 2.39 g/dL) and globulin (1.63 to 1.28 g/dL) compared to chickens fed zingerone at 0, 40 and 80 mg kg^{-1}. The decrease in plasma total protein which consists of albumin and globulin might be attributed to the indigent protein metabolism in the birds. Ciurescu et al. (2019) and Aikpitanyi & Egweh (2020) reported normal plasma total protein, albumin and globulin concentrations of broiler chickens to range from 2.04 to 2.66 g/dL, 1.25 to 1.54 g/dL and 1.36 to 1.66 g/dL, respectively. The results of the present study showed similar plasma total protein, albumin and globulin concentrations within the range of reported normal values. Interestingly, Wen et al. (2020) reported that fortifying broiler chicken diets with gingerol, also a phytochemical derived from ginger, returned similarities in broiler chickens' serum metabolites as counterparts fed the control diet. These findings suggest that zingerone can be used to fortify Cobb 500 broiler chicken diets with no risk of triggering alterations in liver fat accretion and without compromising hepatic synthetic

capacity and microarchitecture. Furthermore, its use did not mediate liver cell (canaliculi and bile duct hepatocyte) damage.

5.3.2 Oxidative stress and systemic antioxidant pool activity

Oxidative stress, in addition to compromising poultry productive performance, causes ill health. It is one of the key factors that mediate the pathogenesis of liver diseases (Cichoz-Lach & Michalak, 2014). An imbalance between the production of reactive oxygen species (ROS) and the systemic antioxidant pool's capacity to quench ROS results in oxidative stress (Lee et al., 2017). Catalase, GSH-Px, GST and SOD mop up free radicals (Büyükkılıç Beyzi et al., 2020) that would otherwise damage cellular organelles resulting in tissue and organ damage. These enzymes, which constitute a systemic antioxidant pool, protect against oxidative stress-induced organ damage (Baird & Dinkova-Kostova, 2011). Plasma MDA is a surrogate marker of oxidative stress (El-Bahr et al., 2020) and its higher than normal concentration is associated with excessive lipid peroxidation that may cause steatohepatitis (He et al., 2020). The present results shows similarities in plasma MDA concentration in the chickens fed the zingerone-fortified diets and counterparts fed the ZnBcn-fortified control diet. Furthermore, the systemic antioxidant pool activity (plasma GSH-Px, GST, SOD and CAT activities) of the zingerone-supplemented chickens did not differ from that of ZnBcn control counterparts. Supplementing broiler chicken diets with ginger powder or extracts has been reported to produce contradictory results in plasma CAT activity and MDA concentration (Zhang et al., 2009; Herrero-Encinas et al., 2023). In the current study, the similarities in the plasma MDA concentration and plasma GSH-Px, GST, SOD and CAT activities of zingerone-supplemented and ZnBcn-supplemented chickens suggest that the zingerone neither caused oxidative stress nor altered and compromised the activity of the broiler chickens' systemic antioxidant pool.

5.4 Conclusion

Zingerone is a bioactive substance with several health beneficial biological activities and its antioxidant properties protect the liver and kidney from ROS-induced oxidative damage. Based on the present study findings it can be concluded that zingerone at 40; 80 and 120 mg kg^{-1} of feed did not affect the kidney and liver function of Cobb 500 broiler chickens. Specifically, it can potentially be used in broiler chicken diets with no risk of perturbing the deposition of fat in the liver and without eliciting oxidative stress and perturbing the systemic antioxidant pool activity, damaging liver cells and causing hepatic steatosis and inflammation.

The next chapter gives a summary of the key conclusions drawn from the study, the limitations of the current study and some recommendations for future studies.

CHAPTER SIX: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusions

This study aimed to investigate the efficacy of zingerone to fortify broiler chicken diets in place of zinc bacitracin by specifically evaluating its effects on the growth performance, feed intake and utilisation efficiency, GIT and GIT accessory organs growth, meat yield and quality and flock health. It is concluded, based on the findings of the current study that zingerone can replace zinc bacitracin in Cobb 500 broiler chicken diets without compromising growth performance, feed utilisation efficiency, GIT viscera and long bone growth and development, meat yield and meat physico-chemical quality. Zingerone, as a dietary supplement in broiler chicken diets, can potentially be used to increase meat redness hence its preference by consumers and furthermore, it can be used to increase the concentration of favourable fatty acids (monounsaturated fatty acids and oleic acid; polyunsaturated fatty acids and linoleic acid) in the meat thus enhance its nutritional value. Dietary zingerone neither elicited oxidative stress nor alter the activities of the systematic antioxidant enzymes. Furthermore, its use did not affect kidney and liver health in Cobb 500 broiler chickens. The effectiveness of Zingerone to replace zinc bacitracin as a grower promoter in broiler Cobb500 diets demonstrates that it can be used to promote "greener and less environmentally abrasive' poultry production. Importantly, it can potentially be used to reduce and or eliminated the public health challenge of antibiotic resistance in both consumers of poultry products and farmed bird species.

6.2 Limitations and recommendations for future studies

In the current study, fewer replications (3 per dietary treatment) were used against 5 to 6 replicates. A limitation in the present study was the low number of replications used. In hindsight, it is realised that the number of birds per replicate could have been reduced in order to increase the number of replicates. The reduced number of replicates, which could have impacted findings, was due to a limitation in research funds and importantly the need to comply with principle of 3Rs in animal research. The Skeletal system integrity is vital in broiler chicken production. In the current study only long bone mass, length and mass:length ratio was determined. These parameters (mass, length and ratio) do not give a complete picture of bone health and skeletal integrity. Determination of bone-breaking strength, bone mineral composition, bone histology and assaying enzymes that regulate bone growth and development would have provided more insight into the effects of supplemental zingerone. It is, therefore, recommended that future studies consider their measurement. Determination of hormones that regulate feed

intake and satiety would have, perhaps helped, confirm findings, especially in view of similarities in feed intake and utilisation efficiency and growth performance.

Although the colour coordinates in broiler chicken meat were measured, due to lack of funds, the myoglobin oxidation state and concentration of different muscle fibre types were not determined to try to explain the increased meat redness at zingerone 40 mg kg^{-1} of feed. Measurement of the myoglobin oxidation state and concentration of different muscle fibre types in the meat might have helped shed more light regarding the observed "improved redness" in the chicken breast meat with zingerone supplementation at 40 mg kg^{-1} feed. The determination of the myoglobin oxidation state in future studies is recommended.

While findings from the current study showed that supplemental zingerone improved meat colour and increased its nutritive value exemplified by it (supplemental zingerone) mediating increase in the meat's oleic, linoleic, total mono- and total poly-unsaturated fatty acid content, sensory evaluation, which constitutes part of quality measurement, would have given information on the effect of dietary zingerone on the eating quality of Cobb 500 broiler chicken meat. Thus, it is recommended that future studies on the efficacy of "test feed additives" consider the inclusion of sensory evaluation of meat.

Although plasma markers of oxidative stress and the response of the systemic antioxidant pool were measured and determining these in the liver kidney and liver would have given tissue-specific effects of zingerone in addition to a generalised systemic overview given by plasma determinations. Further studies should consider determining the effects of dietary interventions on both systemic and tissue levels.

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APPENDICES

Appendix 1: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I **BAYANDA MDODA** (Student number: **1835040**) am a student registered for the degree of **PhD** in the academic year **2023.**

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permissionand/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my ownunaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:_____ Date: 30/03/2023

1

Appendix 2: Animal ethics clearance

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2020/10/02/C

APPLICANT: Mr B Mdoda

School: School of Physiology; Department: N/A; Location: CAS

PROJECT TITLE: Effect of supplemental zingerone on broiler chicken (*Gallus gallus domesticus*) growth, health and meat quality

Category: C; Species and Numbers involved: *150X 1 day old male Broiler Chickens (Gallus gallus domesticus), Commercial chickens (Cobb 308)*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 27 Oct 2020. This approval remains valid until 2 Dec 2022 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4
liaise with CAS in due time for the supply of animals			

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ _____ Date: __04/12/2020____

(Chairperson of the AREC)

I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

Signed: _____ Date: __04/12/2020_____

(Registered Veterinarian)

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Central Animals Service: Dr Kim Jardine

Appendix 3: Modification and extensions to experiments

AESC 2012 M&E

Please note that only type written applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND
ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Bayanda Mdoda (1835040)

b. School and email address: School of Physiology. 1835040@students.wits.ac.za

c. Experiment to be modified / extended **AESC NO**

Original AESC number	**2020**	**10**	**02C**
Other M&Es:			nil

d. Project Title: Effect of supplemental zingerone on broiler chicken (*Gallus gallus domesticus*) growth, health and meat quality

		No.	**Species**
e.	Number and species of animals originally approved:	150	Broiler chicks
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	50	
h.	Number of animals used to date:	50	

i. Specific modification / extension requested:

1. To include: Ms Faith Machabi (1849669) as a co-worker.

j. Motivation for modification / extension:

1. Ms Faith Machabi (1849669) will assist with animal husbandry and laboratory assays.

Date: 03/03/2021 Signature: 

RECOMMENDATIONS

- Addition of Ms Faith Machabi as a co-worker.

Condition: The co-workers must attend the first-time user's course facilitated by WRAF before working with animals. Please contact Lorraine Matjila (lorraine.matjila@wits.ac.za) for details.

Date: 05 March 2021 Signature: Deputy Chair, AESC

Appendix 4: Modification and extensions to experiments

AESC 2012 M&E

Please note that only type written applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND
ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Bayanda Mdoda (1835040)
b. School and email address: School of Physiology. 1835040@students.wits.ac.za
c. Experiment to be modified / extended **AESC NO**

Original AESC number	**2020**	**10**	**02C**
Other M&Es:			nil

d. Project Title: Effect of supplemental zingerone on broiler chicken (*Gallus gallus domesticus*) growth, health and meat quality

		No.	Species
e.	Number and species of animals originally approved:	150	Broiler chicks
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	100	
h.	Number of animals used to date:	100	

i. Specific modification / extension requested:

1. Addition of 10 broiler chicks to replace the outliers.

j. Motivation for modification / extension:

1. Thus far, the experiment that I am running is going well, only one chick has died from the total of 100 chicks that I currently have. From all my dietary treatments I have one chicken which weighs less than 1000g of which the targeted weight gain for this study is between 1500-2500g. The chickens which weigh less than 1000g will jeopardise the end results of this study, thus I kindly request to add 10 broiler chicks to replace those that will weigh less than 1000g.

Date: 08/04/2021 Signature: 

RECOMMENDATIONS

- Addition of 10 broiler chicks to replace the outliers. In each dietary treatment 2 chicks will be added to make a total number of 32 chicks per dietary treatment.

Date: 12/04/2021

Signature: 

Chairman, AESC

Appendix 5: Article acceptance certificate

Links Researchers

Acceptance Certificate

This document certifies that the manuscript listed below is accepted for publication:

Effects of zingerone on growth performance, feed intake and utilisation efficiency, carcass yield and viscera macromorphometry of Cobb 500 broiler chicken

Journal	Journal of Animal Health and Production
Manuscript ID	MH20230118100127-R1
Manuscript Type	Research Article
Area of Interest	Animal Production
Complete List of Authors:	■ Mr Bayanda Mdoda, University of the Witwatersrand, South Africa ■ Ms Faith Machabi, University of the Witwatersrand, South Africa ■ Dr Busisani Lembede, University of the Witwatersrand, South Africa ■ Prof Eliton Chivandi, University of the Witwatersrand , South Africa

This certificate can be verified by the editorial office of the journal using following details:

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Appendix 6: Animate animal health feed premix

Animate
Animal Health

FOR ANIMAL USE ONLY

ZINC BACITRACIN® 15% GRANULAR

Reg. No. G3318 (Act 36 of 1947)
FEED PREMIX

INDICATIONS
Zinc Bacitracin® 15% Granular is recommended for use in sheep, calves, beef cattle, broilers, turkeys and pigs to improve feed efficiency and mass gain. It is also recommended for use in layers for increased egg production.

CAUTION

STORAGE INSTRUCTIONS
Store in a cool, dry place.

COMPOSITION
Zinc Bacitracin 150 g/kg

WARNINGS
Keep out of reach of children, uninformed persons and animals.
Handle with care.
Although this remedy has been extensively tested under a large variety of conditions, failure thereof may ensue as a result of a wide range of reasons. If this is suspected, seek veterinary advice and notify the registration holder.

WITHDRAWAL PERIOD
All species – Zero (0) days

DIRECTIONS FOR USE
Use only as directed. Intended for further processing. To be mixed into feed before use.
Feed continuously at the following levels of inclusion:

Species	Zinc Bacitracin gram active per ton of feed	Zinc Bacitracin® 15% Granular gram premix per ton of feed
Sheep	10 – 50g	67 – 333g
Calves	10 – 100g	67 – 667g
Beef Cattle	10 – 50g	67 – 333g
Broilers	10 – 100g	67 – 667g
Turkeys	10 – 50g	67 – 333g
Pigs	10 – 100g	67 – 667g
Layers	10 – 100g	67 – 667g

BATCH NUMBER:
EXPIRY DATE:

NET WEIGHT: 25 kg

Registration holder: Animate Animal Health (Pty)Ltd. (Co.Reg.No.1973/002312/07)
43 Hornbill Avenue, Rooihuiskraal, 0157; PO Box 66698, Highveld, 0169;
Phone (+27 12) 661 3485; Fax (+27 12) 661 3486; www.animate.co.za

AFRI COMPLIANCE
Biesterfeld
Biesterfeld International GmbH
AFMA SAFE FEED FOR SAFE FOOD

Appendix 7: Certificate of registration for zinc bacitracin

agriculture,
forestry & fisheries

Department:
Agriculture, forestry & fisheries
REPUBLIC OF SOUTH AFRICA

FOR OFFICIAL USE ONLY

CERTIFICATE OF REGISTRATION: STOCK REMEDY

Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947)

1. This is to certify that the stock remedy mentioned below and the label attached hereto comply with the requirements of Act No. 36 of 1947 and the regulations promulgated there-under and that it has been registered by me:

   1.1 Registration Number awarded **G3318**

   1.2 Name of remedy..... **Zinc Bacitracin 15%**

   1.3 Name of applicant..... **Animate Animal Health (Pty) Ltd**

   1.4 Active ingredient (s)..... **Zinc Bacitracin and Calcium Carbonate**

   1.5 Type of product..... **Polypeptide Antibiotic**

2. This registration is further subject to the following conditions:

   2.1 That the registration is only valid for three (3) years and must be renewed **30 June 2023**.

   2.2 That only facsimiles of the attached approved label may be used.

   2.3 The type and container size must conform to the sizes as stated in paragraph 6.1 of the application form.

   2.4 That the container in which the stock remedy is offered for transport shall conform to the applicable packaging specifications as laid down by SABS Code of Practice 0229.

   2.5 That the printed labels, cartons, pamphlets and package inserts be submitted within 3 (three) months from the date of registration.

   2.6 That if the source of active ingredient is changed the Registrar must be informed in writing.

   2.7 That all adverse effects, including adverse reactions, toxicity, misuse, formulation deviation or any other undesirable effect caused by this product must be reported immediately to the Registrar by the registration holder.

3. The granting of this registration does not exempt anybody from the requirements of any other Law.

Dept of Agriculture, Forestry and Visserye
Private Bag X343
DATE 2020 -11- 11
Pretoria 0001
Dept of Agriculture, Forestry and Fisheries

REGISTRAR: ACT NO. 36 OF 1947

Appendix 8: Histology scoring criteria

A. Criteria assessed: 1. hepatocellular ballooning (H and E)
2. Steatosis (H and E)
3. Inflammation (H and E)
Location of the changes: perivenular (zone 3), perisinusoidal (zone 2) and periportal (zone 1). Steatosis can be classified as macrovesicular or microvesicular.

B. Semi quantitative: Grading or Scoring
Scored from 0-3

Steatosis

0- < 5%
1- 5-33%
2- 33-66%
3- > 66%

Inflammation

0- None or no foci per camera field (approx. X200)
1- < 2 foci per camera field
2- 2-4 foci per camera field
3- > 4 foci per camera field

Ballooning

0. None
1. A few cells
2. Many cells and often prominent ballooning

Appendix 9: Elisa protocols

Bioassay Technology Laboratory

Optimize Your Research

Chicken Catalase ELISA Kit

USER INSTRUCTION

Cat.No E0118Ch

Standard Curve Range: 0.5ng/ml - 200ng/ml

Sensitivity: 0.2ng/ml

Size: 96 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Chicken Catalase (also known as CAT) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Chicken CAT antibody. CAT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Chicken CAT Antibody is added and binds to CAT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CAT antibody. After incubation

unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Chicken CAT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Provided

Components	Quantity
Standard Solution (240ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Chicken CAT Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Material Required But Not Supplied

- 37°C±0.5°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- This instruction must be strictly followed in the experiment.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.

- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2 - 8°C within 30 minutes of collection.

Urine Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

Cell Culture Supernatant Collect by sterile tubes when examining secrete components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Tissue Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Note

- Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must **contact us** to determine the optimal sample for their particular experiments.
- Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.

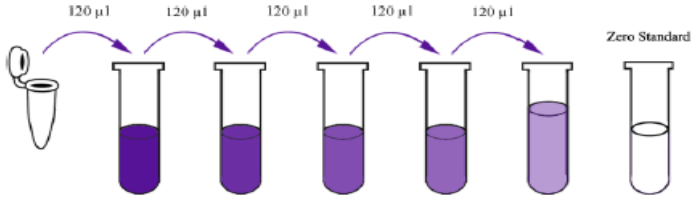
- Centrifuge to collect sample before use.
- Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Sample can't be diluted with this kit. Owing to the the material we use to prepare the kit, the sample matrix interference may falsely depress the specificity and accuracy of the assay.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Standard** Reconstitute the 120μl of the standard (240ng/ml) with 120μl of standard diluent to generate a 120ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (120ng/ml) 1:2 with standard diluent to produce 60ng/ml, 30ng/ml, 15ng/ml and 7.5ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20℃ and used within one month. Dilution of standard solutions suggested are as follows:

120ng/ml	Standard No.5	120μl Original Standard + 120μl Standard Diluent
60ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard Diluent
30ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard Diluent
15ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard Diluent
7.5ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
240ng/ml	120ng/ml	60ng/ml	30ng/ml	15ng/ml	7.5ng/ml

- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently

until the crystals have completely dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. ***Note:*** *Don't add antibody to standard well because the standard solution contains biotinylated antibody.*
4. Add 40µl sample to sample wells and then add 10µl anti-CAT antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Summary

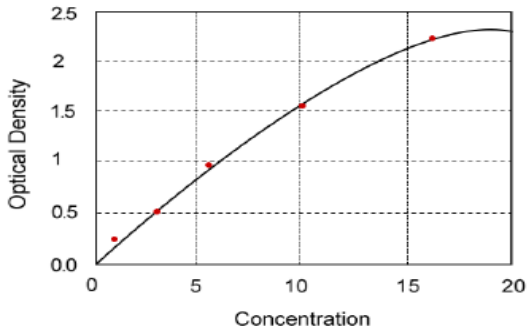
1. Prepare all reagents, samples and standards.
2. Add sample and ELISA reagent into each well. Incubate for 1 hour at 37°C.
3. Wash the plate 5 times.
4. Add substrate solution A and B. Incubate for 10 minutes at 37°C.
5. Add stop solution and color develops.
6. Read the OD value within 10 minutes.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Bioassay Technology Laboratory

Optimize Your Research

Chicken Glutathione Peroxidase ELISA Kit

USER INSTRUCTION

Cat.No E0298Ch

Standard Curve Range: 0.1ng/ml - 40ng/ml

Sensitivity: 0.052ng/ml

Size: 96 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Chicken Glutathione Peroxidase (also known as GPX) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Chicken GPX antibody. GPX present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Chicken GPX Antibody is added and binds to GPX in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated GPX antibody. After incubation

Bioassay Technology Laboratory

Optimize Your Research

Chicken Glutathione S-transferases ELISA Kit

USER INSTRUCTION

Cat.No E0095Ch

Standard Curve Range: 0.5ng/ml - 150ng/ml

Sensitivity: 0.25ng/ml

Size: 96 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Chicken Glutathione S-transferases (also known as GSTs) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Chicken GSTs antibody. GSTs present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Chicken GSTs Antibody is added and binds to GSTs in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated GSTs antibody. After incubation

Bioassay Technology Laboratory

Optimize Your Research

Chicken Superoxide Dismutase ELISA Kit

USER INSTRUCTION

Cat.No E0295Ch

Standard Curve Range: 0.1ng/ml - 40ng/ml

Sensitivity: 0.044ng/ml

Size: 96 wells

Storage: Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

***This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Intended Use

This sandwich kit is for the accurate quantitative detection of Chicken Superoxide Dismutase (also known as SOD) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Chicken SOD antibody. SOD present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Chicken SOD Antibody is added and binds to SOD in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated SOD antibody. After incubation