Effect of fulvic acid inclusion in broiler diets on growth performance, physiological and meat quality parameters

KC Mokubung

orcid.org 0000-0001-5847-4098

Dissertation accepted in fulfilment of the requirements for the Masters (MSc) degree at the North-West University

Supervisor: Prof U Marume

Graduation: July 2024

DECLARATION

I, Keamogetswe Mokubung affirm that this dissertation entitled "The effect of fulvic acid inclusion in broiler diets on growth performance, physiological and meat quality parameters" submitted to the North-West University for master's degree in Agriculture with Animal Science has not been previously submitted to any other university or institution. This dissertation is my own original work. All assistance received in the process of producing this work, as well as all references included herein have been duly acknowledged.

Candidate: Keamogetswe Mokubung

Signed……. Date……25/11/2023…….

Promoter: Prof U. Marume

Signed  ……. Date…**27-11-2023…….**

GENERAL ABSTRACT

The use of non-conventional feed sources in poultry farming has a beneficial impact on sustainably improving nutrient supply in poor communities in South Africa. Fulvic acid (FA) is one of the feed resources which can promote animal performance by improving growth rate, immunity, feed efficiency and reducing the risk of infections, but its utility in broilers has not been fully assessed. This study was therefore, undertaken to investigate the effect of FA inclusion in broiler diets on growth performance, physiological and meat quality parameters. For six weeks, chickens were fed five isonitrogenous and isoenergetic experimental diets formulated as follows: negCON (T1): commercial diets without antibiotics, posCON (T2): commercial diet with antibiotics, FA1 (T3) commercial diet + 1% FA1, FA2 (T4): commercial diet + 2% FA and FA3 (T5): commercial diet with 3% FA. The feeding trial was conducted over 3 feeding phases, Starter (day 1-14); Grower (15-28) and Finisher ration (day 28-42). Birds fed the posCON diet had the highest ($P < 0.05$) ADFI (93.33 g) followed by those fed the FA2 (90.66 g) diet. Dietary inclusion on cumulative weight gain (CWG) in the final week revealed that birds fed the FA2 (1897.34 g) diet gained at the same level as those fed the posCON (1932.74 g) diets and birds in both treatments achieved similar final weights at the end of the feeding trial. Dietary inclusion treatments significantly influenced protein utilization and growth efficiency parameters in all the three growth phases apart from the protein efficiency ratio (PER) in the grower phase, the protein consumed (PC) and the specific growth rate (SPR) at the finisher phase. Chickens offered the negCON (44.05) diet had the highest platelet count, while broilers offered the FA3 diet had the lowest platelet count values. Birds fed the posCON, FA1 and FA2 diets had statistically similar values for platelet counts with FA3 having the least value. Dietary treatments significantly ($P <0.05$) affected the glucose concentration, symmetric dimethylarginine (SDMA), alanine transaminase (ALT), alkaline phosphatase (ALP), phosphorus and lipase concentration. The SDMA was observed to decline

with increasing levels of FA inclusion in diet with the birds fed the FA3 (6.87 μg/dL) diet having the lowest levels of SDMA compared with all other diets. Diet had a significant effect (P <0.05) on all measured carcass characteristic parameters apart from the dressing percentage, chilling loss percentage and the thigh index. Chickens fed the posCON diet (1959.27 g) obtained the highest slaughter weights, followed by those offered the FA2 diet (1942.10 g), while broilers offered the negCON diet had the lowest (1789.25 g) value. Chickens offered posCON (42.61) diet had statistically similar values for lightness as with those fed FA1 (42.62) and FA3 (42.54) diets. However, the birds fed the FA2 (41.90) diets appeared to have darker breast muscle compared to all other treatments. Findings from this study illustrate that the experimental diets cooperated with FA inclusion revealed an increase in total PUFA/SFA ratio compared to the control diets. However, diets consisting of FA reduced the total n-3/n-6 ratio in all the FA treatments with FA2 having a ratio of 5 in the n-3PUFA, resulting in a beneficial impact on the important fatty acid needed for human and animal health maintenance. Results showed that diet had no effect on latency to lie, feather and gait scoring test. The highest latency to lie was observed in broilers fed the positive control diet with the highest standing persistence observed in broilers fed FA2 followed by FA3. Diet showed no dietary influence on all micro and macro minerals apart from phosphorus, iron and molybdenum. Serum calcium levels were higher in all the diets containing FA compared to the control. FA inclusion in diets also increased calcium levels and ash concentration with increment levels. Overall, the inclusion of FA in broiler diets did not compromise the nutrient utilization efficiency, broiler performance, health status, meat quality traits, internal organs, carcass traits and bone development of broiler diets. Collectively, findings from this study may be useful in developing low-cost feed formulas that will benefit customers and enhance future chicken farming systems for growth performance, health status, carcass features, meat quality.

KEYWORDS: Antibiotic growth promoters, Bone development, Broilers, Fulvic acid, Blood parameters, Organic acid.

ACKNOWLEDGEMENTS

My thanks initially must go to God, who gives the ability to do all things, the Almighty father, who has given me courage to pursue my master's studies and to complete this dissertation.

Special gratitude is extended to the National Research Foundation (NRF) MND210602605661, NWU masters bursary and the faculty bursary (FNAS) for the financial support.

I want to sincerely thank my supervisor Professor Upenyu Marume for his patience and to appreciate his guidance and support.

I want to humbly thank Mr Pieter Vorster and the entire team at Ecofarmer (Pty) Ltd, Mr Navin Saydah from Autus Growth for the contribution of fulvic acid, may the Great Lord continue to bless them.

I am very humbled by the gratuitous help from the Animal Science and Animal health undergraduate and postgraduate participants, academic support and staff members; thank you so much for your time and assistance, I am indeed grateful for your help, especially during the time-consuming feeding trials and mixing of feed.

Another special gratitude goes to my family and friends, for their undying love, loyalty and everlasting support throughout the entire journey. My mother Matshediso Mokubung, who served as an inspiration. I strive to put a spotlight on her sacrifices and efforts. *Kea leboga Tshikzozo wam.*

DEDICATION

This work is dedicated to my father, Daniel Macuacua, who met his untimely death the second day of my feeding trial experiment. Continue resting in peace king dad.

Put all your faith in the Lord and acquire knowledge through your own comprehension: in every aspect, acknowledge him and he will straighten your path.
Proverbs 3:5-6

TABLE OF CONTENTS

DECLARATION I
GENERAL ABSTRACT II
ACKNOWLEDGEMENTS V
DEDICATION VI
TABLE OF CONTENTS VII
LIST OF TABLES XI
LIST OF FIGURES XII
LIST OF ABBREVIATIONS XIII
CHAPTER ONE 1
1. GENERAL INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 3
1.3 Justification 3
1.4 Objectives of this study 4
1.5 Hypothesis 4
1.6 References 6
CHAPTER 2: LITERATURE REVIEW 9
2.1 Introduction 9
2.2 Use of feed additives in broiler diets 9
2.3 Alternative feed additives 10
2.4 Humic substances as alternative growth promotants 11
2.5 Potential effects of fulvic acid 13
2.5.1 Immunomodulation effects 13
2.5.2. Oxidative stress 16
2.5.3. Gut health 18
2.5.4 Bone development and incidents of bone disease 20
2.5.5 Potential of fulvic acid as ethnomedicine 20
2.5.6 Influence on blood parameters and health 22
2.5.7 Effect of fulvic acid in meat quality attributes 22
2.6 Summary 23

2.7 References 24

CHAPTER 3 33

EFFECT OF FULVIC ACID ON GROWTH PERFORMANCE, PROTEIN UTILIZATION EFFICIENCY AND BLOOD METABOLITES IN BROILER CHICKENS 33

3.1 Introduction 34

3.2 Materials and methods 35

3.2.1 Description of the study site 35

3.2.2 Sourcing of fulvic acid 36

3.2.3 Dietary treatments and experimental design 36

3.2.4 Animal management 37

3.2.5 Growth performance 42

3.2.6 Protein utilization efficiency 42

3.2.7 Hematology and serum biochemical analysis 43

3.2.8 Statistical analysis 44

3.3 Results 45

3.3.1 Feed intake and growth performance 45

3.3.3 Protein utilization and growth efficiency 49

3.3.3 Haematological parameters 49

3.3.4. Serum biochemistry 52

3.4 Discussion 54

3.5 Conclusion 59

3.6 References 60

CHAPTER 4 67

THE EFFECT OF FULVIC ACID ON CARCASS CHARACTERISTICS AND 67

MEAT QUALITY PARAMATERS IN BROILER CHICKENS 67

4.1. Introduction 68

4.2 Materials and methods 69

4.2.1 Study site, source of diets and animal management 69

4.2.2 Slaughter process 70

4.2.3 Carcass traits 70

4.2.4 Internal organs 71

4.2.5. Meat quality measurements 71

(i) Meat pH and temperature measurements....71
(ii) Meat colour....71
(iv) Meat tenderness....72
(vii) Proximate and fatty acid analysis....73
4.2. 6 Statistical analyses....74
4.3 Results....75
4.3.1 Carcass characteristics....75
4.3.2 Internal organs....77
4.3.3 Meat quality....77
4.3.4 Individual fatty acid profiles....80
4.3.5 Total fatty acids....82
4.4 Discussion....82
4.5 Conclusion....87
4.6. References....89
CHAPTER 5....94
THE EFFECT OF FULVIC ACID ON BONE DEVELOPMENT AND....94
BROILER WELFARE....94
5.1 Introduction....95
5.2 Materials and methods....96
5.2.1 Study site, source of diets and animal management....96
5.2.3 Gait scoring....97
5.2.4 Feather scoring....97
5.2.5 Tibia gross lesion analysis and tibia linear parameters....98
5.2.6. Bone ash and mineral content....98
5.3 Statistical analysis....99
5.4 Results....100
5.4.1 Latency to lie test, gait and feather scores....100
5.4.2 Tibial growth plate analysis....100
5.4.3 Tibia biomechanical parameters....103
5.4.4 Bone ash and mineral content....103
5.5 Discussion....106
5.6 Conclusion....109
5.7 References....110

CHAPTER 6 115
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS 115
6.1 General discussion 115
6.2 Conclusion and recommendations 117
6.3 Future prospects 118
6.4 References 119
7. LIST OF APPENDICES 120
7.1 Ethical certificate 120
7.2. Language editing certificate 121
7.3. Turnitin report 122

LIST OF TABLES

Table 3.1. Ingredient composition (%) of (FA) for starter diet. 38

Table 3.2. Ingredient composition (%) of (FA) for grower diet. 39

Table 3.3. Ingredient composition (%) of FA for finisher diet. 40

Table 3.4. Nutrient composition (%) of formulated experimental diets. 41

Table 3.5. The effect of diets containing FA in average feed intake (g), average daily weight gain and feed conversion ratio of broiler chickens. 46

Table 3.6. The effect of diets containing FA on protein utilization efficiency (g) of Cobb 500 broiler chickens. 50

Table 3.7. The effect of diets containing FA in blood haematology of Cobb 500 broiler chickens.....51

Table 3.8. The effects of diets containing of FA in serum biochemistry of Cobb 500 broiler chickens.....53

Table 4.1. The effect of diets containing FA on carcass characteristics in broiler diets. 76

Table 4.2. The effect of diets containing FA on internal organs in broiler chickens.....78

Table 4.3. The effects of dietary treatments on meat quality traits of broiler chickens.....79

Table 4 4. The effect of diets containing FA on fatty acid composition. 81

Table 4.5. The effect of diets containing FA on fatty acids profiles (total nutritional indices) of broiler meat. 83

LIST OF FIGURES

Figure 2.1. Average molecular structure of HA (source Guan-qun Gong, 2020). 12

Figure 2.2. Compound structure of FA.....12

Figure 2.3. The origin of humic substances and relationship among them 15

Figure 2.4. Potential effects of FA on immune system in different animal species…… 16

Figure 2.5. Potential effects of FA on oxidative stress.....17

Figure 2.6. FA effects on gut health 18

Figure 3. 1. Picture of FA. 36

Figure 3. 2. The effect of dietary FA on cumulative weight gain on broiler chickens. 48

LIST OF ABBREVIATIONS

a*	Redness
L*	Lightness
b*	Yellowness
ADFI	Average daily feed intake
ADWG	Average daily weight gain
AGPs	Antibiotic growth promoters
ALT	Alanine amino transaminase
ALP	Alkaline phosphate
ANFs	Anti nutritional factors
Ca	Calcium
CBZ	Carbamazepine
CCW	Cold carcass weight
COX2	Cyclooxygenase 2
CP_d	Crude protein concentration
CRD	Completely randomized design
ESNP	Eosinophils
FA	Fulvic acid
FAMEs	Fatty acid methyl esters
FCR	Feed conversion ratio
FI	Feed intake
GE	Gain efficiency
GIT	Gastrointestinal tract
GIQLI	Gastrointestinal quality of life index
HA	Humic acid
HS	Humic substance
HCW	Hot carcass weight
HCL	Hydrochloric acid
LTL	Latency to lie
LPS	Lipopolysaccharide
MNC	Monocytes

NegCON	Negative control
OA	Organic acid
P	Phosphorus
PC	Protein consumed
PDIFF	Probability of difference
PER	Protein efficiency ratio
PFOS	Perfluorooctanesulfonate
PGE2	Prostaglandin E2
PMM	Pectoral major muscle
posCON	Positive control
PUE	Protein utilization efficiency
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
RP	Reducing power
SDMA	Symmetric dimethylarginine
SGR	Specific growth rate
SFA	Saturated fatty acid
SOD	Superoxide dismutase
TDPE	Tibia diameter proximal end
TLD	Tibia length diameter
TNF-α	Tumour necrosis factor alpha
TPC	Total phenol content
TWD	Tibia width diameter
TW	Tibia weight
VAS	Visual analogue scale
WBC	White blood cell
WHC	Water holding capacity

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1 Background

Commercial growth promoters and feed additives have been commonly used in extensively large-scale broiler production units to stimulate growth, health and general productivity (Dibner & Richards, 2005). However, their continuous usage is becoming a cause of concern among consumers as they are increasingly inducing persistence of residues in poultry products and increased microbial resistance (Alloui *et al*., 2014). This has resulted in a ban of the use of antibiotic growth promotants (AGPs) by European Union (EU) in 2005. The exclusion of AGPs has stimulated great efforts by researchers to explore effective alternatives to antibiotics (Yanga e*t al*., 2019). Many attempts have now been made in assessing the efficacy of alternatives to commercial growth promotants and performance enhancers including the potential use of natural phytogenic essential oils or phytobiotics, organic acids and pre and probiotics (Mpofu *et al*., 2016; Disetlhe *et al*., 2018). As early as the 1950s, AGPs were commonly used in food-animal production systems all over the world to develop growth and animal performance (Van *et al*., 2020; Zommiti *et al*., 2020). Amid the growing consumer concerns on antibiotics use, numerous alternative feed additives have been suggested including organic acids, which are naturally occurring compounds such as acetic, lactic, propionic, butyric acids and humic elements which includes humic and fulvic acid (Polycarpo *et al*., 2017).

Fulvic acid (FA) is one of the organic humic substances that is receiving much attention as an alternative AGPs due to their chemical composition and characteristics. These include among others, their ability to improve animal production performances by enhancing growth rate, boost feed efficiency and immunity, and weaken the risk of infections (Islam *et al.,* 2005; Vucskits *et al.,* 2010; Kunavue *et al.,* 2012). FA is obtained from humic substances, which are a class of high molecular weight, heterogeneous organic substances that occur naturally (Ukalska-Jaruga *et al*., 2021). The bacterial and chemical degradation of lignin and other structural plant carbohydrates within the subsoil are responsible for forming the intermediate products of humic acid and FA (Kunavue *et al.,* 2012). Several reactive groups functional groups, such as phenols, hydroxyls, carboxyls and quinones are present in these organic substances (Jusadi *et al*, 2020). These reactive groups indicate that FA may select from a variety of advantageous effects, including stress management Plaza *et al.* (2005), immunostimulation, metal chelation, antioxidant mechanism Chang *et al.* (2013), Mao *et al.* (2019), induction of efficient intestinal permeability and improved gut function (Winkler *et al*., 2018). It is therefore, imperative to evaluate the efficiency of FA usage as a potential alternative growth promotant in Cobb 500 chickens.

The effect of dietary FA inclusion in diets on growth performance traits of broilers was assessed. The results showed that inclusion levels of FA in broiler diets had no negative effects on growth performance and protein utilisation efficiency. Diet significantly affected all measured carcass parameters apart from the dressing percentage, chilling loss percentage and the thigh index. Results showed that broilers fed the FA2 and FA3 diets appeared to have the highest standing persistence, indicating that the inclusion of FA in two experimental diets led to the optimum bone strength qualities

1.2 Problem statement

The increasing consumer concerns of the use of AGPs in broiler meat production, which has led to the ban of AGPs in the European Union Yanga *et al.* (2019) has stimulated immense efforts by scientist, researchers, and industries in search of potential alternatives to AGPs, particularly naturally occurring compounds. FA can be used as a potential alternative to the conventional antibiotics. The functional groups in the acid, such as carboxyl, phenol, hydroxyl, amine, and quinine can alter meat quality characteristics in Cobb 500 broilers (Hanim *et al.*, 2012; Gasco *et al.*, 2020). Some research has suggested that FA may be utilized as a natural feed supplement in animal production to protect against infections and poisoning Arief *et al.* (2019) as well as increasing nutrient utilization and growth performance (Winkler *et al.*, 2018). Nevertheless, there is general lack of scientific evidence of their potential use in the broiler industry. Although there is vast amount of evidence of the potential of other organic acids as growth promoters, their effects are rather specific and cannot be generalised because their chemical composition and origins differs. Due to this fact, assessment of the efficacy of FA as a potential growth promoter is imperative.

1.3 Justification

In recent years, concerns about antibiotic usage long term effects on the environment and possible risks to human health have grown (Kadykalo *et al.*, 2018; Marshall & Levy, 2011). Therefore, researchers are in constant pursuit of potential naturally occurring compounds, to eliminate pathogenic resistance found in poultry by products and to improve broiler performance, carcass characteristics, blood parameters and intestinal enzyme activity as a non-antibiotic option. Generally, there is vast amount of empirical evidence of the potential of some organic acids as growth promotants. However, the effects of various organic acids are rather specific because their chemical composition differs and cannot be generalised to all other

untested organic acids. The use of FA as a substitute feed additive in animal production, is still at its infant stage, therefore, it is of importance to gather as much information on the positive effects as it may have on broilers to improve its integration in the poultry production systems. Knowledge of the utilization of FA is expected to provide a sustainable and safe approach to commercial poultry production. The information from this study may also provide ways in which FA can be incorporated in broiler diets for optimum productivity and provide advanced knowledge on the influence of FA on growth traits, physiological, meat quality parameters and bone development in broiler chickens.

1.4 Objectives of this study

The study evaluated the effect of FA inclusion levels in broiler diets, growth performance, physiological parameters, meat quality and bone development in broilers.

Specific objectives

(a) To determine the effect of FA inclusion in diets on growth performance, protein utilization efficiency and health.

(b) To investigate the effect of FA inclusion in diets on carcass characteristics, meat quality characteristics and fatty acids profiles of broiler meat.

(c) To determine the effect of FA inclusion in diets on bone development and welfare in broilers.

1.5 Hypothesis

a) Inclusion of FA in broiler diets does not affect growth performance, and protein utilization efficiency and health.

b) Inclusion of FA in broiler diets does not affect carcass characteristics and meat quality characteristics of broiler meat.

c) Inclusion of FA in broiler diets does not affect bone development, latency to lie, tibia characteristics and bone mineralization.

1.6 References

Alloui, M.N., Agabou, A. & Alloui, N., 2014. Application of herbs and phytogenic feed additives in poultry production-A Review. Global. J. Anim. Sci. Res. 2(3), 234-243.

Arif, M., Alagawany, M., Abd El-Hack, M.E., Saeed, M., Arain, M.A. & Elnesr, S.S., 2019. Humic acid as a feed additive in poultry diets: A review. Iran. J. Vet. Res. 20(3), 167.

Chang, Q.F., Bai, H.X., Shi, B.M. & Shan, A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing-finishing pigs. Livest. Sci. 158, 118-123.

Dibner, J.J. & Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84, 634-643.

Disetlhe, A.R.P., Marume, U. & Mlambo, V., 2018. Humic acid and enzymes inclusion in canola-based diets generate different responses in growth performance, protein utilization dynamics, and hemato-biochemical parameters in broiler chickens. Poult. Sci. 97(8), 2745-2753.

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P.P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G. & Pinotti, L., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Italian. J. Anim Sci. 19(1), 360-372.

Hanim, A.N., Muhamad, A.N., Ahmed, O.H., Susilawati, K. & Khairulmazmi, A., 2012. Physico-chemical properties of indigenous microorganism-composts and humic acid prepared from selected agro-industrial residues. Afr. J. Biotechnol. 11(34), 8456-8463.

Islam, K.M.S., Schuhmacher, A. & Gropp, J.M., 2005. Humic acid substances in animal agriculture. Pak. J. Nutr. 4(3), 126-134.

Jusadi, D., Aprilia, T., Setiawati, M., Suprayudi, M.A & Ekasari, J., 2020. Dietary supplementation of fulvic acid for growth improvement and prevention of heavy metal accumulation in Nile tilapia fed with green mussel. Egypt. J. Aquat. Res. 46(3), 95-301.

Kunavue, N. & Lien, T.F., 2012. Effects of fulvic acid and probiotic on growth performance, nutrient digestibility, blood parameters and immunity of pigs. J. Anim. Sci. Adv, 2(8), 711-721.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M. & Espeisse, O., 2018. The value of anticoccidials for sustainable global poultry production. Int. J. Antimicrob. Agents. 51(3), 304-310.

Mao, Y., 2019. Modulation of the growth performance, meat composition, oxidative status, and immunity of broilers by dietary fulvic acids. Poult. Sci. 98(10), 4509-4513.

Marshall, B.M. & Levy, S.B., 2011. Food Animals and antimicrobials: Impacts on human health. Clin. Microbiol. Rev. 24, 718-733.

Mpofu, D.A., Marume, U., Mlambo, V. & Hugo, A., 2016. The effects of *Lippia javanica* dietary inclusion on growth performance, carcass characteristics and fatty acid profiles of broiler chickens. Anim Nutri. 2(3), 160-167.

Plaza, J.C., Garcia-Gil A. & Polo, N., Senesi, G. & Brunetti, G., 2005. Proton binding by humic and fulvic acids from pig slurry and amended soils. J. Environ. Qual. 34, 1113-1117.

Polycarpo, G.V., Andretta, I., Kipper, M., Cruz-Polycarpo, V.C., Dadalt, J.C., Rodrigues, P.H.M. & Albuquerque, R., 2017. Meta-analytic study of organic acids as an

alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult. Sci. 96(10), 3645-3653.

Ukalska-Jaruga, A., Bejger, R., Debaene, G. & Smreczak, B., 2021. Characterization of soil organic matter individual fractions (fulvic acids, humic acids, and humins) by spectroscopic and electrochemical techniques in agricultural soils. Agronomy, 11(6), 1067.

Van, T.T.H., Yidana, Z., Smooker, P.M. & Coloe, P.J., 2020. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. J. Glob. Antimicrob. Resist. 20, 170-177.

Vucskits, A.V., Hullár, I., Bersényi, A., Andrásofszky, E., Kulcsár, M. & Szabó, J., 2010. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J. Anim. Physiol. Anim. Nutr. 94, 721-738.

Winkler, J. & Ghosh, S., 2018. Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes. Hindawi J. Diabetes Res.

Yanga, F., Zhang, S., Cheng, K. & Antonietti, M., 2019. A hydrothermal process to turn waste biomass into artificial fulvic and humic acids for soil remediation. Sci. Total Environ. 686, 1140-1151.

Zommiti, M., Chikindas, M.L. & Ferchichi, M., 2020. Probiotics—live biotherapeutics: a story of success, limitations, and future prospects—not only for humans. Probiotics Antimicrob. Proteins. 12. 1266-1289.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Broiler meat is one major contributors to the meat basket for consumer and is widely consumed globally. Broilers are nutritionally demanding, albeit highly efficient feed converters, hence their diets are generally formulated to fulfil minimum nutritional requirements for muscle build up (Frempong *et al*., 2019). Antibiotic growth promoters (AGPs) inclusion in broiler feeds has been practiced over years, and this has enhanced optimisation of nutrient utilisation and general broiler performance. However, the continuous use and misuse of AGPs has raised anxiety from consumers, who are wary of the transference of antibiotic residues from the broiler meat to their body systems, affecting their health. Therefore, there has been increased interest in the exploration of alternatives to AGPs in animal nutrition. Among other alternative growth promotants, organic acids have been receiving much attention by researchers. One of the organic acids that has potential to be used in broilers is FA, which is an organic substance found in soil and natural water systems. FA is a subclass of diverse compounds known as humic substances, which are by-products of organic degradation from microorganisms (Senesi *et al*., 1999). These organic substances contain many reactive functional groups, including phenols, hydroxyls, carboxyls and quinones (Jusadi *et al.*, 2020). The presence of these reactive groups means that FA can elicit many beneficial effects, including induction of efficient intestinal permeability and improved gut function (Mao *et al.*, 2019). The use of FA in this review evaluates it's potential to serve as a potential growth promotant alternative in broiler diets.

2.2 Use of feed additives in broiler diets

Use of antibiotics as growth promoters in animal feeds has been permitted in the member states of the European Union during the last 50 years. AGPs have been effectively applied to increase growth rates and productivity in broilers and other livestock (Donoghue, 2003). However,

anxiety over the transference of residues from animal to human and increasing antimicrobial resistance and led to a ban in the use of antibiotics use as growth promoters in the European Union since January 1, 2006 (Swiatkiewicz *et al*., 2018). Over the years, the increased usage of antibiotics as feed additives could cause bacteria to become resistant to the drugs used to treat infections (Banin *et al*., 2017). These resistant gene microbes could be harmful to people if they are assigned to individuals. Because of this, the World Health Organization (1997) and the Economic and Social Committee of the European Union (1998) have determined that the use of antimicrobials in food animals is a matter of public health. Examples of antibiotics often used in broilers and other livestock include penicillin, cephalosporins and aminoglycosides.

2.3 Alternative feed additives

In recent times, there has been an increase in livestock industry's interest in the use of natural compounds either for direct addition or for use in synergy with other compounds in animal diets (Islam *et al.,* 2005; Vucskits *et al.,* 2010; Kunavue *et al.,* 2012). Many attempts have now been made in assessing the efficacy of alternatives to commercial growth promotants and performance enhancers including the potential use of natural phytogenic essential oils or phytobiotics, organic acids and pre and probiotics (Mpofu *et al*., 2016; Disetlhe *et al*., 2018). It has been accepted that organic acids, as well as combinations of these acids and phytogenic feed additives, may serve as potential substitutes with unique mechanisms of actions. Organic acids are effective antimicrobials that have been illustrated to stop the growth of pathogenic bacteria in the gut and support a balanced gut microbiota (Islam *et al.,* 2005; Vucskits *et al.,* 2010; Kunavue *et al.,* 2012), while selected phytogenic feed additives including humic substances proved to be able to reduce inflammation and oxidative stress in animals, improving herewith the nutrient digestibility.

2.4 Humic substances as alternative growth promotants

Humic acid (HA) and FA are two humic substances that have been suggested as alternatives to the antibiotics. These two acids belong to a group of organic humic substances that are receiving much attention as alternatives to AGPs because of their characteristics and chemical composition (Nardi *et al*., 2021). Based on its possible anti-inflammatory capabilities, studies show that HA has been employed as a neuron- or nephron-protective drug in clinical therapy (Ozkan *et al*., 2015). Recent research established that HA had substantial antiviral motion against the human immunodeficiency virus, which is currently untreated due to the absence of appropriate treatments (Dai *et al*., 2020). HA may cure certain clinical symptoms including diarrhoea, dyspepsia, and acute intoxication in horses, ruminants, swine, and poultry in veterinary medicine (Pettit, 2004). On the other hand, FA has also been utilized to boost growth rates, enhance the efficiency of feed and boost immunity, as well as elevating the economic and environmental advantages of animal production (Dai *et al*., 2020). Peat and various coal types, including lignite and weathered coal, are sources of HA and FA, it can increase animal performances by improving growth rate, feed efficiency, immunity and weakening the risk of infections (Vucskits *et al.*, 2010; Kunavue *et al*., 2012). FA is obtained from the breakdown products of plant and animal remnants that have been extracted in an alkaline solution (humic Substance) Ukalska-Jaruga *et al*. (2021), which have low molecular weight component of humic substance, a short-chain molecule that dissolves in acids and bases (Hasan *et al*., 2020).

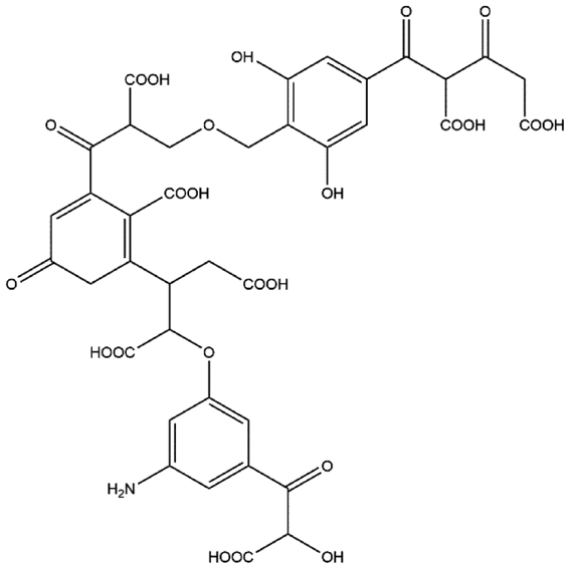


Figure 2. 1. Average molecular structure of HA (source Guan-qun Gong, 2020).

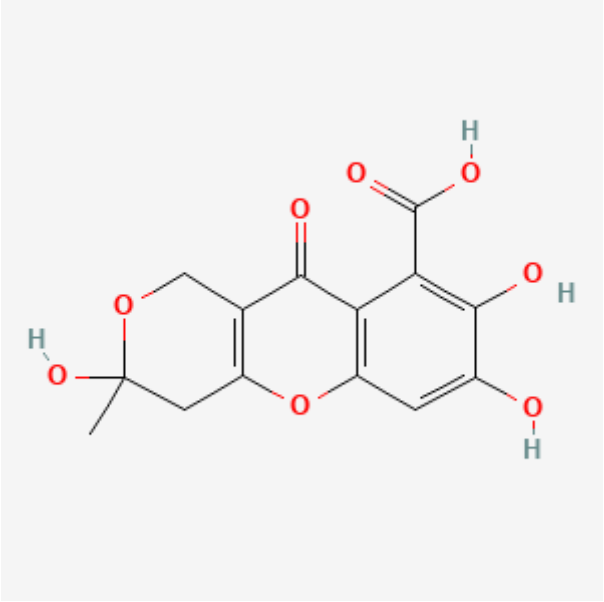


Figure 2. 2. Compound structure of FA.

Subbituminous coals, ignites, peat, soil, composts, and raw organic wastes are only a few of the places, where they humic substances can be found (Rose *et al.*, 2014). The bacterial and chemical degradation of lignin and other structural plant carbohydrates within the subsoil are liable for creating the intermediate products of HA and FA (Schellekens *et al*., 2017). Jusadi *et al*. (2020) stated that organic substances have many reactive functional groups, including quinine, phenol, hydroxyl, carboxyl and amine as well as the ability to combine heavy metals. However, the occurrence of these reactive groups in FA can stimulate countless valuable effects, including metal chelation Plaza *et al.* (2005), antioxidant action, stress management, immunostimulation, induction of efficient intestinal permeability, improved gut function and anti-inflammatory activities Chang *et al.* (2013) and Mao. (2019). However, the effects of FA as a natural feed additive have not been extensively researched in agriculture. Several studies pertaining to the positive effects of FA relate to their effects on aquatic animals; however, studies related to poultry production is limited. It is, therefore, important to evaluate the efficiency of use of FA as a potential alternative growth promoter in broiler chickens.

2.5 Potential effects of fulvic acid

2.5.1 Immunomodulation effects

The most sufficiently investigated claim of FA is its capability to regulate the immune system (Winkler *et al.*, 2018). Numerous diseases, including eczema, allergies, and asthma, can be brought on by immune cells that are overactive (Ngoc *et al*., 2005; Winkler *et al*., 2018). Anti-inflammatory medications are essential in these situations for alleviating symptoms. According to several studies, FA can reduce inflammation by preventing cells from releasing pro-inflammatory mediators. Following exposure to the endotoxin Lipopolysaccharide (LPS), differentiated human monocytes (U937) have been demonstrated to express less tumour

necrosis factor alpha (TNF-alpha) when FA, at 200 g/mL, is added (Junek *et al*., 2009). FA has also been demonstrated to decrease the secretion of prostaglandin E2 (PGE2) and cyclooxygenase 2 (COX2) in prime human monocytes after homocysteine stimulation (Chien *et al*., 2015; Winkler *et al*., 2018). FA from solubilized sludge (SS-FA) has been found to decrease B-hexosaminidase and histamine production in mast cells and basophil cells that have been immunoglobulin-E-sensitized (Winkler *et al*., 2018). These findings imply that FA may have anti-inflammatory and anti-allergenic properties. According to a pilot clinical research, oxifulvic acid, which is generated from coal, at 4.5% (w/w), lowers wheal and flare size after allergen challenge in people (Snyman *et al*., 2002). A decrease in FA produces effects comparable to those of 1% hydrocortisone.

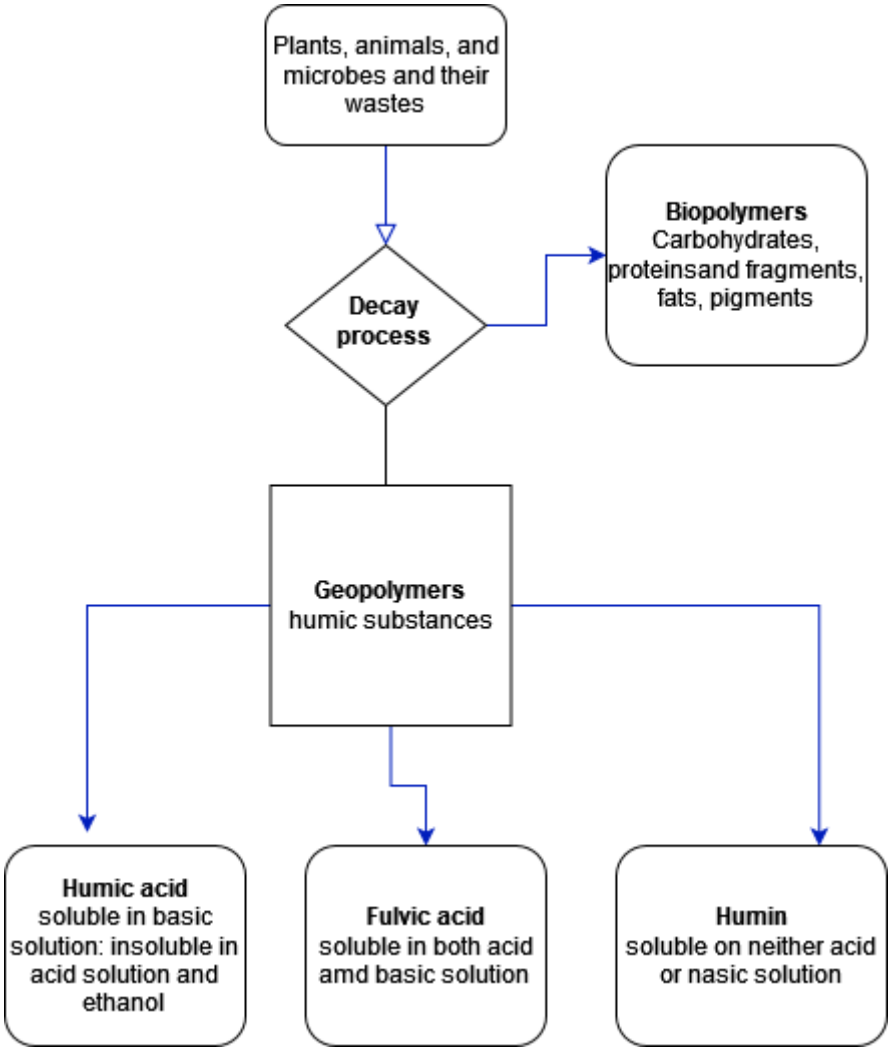


Figure 2. 3. The origin of humic substances and the relationship among them.

Mice have also demonstrated FAs anti-inflammatory effects (Van Rensburg *et al*., 2001; Winkler *et al*., 2018). According to Gandy *et al*. (2011), topical treatment of CHD-FA has been verified in a randomized clinical trial, to significantly lessen eczema rash in people. Additionally, oral administration of the South African CHD-fulvic acid at a dose of 100 mg/kg, can diminish paw oedema in rats at a level comparable to that of nonsteroidal anti-inflammatory medicines (Sabi *et al*., 2012; Winkler *et al*., 2018). The afore mentioned research suggests that FA may be used to treat autoimmune diseases, particularly eczema (Winkler *et al*., 2018).

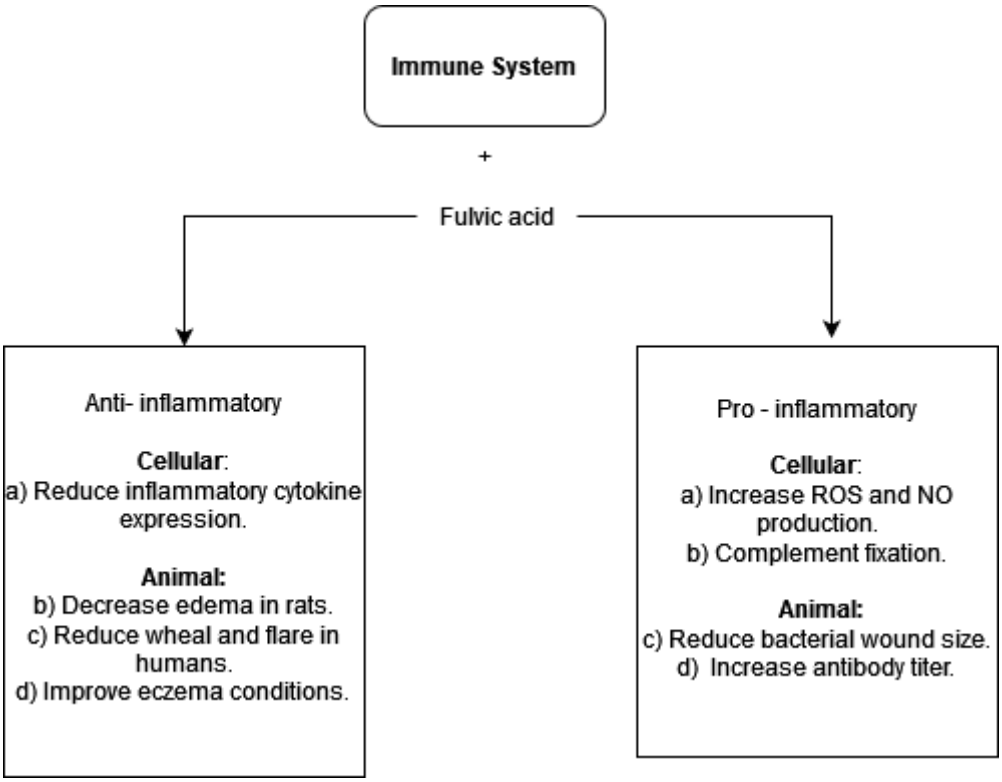


Figure 2.1. Potential effects of fulvic acid on immune system in different animal species.

2.5.2. Oxidative stress

According to Reuter *et al.* (2010), oxidative stress and chronic inflammatory disorders are strongly associated. Metcalfe *et al.* (2010), defined oxidative stress as the disparity among highly reactive oxygen species (ROS) and antioxidants. Endogenous antioxidants like glutathione (GSH) and superoxide dismutase (SOD), lose out as the cellular balance swings towards higher ROS. According to Dotan *et al.* (2004), this results in cellular malfunction, lipid peroxidation, and perhaps, even cell death. Figure 2.5 summarizes the effects of FA on the oxidative state in cells and animals.

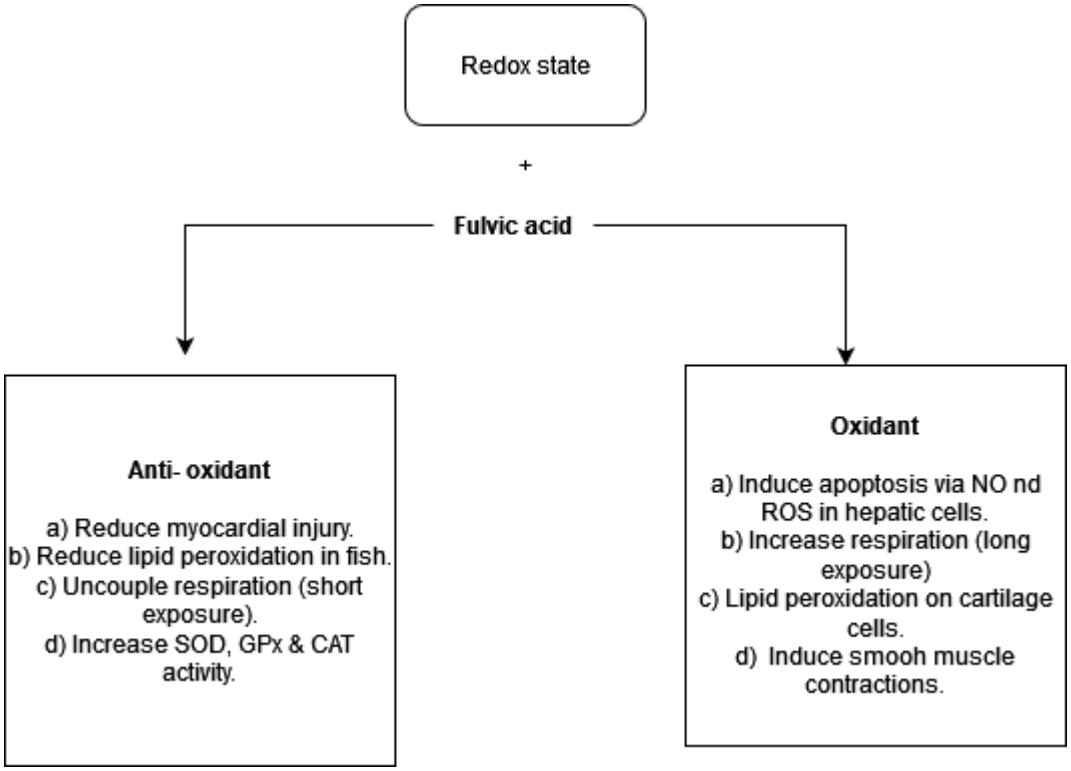


Figure 2.2. Potential effects of FA on oxidative stress.

Superoxide radicals and other ROS have been shown to be retained by FA outside of the cell (Rodrguez *et al*., 2011). However, FA may dissociate the electron transport chain in the liver mitochondria inside the cell, which is related to a reduction in ROS production (Klaine *et al*., 2008). In addition, the decrease in oxidative stress indicators following isoproterenol (ISO), caused cardiac injury in rats, which is the most encouraging thing *in vivo* studies in terms of the antioxidant capacity of FA. According to Shikalgar & Naikwade (2018), feeding chickens FA at doses of 300 mg/kg/day for the duration of 30 days, reduces cardiac damage and lipid peroxidation markers following ISO while also markedly raising SOD levels. Another study supports this finding by showing that when FA was administered to fish for 60 days, lipid

peroxidation was reduced and the production of SOD, CAT, and glutathione peroxidase was increased (Gao *et al*., 2017).

2.5.3. Gut health

The gut serves as a transitional zone between the outside world, the microbiome, and the host. Inflammation and disease are linked to poor gut health, according to literature (Round & Mazmanian, 2009: Winkler *et al.*, 2018). FA has been demonstrated to promote gut flora, nutritional absorption, and the healing of gut disorders (Winkler *et al*., 2018). Regarding the microbiota, limited literature exists in animals. Gao *et al*. (2017) reported that FA at 1.5% (w/w), can alter the intestinal flora of the loach (Paramisgurnus dabryanus fish). Proteobacteria phyla are less prevalent and firmicute levels are higher in the colon after 60 days of feeding. In addition, FA therapy affected 10 different bacteria genera. The bacteria *Variovorax*, *Lactococcus* and *Lactobacillus* have increased, while *Serratia* and *Acinetobacter* have decreased (Winkler *et al*., 2018). The data that is now available on the impact of FA on intestinal health is summarized in Figure 2.6 below.

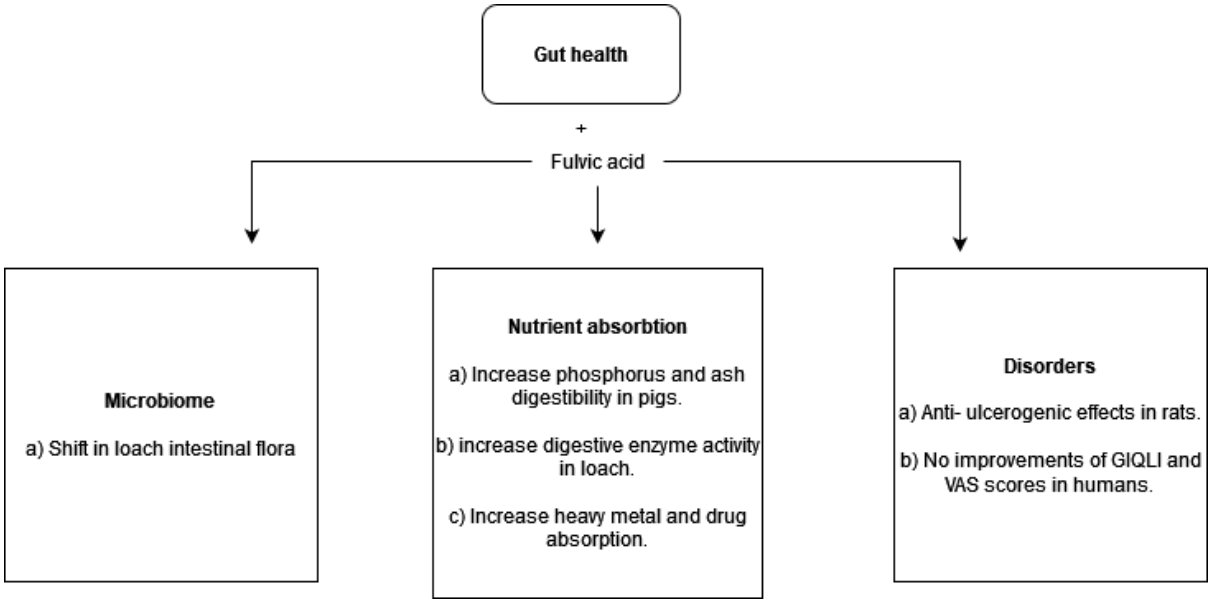


Figure 2.3. FA effects on gut health.

The movement of gastric enzymes such lysozyme, proteases, and acid or alkaline phosphatases in fish is increased by FA (Gao *et al*., 2017). A thorough investigation of nutritional digestibility in pigs (Kunavue *et al*., 2012) lends support to this. In animal models, FA has also been demonstrated to affect the bioavailability of heavy metals. FA can both increase copper absorption and decrease lethal copper in swine oviductal epithelial cells (Sanmanee & Areekijseree, 2010). FA has also been demonstrated to mediate medication transport in rats in addition to nutrients (Mirza *et al*., 2011). Common anticonvulsant carbamazepine (CBZ) has a limited bioavailability, but when it is coupled with FA, absorption across an everted rat intestinal sac rises along with blood plasma levels of CBZ.

According to the latest research by Winkler *et al*. (2018), FA has been shown to boost nutrient and medication absorption, making it dangerous for contaminants and toxins to enter the bloodstream. However, according to Qiang *et al*. (2016), FA did not escalate the immersion of perfluorooctanesulfonate (PFOS) in carp. This finding was made in light of the difference between the concentration of PFOS in fish faeces and controls. There is a preliminary clinical study looking at the effectiveness of probiotics combined with FA in treating gastrointestinal (GI) diseases (Kim *et al*., 2006). Unfortunately, neither the gastrointestinal quality of life index (GIQLI) nor the visual analogue scale (VAS) scores for GI symptoms increase in any group, even those who get FA. However, FA had no impact in the previous investigation, FA herein its extraction from Shilajit showed promise as an antiulcerogenic agent in a battery of albino rat battery tests (Winkler *et al*., 2018).

2.5.4 Bone development and incidents of bone disease

Organic acids, such as FA, have also been shown to enhance mineral assimilation and bone matrix mineralization during digestion (Nkukwana *et al*., 2014). Poor bone growth can frequently be caused by a diet low in certain minerals, such as calcium and phosphates (Muszyński *et al*., 2018). Additionally, inadequate dietary calcium can cause blood hypocalcemia, which can impair bone mineralization and strength. The metabolism and structure of bones are significantly influenced by phosphorus and calcium, and phosphorus is crucial for the storage of energy (Ciosek *et al*., 2021). The broiler industry, particularly in rapid growth chickens, has recognized bone conditions related to leg weakness as a serious issue, because of the reduction in productive efficiency brought on by mortality and culling, which reduced economic profits and raised questions about the welfare of chickens (Selaledi *et al*., 2020). According to Selim *et al*. (2022), bone related issues is one of the major concerns for poultry breeders of broiler chickens. Tibia dyschondroplasia rickets and valgus deformities, leg breakage and osteoporosis are some of the disorders, which results in lameness of chickens, resulting in poor performance on broiler chickens (Fleming, 2008). According to Barve *et al.* (2017), the origin of the broiler weakness on legs and reduced movement is not well understood, as it is associated with numerous issues including, amino acid (AA) metabolism, genetic modification, management and insufficient nutrition by pathological aliment of different organs.

2.5.5 Potential of fulvic acid as ethnomedicine

For around 3000 years, FA has been used gradually in Ayurvedic therapy in India for both *Homo sapiens* and animals (Wilson *et al*., 2011). Shilajit is a tar-like exudate from the Himalayas that is used medicinally and contains about 15-20% FA. Shilajit may have immunomodulatory, antioxidant, diuretic, antihypertensive and hypoglycaemic properties,

according to ancient writings (Wilson *et al*., 2011). It has been reported to have analgesic and antimicrobial properties when used externally (Batiha *et al*., 2020). Stohs (2014) suggest that the intake of Shilajit is safe, although Wilson *et al*. (2011) note that the pharmacological dosage of such compounds is still unclear. Despite this lack of knowledge, Shilajit/FA is being currently offered to the general population as a nutraceutical (Pena-Mendez *et al*., 2005). The goal of recent scientific study has been to increase poultry production (Elnesr *et al*., 2022). The diet of poultry includes various feed additives, such as humate compounds (HS). The effects of FA supplementation on productivity and intestinal morphology appeared beneficial (Hajiaghapour & Rezaeipour, 2018; Hazrati *et al*., 2020). Increasing usage of artificial growth promoters and antibiotics has always been linked to increased broiler production (Mehdi *et al*., 2018). As a result of the linked health conditions, the safety and quality of meat are starting to be questioned by the public, this has therefore, made it necessary to evaluate how natural antibiotics are used in poultry production. According to Gao *et al*. (2018), humic compounds include FA, which is a kind of humic acid. Humic substances are straw-coloured, aquaphobic organic acids produced mostly from soil humus and plants, and they often make up a significant amount of the natural organic material found in soils, residues and waters (Jusadi *et al*., 2020). Humic compounds are utilized in animal husbandry methods as alternative feed additives to boost animal output by enhancing growth rates, feed efficiency, and immunity. Chosen phytogenic feed additives, including humic compounds, have shown to lower inflammation and oxidative stress in animals, Organic acids are strong antimicrobial constituents Vucskits *et al.* (2010) and Kunavue *et al.* (2012), which play a crucial role in enhancing the nutritional digestibility while also preventing the formation of pathogens in the gut and supporting a healthy gut microbiota.

2.5.6 Influence on blood parameters and health

The primary factors influencing the sustainability of poultry production are diseases control measures and performance. Therefore, it is of economic importance to improve livestock performance, particularly in broiler production, without compromising their health status, specifically blood metabolites. Poultry farmers frequently look for less expensive alternative ingredients to feed their chickens without considering the anti-nutritional factors (ANFs) present in different diets that can have a negative influence on the haematological parameters. According to Marcin *et al*. (2021), humic substances administered as feed additives, can positively alter parameters of animals. Enriching the ability of animals to grow depends on HA and FA sources found in humic substances. Published results demonstrated various observations of the use of humates in the improvement of broiler production in several parameters including blood metabolites and their potential benefits in improving the health status and wellbeing in animals. According to Wang *et al*. (2008), humic substances positively affect the animal's immune system. Humic substances' action mechanism is linked to their ability to form compound saccharides in animal bodies, which are important regulators of intercellular interaction. These compounds regulate the immune system development and balance, preventing insufficient reactions. HA promotes the immunocompetence resulting in increased phagocytic activity. Terratol (2002) also reported that HA may increase the production of glycoproteins, which can synchronise the immune system by maintaining the balance of T and B cells.

2.5.7 Effect of fulvic acid in meat quality attributes

FA has the ability to elevate the absorption of nutrients and quicken digestive roles that can change and control the metabolism to improve animal carcass traits (Bai *et al*., 2013). According to Ozturk *et al*. (2012), the body and carcass weights increased when HA

compounds were added to the broiler diet. According to Macit *et al*. (2003), water holding capacity is associated with lipid peroxide contents in the meat muscle. Lipid peroxidation is defined as the loss of unsaturated lipids by peroxidation, which results in lipid degradation and membrane disruption. Reports by Beer *et al*. (2000) specified that FA has a stimulating effect on the smooth muscle tissues' impulsive contractile activity. According to Mir *et al*. (2017), meat colour, texture, drip loss and cook loss are all common meat qualities. These characteristics are inclined by the pH of the meat at the time of slaughter.

2.6 Summary

Ensuring safety for poultry in the feed is the industry's overall trend. The goal of recent scientific research has been to increase poultry production (Elnesr *et al*., 2022). The diet of poultry includes various feed additives, including antibiotics. Due to the associated health issues, people are starting to doubt the quality and safety of meat, leading to intense efforts to assess the utilization of natural feed additives in poultry production. Select phytogenic feed additives, including humic substances, have shown to decrease inflammation and oxidative stress in animals, improving nutrient digestibility in the process. FA belongs to a group of chemicals known as humic substances, which are by-products of microbes' degrading organic matter. The presence of these reactive groups allows FA to produce a variety of beneficial effects, including metal chelation (antioxidant activity, stress management and immunostimulant. This study, therefore, sought to assess the efficiency of FA in broiler diets as a growth factor in place of conventional antibiotics.

2.7 References

Al-Hijazeen, M., Lee, E.J., Mendonca, A. & Ahn, D.U., 2016. Effects of tannic acid on lipid and protein oxidation, color and volatiles of raw and cooked chicken breast meat during storage. Antioxidants. 5(2), 19.

Batiha, G.E.S., Alkazmi, L.M., Wasef, L.G., Beshbishy, A.M., Nadwa, E.H. & Rashwan, E.K., 2020. *Syzygium aromaticum L. (Myrtaceae*): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 10(2).

Bai. H.X., Chang. Q.F., Shi. B.M. & Shan. A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing- finishing pigs. Livest. Sci. 158(13), 118─123.

Banin, E., Hughes, D. & Kuipers, O.P., 2017. Bacterial pathogens, antibiotics and antibiotic resistance. FEMS. Microbiol Rev. 41(3), 450-452.

Barve, S., Chen, S.Y., Kirpich, I., Watson, W.H. & McClain, C., 2017. Development, prevention, and treatment of alcohol-induced organ injury: the role of nutrition. Alcohol research. Curr. Rev. 38(2), 289.

Beer, A.M., Lukanov, J. & Sagorchev, P., 2000. The influence of fulvic and ulmic acids from peat, on the spontaneous contractile activity of smooth muscles. Phytomedicine. 7, 407-415.

Chang, H., Wang, Q., Xu, X., Li, C., Huang, M., Zhou, G. & Dai, Y., 2011. Effect of heat-induced changes of connective tissue and collagen on meat texture properties of beef semitendinosus muscle. Int. J. Food Prop. 14(2), 381-396.

Chien S, Chen T, Kuo H, Chen H, & Chang S., 2015. Fulvic acid attenuates homocysteine-induced cyclooxygenase-2 expression in human monocytes. BMC Compl. Alt. Med. 15(1), 61.

Ciosek, Ż., Kot, K., Kosik-Bogacka, D., Łanocha-Arendarczyk, N. & Rotter, I., 2021. The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue. Biomolecules, 11(4), 506.

Dai, C., Xiao, X., Yuan, Y., Sharma, G. & Tang, S., 2020. A comprehensive toxicological assessment of fulvic acid. *Evidence-Based Complementary and Alternative Medicine*. vol. 2020, Article ID 8899244, 11 pages, 2020. https://doi.org/10.1155/2020/8899244.

Donoghue, D.J., 2003. Antibiotic residues in poultry tissues and eggs: human health concerns. Poult. Sci. 82(4), 618-621.

Dotan, Y., Lichtenberg, D. & Pinchuk, I., 2004. "Lipid peroxidation cannot be used as a universal criterion of oxidative stress," Progress in Lipid Research. 43(3), 200-227.

Disetlhe. A.R.P., Marume. U., Mlambo. V. & Hugo. A., 2018. Effects of dietary humic acid and enzymes on meat quality and fatty acid profiles of broiler chickens fed canola-based diets. 32(2), 711-720.

Elnesr, S.S., Abdel-Razik, A.R.H. & Elwan, H.A., 2022. Impact of humate substances and Bacillus subtilis PB6 on thyroid activity and histomorphometry, iron profile and blood haematology of quail. J Anim. Phys & Anim. Nutri. 106(1), 110-117.

Fleming, R.H., 2008. Nutritional factors affecting poultry bone health: Symposium on 'Diet and bone health'. Proceedings of the Nutrition Society, 67(2), 177-183.

Gao, Y., He, J., He, Z., Li, Z., Zhang, B., Mu, Y., Lee, J. & Chu, Z., 2017. Effects of fulvic acid on growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus (Sauvage),* Fish Shellfish Immunol. 62, 47–56.

Gao, Y., Zhu, J., Bao, H., Hector, V., Zhao, B. & Chu, Z., 2018. Effect of lignite fulvic acid on growth, antioxidant ability and HSP70 of Pacific white shrimp, *Litopenaeus vannamei*. J. Aquac Int. 26(6), 1519-1530.

Gonzalez-Rivas, P.A., Chauhan, S.S., Ha, M., Fegan, N., Dunshea, F.R. & Warner, R.D., 2020. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Sci. 162, 108025.

Hajiaghapour, M. & Rezaeipour, V., 2018. Comparison of two herbal essential oils, probiotic, and mannan-oligosaccharides on egg production, hatchability, serum metabolites, intestinal morphology, and microbiota activity of quail breeders. Livest. Sci. 210, 93-98.

Hassan, A.A., Salem, A.Z.M., Elghandour, M.M.Y., Hafsa, S.A., Reddy, P.R.K., Atia, S.E.S. & Vidu, L., 2020. Humic substances isolated from clay soil may improve the ruminal fermentation, milk yield, and fatty acid profile: A novel approach in dairy cows. Anim. Feed Sci. Tech. 268, 114601.

Hazrati, S., Rezaeipour, V. & Asadzadeh, S., 2020. Effects of phytogenic feed additives, probiotic and mannan-oligosaccharides on performance, blood metabolites, meat quality, intestinal morphology, and microbial population of Japanese quail. Br. Poult. Sci. 61(2), 132-139.

Islam, K.M.S., Schuhmacher, A. & Gropp, J.M., 2005. Humic acid substances in animal agriculture. Pak. J. Nutr. 4(3), 126-134.

Junek, R. & Schoenherr, M.R., 2009. Bimodal effect of humic acids on the LPS-induced TNF-α release from differentiated U937 cells, Phytomedicine. 16(5), 470–476.

Jusadi. D., Aprilia. T., Setiawati. M., Suprayudi. M.A & Ekasari. J., 2020. Dietary supplementation of fulvic acid for growth improvement and prevention of heavy

metal accumulation in Nile tilapia fed with green mussel. Egypt. J. Aquat. Res. 46(3), 95─301.

Kim, S., Hilli, L., Orlowski, L., Kupperman, J. L., Baral, F. & Waters, R., 2006. Efficacy of probiotics and nutrients in functional gastrointestinal disorders: a preliminary clinical trial. J. Dig. Sci. 51, 2134-2144.

Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J. & Lead, J.R., 2008. Nanomaterials in the environment: behaviour, fate, bioavailability, and effects. Environ. Toxicol. Chem: An Int. J. 27(9), 1825-1921.

Kunavue, N. & Lien, T.F., 2012. Effects of fulvic acid and probiotic on growth performance, nutrient digestibility, blood parameters and immunity of pigs. J. Anim. Sci. Adv, 2(8), 711-721.

Macit, M., Aksakal, V., Emsen, E., Aksu, M.L., Karaoglu, M. & Esenbuga, N., 2003. Effects of vitamin E supplementation on performance and meat quality traits of Morkaram male lambs. Meat Sci. 63, 51-55.

Mao, ying ming., 2019. Modulation of the growth performance, meat composition, oxidative status, and immunity of broilers by dietary fulvic acids, J. Poult Sci. 98, 4509-4513.

Marcin, A., Nad, P., Skalicka, M., Bujnak, L. & Gancarčíkova, S., 2021. Effect of humic acid substances on proteolytic activity in intestine, digestibility of crude protein and protein content in the blood of broiler chickens. Magnesium. 2(3), 3-11.

Matshogo, T.B., Mlambo, V., Marume, U. & Sebola, N., 2017. Growth performance, blood parameters, carcass characteristics and meat quality of Potchefstroom koekoek chickens supplemented with *Lippia javanica* leaf meal.

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Côté, C., Ramirez, A.A. & Godbout, S., 2018. Use of antibiotics in broiler production: Global impacts and alternatives. J Anim. Nutri. 4(2), 170-178.

Metcalfe, N.B. & Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct. Ecol. 24(5), 984-996.

Mir, N.A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54(10), 2997-3009.

Mirza, M.A., Agarwal, S.P. & Iqbal, Z., 2011. Effect of fulvic acid on oral delivery of Carbamazepine. Sci. Adv. Mater. 3(2), .223-232.

Mpofu, D.A., Marume, U., Mlambo, V. & Hugo, A., 2016. The effects of *Lippia javanica* dietary inclusion on growth performance, carcass characteristics and fatty acid profiles of broiler chickens. Anim Nutri. 2(3). 160-186.

Muszyński, S., Tomaszewska, E., Kwiecień, M., Dobrowolski, P. & Tomczyk, A., 2018. Effect of dietary phytase supplementation on bone and hyaline cartilage development of broilers fed with organically complexed copper in a Cu-deficient diet. Biol. Trace Elem. Res. 182, 339-353.

Nardi, S., Schiavon, M. & Francioso, O., 2021. Chemical structure and biological activity of humic substances define their role as plant growth promoters. Molecules, 26(8), 2256.

National Research Council (NRC), 1994. Nutrient Requirements of Poultry. 9th revised ed. National Academy Press, Washington. DC.

Ngoc, L., Gold, D., Tzianabos, A., Weiss, S. & Celedón, J., 2005. Cytokines, allergy, and asthma, Current Opinion in Allergy and Clinical Immunology. 5(2), 161–166.

Nkukwana, T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. SA. J. Anim. Sci. 48(5), 869-884.

Owens, C.M. & Sams, A.R., 2000. The influence of transportation on turkey meat quality. Poult. Sci. 79(8), 1204-1207.

Ozkan, A., Sen, H.M. & Sehitoglu, I. 2015. "Neuroprotective effect of humic acid on focal cerebral ischemia injury: an experimental study in rats. Inflammation. 38(1), 32–39.

Ozturk, E., Ocak, N., Turan, A., Erener, G., Altop, A. & Cankaya, S., 2012. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. J. Sci. Food. Agric. 92, 59–65.

Pena-Mendez, E.M., Havel, J. & Patocka, J., 2005. Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. J. Appl. Biomed. 3(1), 13-24.

Pettit, R.E. 2004. Organic Matter, Humus, Humate, Humic Acid, Fulvic Acid, and Humin: Their Importance in Soil Fertility and Plant Health, Texas A&M University, Texas, USA.

Plaza, J.C., Garcia-Gil A. & Polo, N., Senesi, G. & Brunetti, G., 2005. Proton binding by humic and fulvic acids from pig slurry and amended soils. J. Environ. Qual. 34, 1113-1117.

Qiang, L., Chen, M., Zhu, L., Wu, W. & Wang, Q., 2016. Facilitated bioaccumulation of perfluorooctanesulfonate in common carp (*Cyprinus carpio*) by graphene oxide

and remission mechanism of fulvic acid. Environ. Sci. Techn. 50(21), 11627-11636.

Reuter, S., Gupta, S.C., Chaturvedi, M.M. & Aggarwal, B.B., 2010. Oxidative stress, inflammation, and cancer: how are they linked. Free radic. Boil. Med 49(11), 1603-1616.

Rodriguez, N.C., Urrutia, E.C., Gertrudis, B.H., Chaverri, J.P. & Mejía, G.B., 2011. Antioxidant activity of fulvic acid: A living matter-derived bioactive compound. J Food Agri Environ. 9(3), 123-7.

Rose, M.T., Patti, A.F., Little, K.R., Brown, A.L., Jackson, W.R. & Cavagnaro, T.R., 2014. A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. In Advances in agronomy. 124, 37-89. Academic Press.

Round, J.L. & Mazmanian, S.K., 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9(5), 313-323.

Sabi, R., Vrey, P. & van Rensburg, C.E.J., 2012. Carbohydrate-derived Fulvic acid (CHD-FA) inhibits Carrageenan-induced inflammation and enhances wound healing: efficacy and Toxicity study in rats. Drug Dev. Res. 73(1), 18-23.

Sanmanee, N. & Areekijseree, M., 2010. The effects of fulvic acid on copper bioavailability to porcine oviductal epithelial cells. Biol. Trace Elem. Res. 135, 162-173.

Schellekens, J., Buurman, P., Kalbitz, K., Zomeren, A.V., Vidal-Torrado, P., Cerli, C. & Comans, R.N., 2017. Molecular features of humic acids and fulvic acids from contrasting environments. Environ. Sci. Technol. 51(3), 1330-1339.

Selaledi, A., Mohammed H. L., Z., Manyelo, T.G. & Mabelebele, M., 2020. The current status of the alternative use to antibiotics in poultry production: An African perspective. Antibiotics. 9(9), 594.

Selim, S., Abdel-Megeid, N.S., Khalifa, H.K., Fakiha, K.G., Majrashi, K.A. & Hussein, E., 2022. Efficacy of various feed additives on performance, nutrient digestibility, bone quality, blood constituents, and phosphorus absorption and utilization of broiler chickens fed low phosphorus diet. Animals. 12(14), 1742.

Shikalgar, T.S. & Naikwade, N.S., 2018. Cardioprotective effect of fulvic acid on doxorubicin induced cardiac oxidative stress in rats. Int. J. Pharm. Sci. Res, 9(8), 3264-3273.

Stohs, S.J., 2014. Safety and efficacy of shilajit (mumie, moomiyo). Phytother. Res. 28, 475-479.

Swiatkiewicz, S., Arczewska-Włosek, A., Szczurek, W., Calik, J., Krawczyk, J.& Józefiak, D., 2018. The influence of selected feed additives on mineral utilisation and bone characteristics in laying hens. Anim. Sci. 18(3), 781.

Terratol, L.L.C., 2002. Effects of Humic Acid on Animals and Humans. http://www.terratol.com/sitebuildercontent/sitebuilderfiles/EffectsofHumicAcidonAnimals and Humans.pdf.

Van Rensburg, C.E J., Malfeld, S.C.K. & Dekker, J., 2001. Topical application of oxifulvic acid suppresses the cutaneous immune response in mice. Drug Dev. Res. 53(1), 29–32.

Vucskits, A.V., Hullar, I., Bersényi, A., Andrásofszky, E., Kulcsár, M & Szabó, J., 2010. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J. Anim. Physiol. Anim. Nut. 94(6). 721–728.

Wang, Y., Xu, Z., Bach, S. J. & McAllister, T. A., 2008. Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on *in vitro* ruminal digestion of mixed forage or barley grain. Anim. Feed Sci. Tech. 145 (1/4), 375-395.

Wilson, E., Rajamanickam, G.V., Dubey, G.P., Klose, P., Musial, F., Saha, F.J., Rampp, T., Michalsen, A. & Dobos, G.J., 2011. Review on shilajit used in traditional Indian medicine. J. Ethnopharmacol. 136(1), 1-9.

Winkler, J. & Ghosh, S., 2018. Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes. J. Diabetes Res.

CHAPTER 3

EFFECT OF FULVIC ACID ON GROWTH PERFORMANCE, PROTEIN UTILIZATION EFFICIENCY AND BLOOD METABOLITES IN BROILER CHICKENS

Abstract

This study was conducted to investigate the effect of fulvic acid (FA) inclusion levels on growth performance, protein utilization efficiency, haematology, and serum biochemistry of Cobb 500 broiler chickens. A total of 400, day-old, unsexed chicks were randomly and equally allocated to five dietary treatments distributed across 8 replicates with each pen carrying 10 chicks. The five isonitrogenous and isoenergetic diets were formulated as follows: NegCON (T1): commercial diets without antibiotics, posCON (T2): commercial diet with antibiotics, FA1 (T3) commercial diet + 1% FA1, FA2 (T4): commercial diet + 2% FA and FA3 (T5): commercial diet with 3% FA. The feeding trial was conducted over 3 feeding phases, Starter (day 1-14); Grower (15-28) and Finisher ration (day 28-42). Chicks had *ad libitum* access to freshwater and feed throughout a period of 6 weeks. Blood was collected for haematology and serum biochemistry 2 days before the chicks were humanly slaughtered at a local abattoir. From the results, it was revealed that dietary treatment only affected ($P < 0.05$) the FCR in the starter phase. Birds fed FA3 diet had the lowest FCR when compared to posCON diet. In the Grower phase, diet had a significant effect on all the growth performance parameters. Birds fed the posCON diet had the highest ($P < 0.05$) ADFI (93.33 g) followed by those fed the FA2 (90.66 g) diet. Dietary inclusion on cumulative weight gain (CWG) in the final week, revealed that birds fed the FA2 (1897.34 g) diet, gained at the same level as those fed the posCON (1932.74 g) diets and birds in both treatments achieved similar final weights at the end of the feeding trial. Overall, ADG was higher in posCON diets (49.37 g/d) followed by FA2 diet (45.17 g/d), whilst negCON had the least. Dietary inclusion treatments significantly ($P <0.05$) influenced protein utilization and growth efficiency parameters in all three growth phases apart

from the protein efficiency ratio (PER) in the grower phase, the protein consumed PC and the specific growth rate (SPR) at the finisher phase. Dietary treatment had no effect on all of the haematology parameters apart from platelet count. The highest (P <0.05) platelet count was observed in chickens offered the negCON diet, while broilers offered the FA3 diet had the lowest platelet count values, Birds fed the posCON, FA1 and FA2 diets had statistically similar values for platelet counts with FA3 having the least value. Diet had similar (P <0.05) effects on the glucose concentration, symmetric dimethylarginine (SDMA), alanine transaminase (ALT), alkaline phosphatase (ALP), phosphorus and lipase concentration. The SDMA concentrations declined as the levels of FA inclusion in diet increased with the birds fed the FA3 (6.87 μg/dL) diet having the lowest levels of SDMA compared with all other diets. Therefore, it was concluded that supplementing FA up to 3%, improves the growth performance of broiler chickens without affecting their physiological and health status.

Keywords: Feed additive; Feed intake; Haematology; Health status; Humic substance; Performance traits.

3.1 Introduction

Over the years, poultry researchers have successfully developed fast-growing broiler strains through genetic selection, while learning more about the metabolism and utilization of nutrients and how those nutrients affect gene expression (Nkukwana, 2018). In addition, the usage and development of antibiotic growth promoters (AGPs) had enhanced the welfare of the birds and significantly increased feed efficiency and broiler performance (Oladukan & Adewole, 2020). Nevertheless, the continued use of antibiotics in broiler diets has been increasing causing anxiety among consumers due to the fear of residues in meat and antimicrobial resistances, which may affect consumer health (Gallo *et al*., 2020). Consequently, a ban was imposed on

the use of antibiotics in broiler production in developed countries and partially in South Africa. Efforts are therefore, being made to explore alternatives to antibiotics as well as the use of natural organic acids, phytogenic essential oils, and probiotics among others, for safe and sustainable broiler production.

Humic substances, such as humic acid (HA) and fulvic acid (FA), are straw-coloured, hydrophobic organic acids produced mostly from soil humus and plants, and they often make up a significant amount of the organic material found in water, soil and sediments (Jusadi *et al*., 2020). FA contains some reactive groups that have the effects of improving growth performance, feed utilisation and ultimately meat quality in animals by stimulating the gut health and immune system development (Bai *et al*., 2013). According to Chang *et al*. (2013), gut health is critical as gut microbiota have an influence on animal cell functions which affects the physiological, developmental, nutritional and immunological processes of the host. Gao *et al*. (2017) also observed that FA improved the growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus* (Sauvage). Whilst there is quite ample knowledge regarding HA influence on animal performance, limited information is known on the influence of FA as a possible replacement of antibiotics on performance in broilers. Therefore, the study assessed the influence of FA inclusion in diet on growth performance, protein utilisation efficiency and blood metabolites in broilers.

3.2 Materials and methods

3.2.1 Description of the study site

The study was carried out at Rooigrond, Braaikuikens located in the Northwest province of South Africa at coordinates of 25° 08′ 0″ South and 25° 05′ 0″ east. The experiment was conducted during the winter season from April until the end of May 2022.

3.2.2 Sourcing of fulvic acid

FA was donated from Ecofarma Southern Africa, Pretoria (Pty) Ltd – 2016/388451/07, a biotech company involved in the production of bio stimulants including the production of FA and other humus substances such as HA for the agricultural sector.



Figure 3. 1. Picture of FA.

3.2.3 Dietary treatments and experimental design

Five isonitrogenous and isoenergetic diets were formulated in accordance with the nutrient requirements of the broilers for the different growth phases. Formulation of dietary treatments was done with the inclusion of FA in diets in place of conventional antibiotics as follows: Negative control (T1): Commercial diet without antibiotics (NegCON). Positive control (T2): Commercial diet with antibiotics (posCON) FA1 (T3): Commercial diet + 1% FA, FA2 (T4): Commercial diet + 2% FA, and FA3 (T5): Commercial diet + 3% FA). The five dietary treatments were randomly allotted to a total of 40 pens. Each dietary treatment had eight replicate pens and each pen holding 10 birds, was considered as the experimental unit. The pen size was 3.5 m long x 1.0 m wide x 1.85 m high, which were designed in accordance with

standards ensuring welfare of chickens. Dietary ingredients and treatments are shown in Tables 3.1 to 3.3.

3.2.4 Animal management

A total of 400-day old chicks were purchased from Chicken Ranch Animal Feeds and Agricultural Supplies at Gauteng. On arrival, birds were arranged randomly and equally in broiler pens under brooding conditions. Broiler units were maintained at brooding temperatures of between 32.5°C and 33°C through the provision of heat from the installed infra-red lights. Before being placed in pens, the chicks were weighed to determine the initial weight. From day 1 to day 3, chicks received stress pack for the development of immune system and a layer of wood shavings were used as bedding of each pen. Chicks had *ad-libitum* access to feed and freshwater, provided by tube feeders and drinkers, respectively. Chicks were fed in a 3-phase feeding programme: Starter (day 1-14); Grower (15-28) and Finisher ration (day 28-42). Chickens were regularly monitored for general behaviour and health with a weekly monitoring report completed. Any sick chickens removed from the study and any mortalities recorded. Ethics approval was sought before commencement of the experiments from NWU-ANIMPROD REC and the following ethics number was given: NWU-00815-21-A5. Refer to 7.1 in page 120.

Table 3.1. Ingredient composition (%) of (FA) for starter diet.

Dietary treatments					
Ingredients	negCON	posCON	FA1	FA2	FA3
Fulvic acid (ml)	-	-	0.10	0.20	0.30
Fine-Yellow maize	63.20	55.40	53.4	53.4	53.40
Prime gluten (60)	1.20	1.20	1.20	1.20	1.20
Soybean meal	31.70	31.70	31.70	31.70	31.70
Full fat soya	0	0	0	0	0
Limestone (powder)	1.62	1.62	1.62	1.62	1.62
MCP	0.90	0.90	0.90	0.90	0.90
Fine Salt	0.34	0.34	0.34	0.34	0.34
Koeksoda	0.18	0.18	0.18	0.18	0.18
Choline (powder)	0.08	0.08	0.08	0.08	0.08
Lysine	0.25	0.25	0.25	0.25	0.25
Threonine	0.048	0.048	0.048	0.048	0.048
Methionine	0.23	0.23	0.23	0.23	0.23
BrStr +phytase	0.17	0.17	0.17	0.17	0.17
Coxistac (12)	-	0.05	-	-	-
Olaquindox	-	0.04	-	-	-

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2] Ingredients: BrStr = Broiler Starter; BrGr = Broiler Grower; BrFin = Broiler Finisher.

Table 3.2. Ingredient composition (%) of (FA) for grower diet.

	Dietary treatments				
Ingredients	negCON	posCON	FA1	FA2	FA3
Fulvic acid (ml)	-	-	0.10	0.20	0.30
Fine - Yellow maize	69.90	59.50	59.50	59.50	59.50
Prime gluten (60)	1.80	2.40	2.40	2.40	2.40
Soybean meal	19.70	2.22	2.22	2.22	31.70
Full fat soya	5.10	5.10	5.10	5.10	5.10
Limestone (powder)	1.46	1.22	1.22	1.22	1.22
MCP	0.72	0.5	0. 576	0. 56	0. 56
Fine Salt	0.32	0.32	0.32	0.32	0.32
Koeksoda	0.17	0.16	0.16	0.16	0.16
Choline (powder)	0.08	0.08	0.08	0.08	0.08
Lysine	0.28	0.28	0.28	0.29	0.29
Threonine	0.04	0.00	0.00	0.00	0.00
Methionine	0.23	0.23	0.23	0.23	0.23
BrGr+ phytase	0.17	0.17	0.17	0.17	0.17
Coxistac (12)	-	0.05	-	-	-
Olaquindox	-	0.04	-	-	-

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2] Ingredients: BrStr = Broiler Starter; BrGr = Broiler Grower; BrFin = Broiler Finisher.

Table 3.3. Ingredient composition (%) of FA for finisher diet.

	Dietary treatments				
Ingredients	negCON	posCON	FA1	FA2	FA3
Fulvic acid (ml)	-	-	0.10	0.20	0.30
Fine - Yellow maize	72.20	72.20	72.20	72.20	72.20
Prime gluten (60)	1.53	1.61	1.60	1.59	1.80
Soybean meal	18.00	0.50	0.60	0.5	0.5
Full fat soya	1.53	1.61	1.60	1.59	1.54
Limestone (powder)	1.30	1.07	1.07	1.07	1.07
MCP	0.50	0.33	0.33	0.33	0.33
Fine Salt	0.33	0.33	0.33	0.33	0.33
Koeksoda	0.13	0.12	0.12	0.12	0.12
Choline (powder)	0.08	0.08	0.08	0.08	0.08
Lysine	0.26	0.27	0.2	0.257	0.27
Threonine	0.03	0.00	0.00	0.00	0.00
Methionine	0.16	0.09	0. 09	0. 09	0. 09
BrFin +phytase	0.17	0.17	0.17	0.17	0.17
Coxistac (12)	-	0.05	-	-	-
Olaquindox	-	0.04	-	-	-

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal

commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.
[2] Ingredients: BrStr = Broiler Starter; BrGr = Broiler Grower; BrFin = Broiler Finisher.

Table 3.4. Nutrient composition % (unless otherwise indicated) of formulated experimental diets of starter, grower and finisher for broilers.

	Phase		
Parameters	Starter	Grower	Finisher
Volume	100	100	100
Moisture	11.56	11.35	11.58
ME (mg/kg)	11.40	12.10	12.30
Protein	20.78	18.00	16.01
Fat	3.13	4.16	3.67
Fibre	2.32	2.31	2.21
ASH	5.61	4.81	4.22
Calcium	0.95	0.84	0.75
Phosphorus	0.56	0.49	0.44
Sodium	0.19	0.18	0.17
Chlorine	0.30	0.29	0.3
Potassium	0.91	0.76	0.68

3.2.5 Growth performance

The birds were offered measured amounts of feed each day. Refusals were collected every morning before the subsequent feeding. The ADFI for each feeding phase was calculated as follows:

$$ADFI = \frac{Feed\ offered - feed\ refusal}{14\ days}$$

The birds were weighed initially to obtain the start weight. Subsequently, all birds in each replicate per treatment were weighed on weekly basis, using a commercial weighing scale and average daily gain (ADG) and feed conversion ratio (FCR) were calculated as follows:

$$ADG = \frac{Finish\ weight - start\ weight}{Age(days)}$$

Feed conversion ratio was calculated as the average daily feed intake divided by weight gain.

$$FCR = \frac{ADFI\ (g)}{ADG(g)}$$

3.2.6 Protein utilization efficiency

The quantity of Protein consumed (PC g/bird) was calculated by multiplying the feed intake during the feeding phase by the crude protein (CP_d) concentration in the diet (g/kg DM

consumed), which is obtained from the diet sheet formula. Protein efficiency ratio (PER g/kg) was determined as the ratio of the mean body weight gain (BWG) over the mean protein consumed. Specific growth rate (SGR), representing the proportion of growth per feeding phase and growth efficiency (GE), were determined as follows:

$PC = FI \times CP_d$

$PER = \frac{BWG}{PC}$

$SGR = \frac{In\ final\ weight - In\ initial\ weight}{14\ days} \times 100$

$GE = \frac{BWG}{Initial\ Weight}$

3.2.7 Hematology and serum biochemical analysis

Two days prior to the end of the experiment (day 40), 2 chickens were randomly selected for blood collection from each pen. Blood samples (4 ml) were collected from chickens bled via puncture of the brachial vein of birds into two separate vacutainer tubes (purple tubes which contained anti-coagulant for haematological parameters and red tubes with Anti-coagulant for serum biochemical analysis). Samples for haematological analysis were stored in an ice cooler and transported to IDEXX Laboratories Inc., (Gauteng, South Africa), where full blood analysis was done. An automated IDEXX LaserCytehd Haematology equipment was used to determine haematological parameters. The red top tubes were transported to the laboratory and centrifuged at 3000 g for 10 min at room temperature for serum separation. Pure serum samples were aspirated by pipette and stored in 1.5 ml Eppendorf tubes at -80^{0}C after, which the tubes were thawed at 4^{0}C pending biochemical analysis. After thawing, serum concentrations of albumin, total protein, glucose, chlorine, sodium, potassium, cortisol levels, urea and serum enzymes; alanine amino transferase and aspartate amino transferase were determined by using

commercial kits. Total cholesterol and triglycerides in serum were determined enzymatically using commercial reagent assay kits (ASAN total cholesterol, aminotransferase (AM 202-K) and ASAN triglyceride (TG-S, AM 157S-K), respectively, ASAN Pharm. Co. Ltd., Whasung, Korea).

3.2.8 Statistical analysis

Data on growth performance, protein utilization efficacy, serum biochemistry and haematology were analysed using the general linear model procedure of (SAS, 2010) with diet treatment being the only fixed effect. Repeated data on cumulative weight gain was analysed using the mixed procedure of (SAS, 2010) for repeated measures considering the effect of both the dietary treatment and week. The employed linear model is as follows:

$$Y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where Y_{ij} = the observation of the dependant variables (growth and blood parameters). μ = the overall mean (general mean) constant to all observations. t_i = the i^{th} fixed effect of the diet level. ε_{ij} = random error or residual effect associated with ij^{th} observation.

$$Y_{ijk} = \mu + T_i + W_j + (T * W)_{ij} + E_{ijk}$$

Where: Y_{ij} = observation of dependant variables (cumulative weight gain parameter)

μ = population mean constant to all observations. T_i = effect of diet. $T*W_{ij}$ = effect of diet interacting with week E_{ij} = random error term associated with observation *ijk*, assumed to be normally and independently distributed.

3.3 Results

3.3.1 Feed intake and growth performance

Table 3.5 shows the effects of FA incorporation in diets on growth traits in broilers in different feeding phases. From the results, dietary treatment only affected (P <0.05) the FCR in the Starter phase. Lowest FCR value was observed in birds fed FA3, whilst those offered the posCON diet had the highest. In the Grower phase, diet had a significant impact on all the growth performance traits. Birds offered the posCON diet had the highest (P < 0.05) ADFI, followed by those offered the FA2 diet. Birds offered the negCON diet had the least ADFI. Similarly, the birds fed the posCON and FA2 diets had the highest (P < 0.05) ADG with those fed the FA3 diet having the lowest. With regards to the FCR, the birds fed the FA diets had significantly (P < 0.05) lower values compared to the control diets. In the Finisher phase, dietary treatment affected the ADG and the FCR. The birds offered the posCON diet had higher FCR values followed by those offered the FA2 diet. Broilers offered the posCON diet also contained the lowest FCR accompanied by those offered the FA2 diet. Overall, ADG was higher in posCON (49.37 g/d) followed by FA2 diet (45.17 g/d), whilst negCON diets had the least. Moreover, the FCR was the lowest in the posCON diet followed by the FA2 diet. In all instances, the ADG and FCR values for the posCON and the FA2 diet were not different in all feeding phases and overall. Across the different feeding phases and overall, diet had no effect on ADFI.

Figure 3.1 demonstrates the effect of dietary FA on cumulative weight gain (CWG). Between weeks 1 and 3, there was no differences in weight gain among the treatments. From week 3 onwards, the birds in the posCON treatment gained more that the birds in other treatments. In

the final week, birds fed the FA2 diet gained at the same level as those fed the posCON diets and birds in both treatments achieved similar final weights towards the termination of the

Table 3.5. The effect of diets containing FA in ADFI (g), ADG and FCR of Cobb 500 broiler chickens.

	Dietary treatments						
[2]Parameters	negCON	PosCON	FA1	FA2	FA3	SEM	*P*-values
Starter							
ADFI (g)	33.44[a]	33.57[ab]	33.28[a]	33.31[a]	33.77[b]	0.44	0.0001
ADWG (g/d)	20.82[a]	21 .18[b]	20.89[a]	20.90[a]	20.76[a]	0.36	0.0001
FCR	1.61[ab]	1.69[b]	1.59[ab]	1.57[a]	1.62[ab]	0.04	0.0001
Grower							
ADFI (g)	88.99[a]	93.33[b]	90.02[ab]	90.66[ab]	87.07[a]	1.34	0.0001
ADWG (g/d)	52.35[a]	54.78[b]	53.31[ab]	54.09[b]	52.10[a]	1.32	0.0001
FCR	1.70	1.80	1.69	1.68	1.67	0.05	NS
Finisher							
ADFI (g)	144.82	145.02	144.41	144.95	147.05	0.97	NS
ADWG (g/d)	51.43	72.17	53.54	60.25	54.24	4.30	NS
FCR	2.90	1.82	2.93	2.50	2.84	0.28	NS
Overall							
ADFI (g)	89.08[a]	89.97[b]	89.24[a]	89.64[a]	89.30[a]	0.48	0.0001
ADWG (g/d)	41.53[a]	49.37[b]	42.58[a]	45.17[ab]	42.37[b]	1.29	0.0001
FCR	2.07[b]	1.77[a]	2.08[b]	1.92[a]	2.04[b]	0.06	0.0002

[a,b,] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

SEM= standard error of means

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2]Parameters: ADFI = average daily feed intake, ADWG = average daily weight gain, FCR = feed conversion ratio.

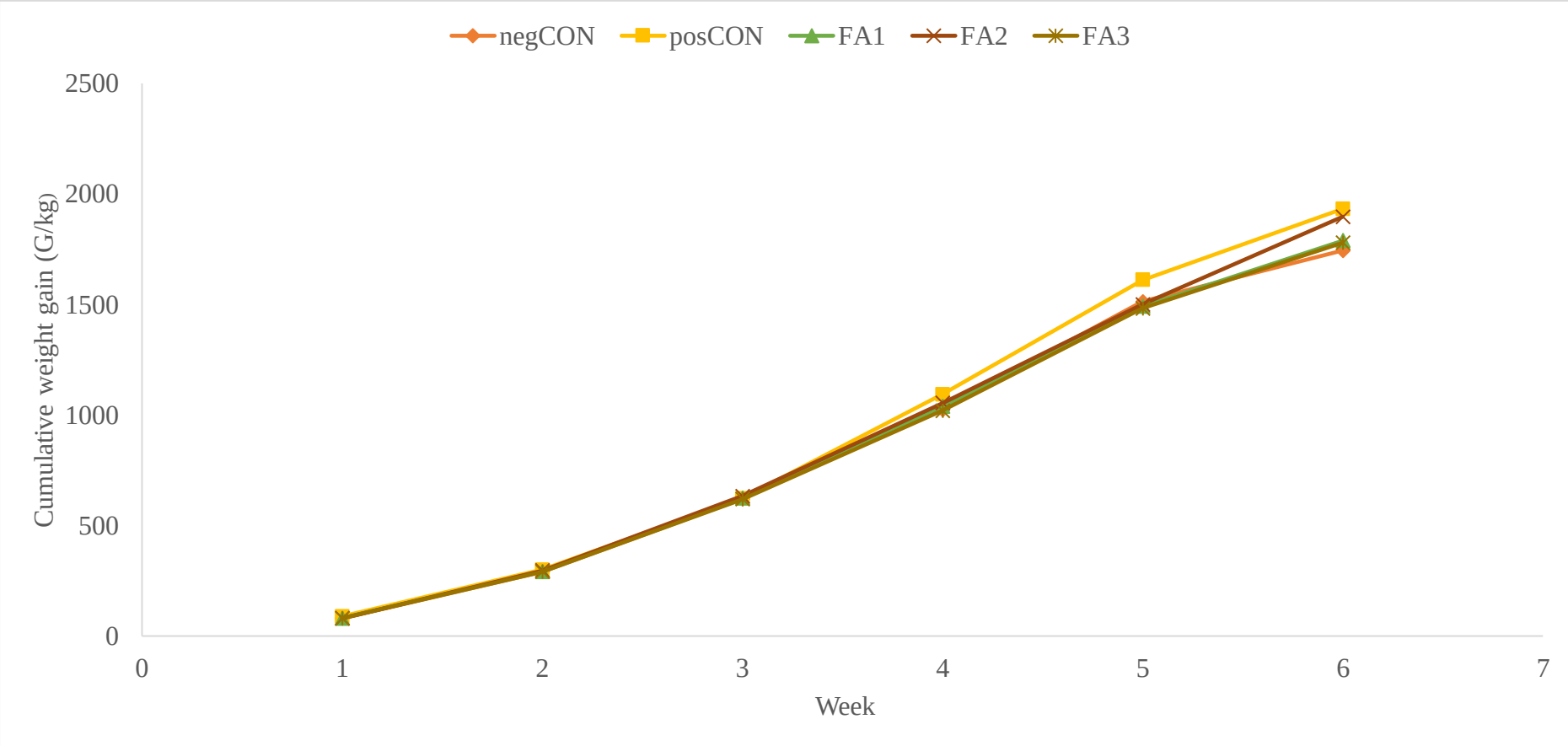


Figure 3. 2. The effect of dietary FA on cumulative weight gain on broiler chickens.

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

feeding trial. Generally, all diets showed a clearly linear increase in weight gain over the three feeding phases of the experiment. The final weight of broilers fed the negCON diet (1789.25 g/w) was the lowest, whereas for those fed posCON (1959.27 g/w) was the highest.

3.3.3 Protein utilization and growth efficiency

Table 3.6 shows the results of protein utilization and growth efficiency. Dietary treatments significantly (P<0.05) influenced protein utilization and growth efficiency parameters in all the three growth phases apart from the protein efficiency ratio (PER) in the grower phase, the protein consumed (PC) and the specific growth rate (SPR) at the Finisher phase. Diet influenced (P <0.05) all the protein utilization and growth efficiency parameters in the Starter phase and PC in the Grower phase. In the Starter phase, PC and PER were highest in birds fed the positive control diet. The birds fed the negCON and the FA3 diets, had the lowest PER. In the Grower phase, PC was also highest in birds fed the posCON diet although the values were not different from those of birds fed the FA1 and FA2 diets. Diet did not alter (P >0.05) on all parameters at the Finisher phase. Nevertheless, overall, PC was highest in birds fed the posCON diet followed by those offered the FA2 diet. Similarly, PER was observed to be high in broilers offered the posCON diet accompanied by those offered the FA2 diet. However, diet had no effect on overall SGR and GE.

3.3.3 Haematological parameters

Table 3.7 presents the effect of FA inclusion in diets on haematological parameters in broiler chickens. As indicted in the table, dietary treatments had no effect on all the haematology parameters apart from platelet count. Birds offered the negCON diet had the highest (P < 0.05) platelet count. Chickens offered the posCON, FA1 and FA2 diets had statistically similar values for platelet counts with FA3 having the least value.

Table 3.6. The effect of diets containing FA on protein utilization efficiency (g) of Cobb 500 broiler chickens.

	[1]Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	SEM	*P*-values
Starter							
PC (g)	6.95[a]	8.92[b]	6.92[a]	6.92[a]	7.02[ab]	0.09	0.001
PER	2.99[a]	4.55[b]	3.01[a]	3.06[a]	2.95[a]	0.06	0.001
SGR (% day [1])	14.39[a]	14.40[a]	14.44[b]	14.39[a]	14.44[b]	36.86	0.001
GE	0.46[a]	0.47[b]	0.46[a]	0.46[a]	0.46[a]	0.00	0.001
Grower							
PC (g)	16.01[b]	17.66[c]	16.20[b]	16.31[b]	15.67[a]	0.24	0.001
PER	3.27	3.39	3.29	3.31	3.33	0.13	NS
SGR (% day [1])	8.27	8.49	8.34	8.34	8.26	0.17	NS
GE	0.16	0.17	0.16	0.16	0.16	0.00	NS
Finisher							
PC (g)	23.17	24.88	23.11	23.19	23.52	0.15	NS
PER	2.82	3.09	2.87	2.99	2.86	0.19	NS
SGR (% day [1])	3.66	4.27	3.73	4.07	3.83	0.28	NS
GE	0.05	0.06	0.05	0.05	0.05	0.00	NS
Overall							
PC (g)	15.38[a]	17.15[b]	15.40[a]	15.47[a]	15.40[a]	0.08	0.001
PER	3.02[a]	3.68[b]	3.05[a]	3.12[b]	3.05[a]	0.06	0.001
SGR (% day [1])	0.09	0.08	0.09	0.09	0.08	0.00	NS
GE	0.22	0.23	0.22	0.23	0.22	0.00	NS

[a,b,c,] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

SEM = standard error of means (n = 6). [1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA. [2]PC = protein consumed, PER = protein efficiency ratio, SGR = specific growth rate, GE = gain efficiency.

Table 3.7. The effect of diets containing FA in blood haematology of Cobb 500 broiler chickens.

	[1]Dietary treatments						
Parameters	NegCON	PosCON	FA1	FA2	FA3	SEM	*P*- values
Haematocrit(g/dl)	34.00	34.66	34.83	33.33	36.16	1.38	NS
WBC($\times 10^9$/l)	18.29	17.72	18.12	14.04	15.88	2.15	NS
Platelet count (x 10^9/l)	44.05^{c}	31.21^{b}	32.16^{b}	30.25^{b}	25.41^{a}	6.34	0.03
Heterophils($\times 10^9$/l)	73.33	70.50	73.00	66.83	72.00	4.53	NS
Lymphocytes (x 10^9/l)	18.66	19.66	16.83	23.83	20.33	3.86	NS
Monocytes ($\times 10^9$/l)	8.16	8.66	9.50	9.50	8.83	1.94	NS
Eosinophils (x 10^9/l)	0.00	0.00	0.16	0.00	0.00	0.07	NS

[a, b,c,] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

SEM = standard error of means (n = 6)

P-values are the effects of dietary FA in blood haematology.

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2]Parameters: WWC = white blood cells, MNC = monocytes, ESNP = eosinophils.

3.3.4. Serum biochemistry

The effects of FA inclusion in diets on serum biochemistry are presented in Table 3.8. The inclusion of FA in diets had no effect ($P > 0.05$) on urea, total protein, calcium, chlorine, amylase lipase, bilirubin, and creatinine concentration. However, diet had similar impact ($P < 0.05$) on glucose concentration, symmetric dimethylarginine (SDMA), alanine transaminase (ALT), alkaline phosphatase (ALP), phosphorus and lipase concentration. Broilers offered the FA diets had higher glucose concentration compared with those fed the positive control. SDMA levels were highest in chickens offered the negCON diet followed by those offered the posCON diet. The SDMA concentration declined as the levels of FA inclusion in diet increased with the chickens given the FA3 diet having the lowest levels of SDMA compared with all other diets. Regarding the liver enzyme activity, diet had the ALT (129.12 IU/L) concentrations were highest levels ($P < 0.05$) in broiler chickens fed posCON diet followed by negative control (126.12 IU/L). The least ALT concentration (107.75 IU/L) was observed in birds fed the FA3 diet. The ALT level also declined with the inclusion of increasing levels of FA in diets. The broilers offered the posCON diet obtained the highest levels of ALP whilst the birds fed the FA diets had lower levels. Lipase concertation was highest in chickens offered the FA3 diet (66.62 IU/L) followed by chickens offered FA2 diets (54.75 IU/L) whilst the birds fed FA1 diet had the lowest levels (36.00 IU/L).

Table 3.8. The effects of diets containing of FA in serum biochemistry of Cobb 500 broiler chickens.

	[1]Dietary treatments						
[2]Parameters	NegCON	posCON	FA1	FA2	FA3	SEM	*P*-values
Glucose (mmol/L)	11.49[b]	10.86[a]	11.73[b]	11.04[ab]	11.26[ab]	0.20	0.03
SDMA (µg/dL)	8.00[b]	7.87[ab]	7.62[ab]	7.42[ab]	6.87[a]	3.40	0.001
Creatine (µ/L)	9.00	9.00	9.00	9.00	9.00	0.00	NS
Urea (mmol/L)	1.51	1.33	1.42	2.32	1.37	0.43	NS
Phosphorus (mmol/L)	4.50[a]	4.94[b]	4.97[b]	4.89[b]	5.20[c]	0.00	0.001
Calcium (mmol/L)	1.44	1.25	1.31	1.40	1.44	0.09	NS
TP (g/L)	120.00	230.25	120.00	113.50	114.00	51.25	NS
Albumin (g/L)	59.62	54.62	60.00	50.37	56.87	2.87	NS
ALT (IU/L)	126.12[ab]	129.12[b]	124.12[ab]	114.12[ab]	107.75[a]	7.37	0.042
ALP (IU/L)	34.12[b]	85.12[c]	19.25[a]	22.25[a]	22.36[ab]	39.76	0.012
Bilirubin (µmmol/L)	458.25	396.75	459.37	352.57	394.37	39.39	NS
Cholesterol (mmol/L)	1.30	1.56	1.47	1.58	1.68	0.20	NS
Amylase (IU/L)	396.25	392.62	438.87	371.62	357.62	29.35	NS
Lipase (IU/L)	53.00[b]	53.12[b]	46.00[a]	54.75[b]	66.62[c]	9.16	0.001

[a.b.c] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

SEM = standard error of means. Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA. [2]Parameters: ALT = alanine amino transaminase. ALP = alkaline phosphatase. TP = total protein; SDMA = symmetric dimethylarginine.

3.4 Discussion

Previous studies relating to the biological significance of humic substance as dietary feed additive have shown their potential in enhancing performance in livestock particularly in poultry production. Studies by Bai *et al*. (2013); Disetlhe *et al*. (2018), Arif *et al*. (2019) and Tang *et al*. (2023) showed that humic substances can promote animal growth and enhance feed efficiency in broilers. The objective of the current study was to investigate the effect of dietary FA, which is one of the humic substances, as an alternative growth promotant in place of commercial antibiotics in Cobb 500 broiler diets. In the current study, the effect of FA inclusion in diet on parameters was apparent. Whilst the broilers offered the posCON diet obtained the highest ADFI, ADG and lowest FCR, it appears that adding FA in diets at 2% had the same effect. Statistically, chickens offered the FA2 diets had similar values for growth performance parameters as those fed the posCON diet. Moreover, while the performance in birds fed the other FA diets was lower than those of the posCON and FA2 diets, the differences in performance appeared to be subtle. This, therefore, confirms that the inclusion of FA in diets had the same effect as the inclusion of antibiotics in diets. This is consistent with the findings by Bai *et al*. (2013) and Wang *et al*. (2020), who observed that the inclusion of humic substance enhanced growth performance in growing pigs, improved immunity in growing swine, Change *et al*. (2013) stimulated mice immune response, Vuckits *et al*. (2015); enhanced the immune system of *Litopenaeus vannamei* Guti´errez-Dagnino *et al*. (2015) and positively altered growth performance in juvenile loach *Paramisgurnus dabryanus* (Sauvage) (Gao *et al*., 2017). In addition, Pistová *et al*. (2016); Jad'uttová *et al*. (2019) and Disethle *et al*. (2018) also observed enhanced growth performance in broilers fed diets containing HA.

The results from this study are consistent with the study conducted by Tang *et al*. (2023), who observed an increase with 0.5% FA inclusion in FI, FCR and gain in the first 7 days in broiler

diets. Where 0.3% increased feed intake and FCR at the grower phase with FA having no effect on growth traits for the rest of the feeding days. Nevertheless, Tang *et al*. (2023) used lower FA inclusion levels than those used in this study. Morales *et al*. (2011) and Tang *et al*. (2023) hypothesized that the mechanism of action of the FA could be through its ability to significantly boost the processes of mineral ion ingestion, absorption in a particular organ or fluid, and animal excretion, which could account for the positive impact on broiler weight gain with the incorporated of FA diets. Prayogi (2011) and Bahadori *et al*. (2017) also suggested that this could be attributed to the ability of substances in stabilizing the intestinal microflora, which ensures improved utilization and availability of nutrients from the diet, making them more absorbable.

The protein utilization efficiency concept is often applied in the determination of the closeness of the relationships between amino acids dietary supply and the needs of the animal (Ismali *et al*., 2020). Generally, apart from fish and rats, there is limited literature available regarding the utilization of the parameters in assessing the feed value of various protein sources, or how certain feed additives may increase the biological value of protein in animals. It is widely acknowledged that the growth rate of weanling rats grown in controlled environments are reliable indicators of the dietary protein value (Wang *et al*., 2018). In the current study, there was very little effects of diet on the protein utilization efficiency parameters in all the feeding phases apart from the overall PC and PER. The fact that the birds fed the posCON diet obtained the highest PC and PER values showed that the antibiotics were more effective in increasing the biological protein value than the FA; PER is a popular technique for assessing the protein content of feed, it has been the benchmark used by the feed industry to assess protein feed. The value of a protein source in meeting requirements is determined by its nutritional value.

Although not affected by the diet specific growth rate and protein efficiency ratio of broilers in the 1.0%, 2.0 % and 3.0%, FA supplemented diets rapidly increased in the first feeding phase compared to the negative control group. There were no exceptional SGR differences between the control and 3% FA inclusion group in the last two growth phases of the experiment. Therefore, data from the current study is in line with observations by Gao *et al*. (2017), who showed increased levels of specific growth rate in juvenile loach supplemented with FA. Additionally, a decline in PC concentration, elevates protein efficiency ratio, which may indicate that more necessary amino acids in the diet become deficient, which can result in low gain efficiency (Walk & Olukosi, 2019). Currently, there are very few attempts made to investigate the use of protein utilisation efficiency parameters in animal nutrition studies.

Haematology is used as one of the tools used to assess immunological, physiological and health status of an animal (Faye & Bengoumi, 2018). According to Swennen *et al*. (2005) and Abd El-Hack *et al*. (2019), it has been affirmed that livestock nutrition also affects haematological parameters. The current study demonstrated that all full blood counts apart from PC were not influenced by the experimental diets. Chickens fed the negCON diet with no addition of antibiotics, had the highest concentration of PC compared to the other dietary treatments. This could be in response to the unprotected immune system due to the absence of the antibiotics or FA in the diets. Haematocrit is closely related to haemoglobin concentration and high levels of haematocrit illustrate greater erythrocyte levels. Haematocrit levels which are lower than accepted levels, illustrate insufficient concentrations of red blood cells. Although there was no dietary effect, the haematocrit levels appeared to increase with increasing levels of FA in the diets. This could attribute to the effects of FA in increasing eryptosis activity in birds as observed in other studies (Disetlhe *et al*., 2018). Granulocytes (neutrophils, eosinophils and heterophils) and T and B lymphocyte cells (T and B cells) play an important role in immunity

development, they are white blood cells that act as a defence mechanism by attacking diseases, viruses and bacterial infections invading the body (Glen & Armstrong 2019). Although the increase had no effect of diet on the white blood cell, a marginal decreased in heterophils levels was observed in birds offered FA2, resulting in depressed percentage of heterophils and increased lymphocytes percentage compared to the control groups.

The results are inconsistent to Akaich *et al*. (2022) who observed increased heterophils percentage in diets supplemented with 0.1% HA, resulting in declined lymphocyte percentage, leading to increased heterophil/lymphocyte ratio compared to the control group. On the contrary, results are similar to findings by Rath *et al*. (2006) and Arafat *et al*. (2017) who affirmed that HA (1.0% or 2.5%) did not alter white blood cell, red blood cell, monocyte and lymphocyte counts, or haematocrit values. All haematological results from this study were within the normal range for broiler chickens in good health (Kumanda *et al*., 2019; Van Niekerk *et al*., 2020; Matshogo *et al*., 2020). This shows that experimental diets were nutritionally acceptable for the Cobb 500 chickens and did not compromise their health status. Additionally, these observations can designate that tested FA dosage levels can depress stressful incidences in birds (Ipek *et al*., 2008). Therefore, data from this study illustrated that FA may have a positive impact on immune systems of broiler chickens.

Serum biochemistry profiles in broilers can also be used to assess, the physiological state, health and nutritional status in birds (Alagawany *et al*., 2020). Serum biochemistry can thus be helpful in the diagnosis of infections and tracking chicken responses to the infections through exhibition of clinical symptoms. According to Hall & Cash (2012), ALP and ALT are liver enzymes that can be utilized to assess the homeostatic responses of liver metabolites passing through the blood portal. They are also indicators of liver diseases and liver enzyme activities,

which are subtle to toxic substances in the feed (Gudiso *et al*.,2019; Ahamefule *et al*., 2006). FA results of ALT and ALP concentrations in this study, confirmed a positive effect of FA in diet in reducing ALT and ALP concentrations in the broiler blood serum compared to the control groups. Thus, confirming no liver damage in broilers with the inclusion of FA in diets. According to Rajman *et al*. (2006), ALP has been linked with calcium and phosphorus metabolism and its mechanism has been implicated in the specific roles in regulating chondrogenic and osteoblastic activities in the growth process of birds.

Literature on broilers describes the normal acceptable ALP value to be between 8 and 108 IU/L (Fuge, 2003). Therefore, results from the present study fall within the acceptable normal reference range (19.25 IU/L – 85.12 IU/L) of ALP poultry levels. Increasing levels of ALT/ALK in serum biochemistry can be attributed to exposure of toxins resulting to toxaemia, alteration in permeability of hepatocellular membrane and inflammation (Khanyile *et al*., 2016). High levels of phosphate and calcium were observed in diets supplemented with FA compared to the control groups. The highest calcium: phosphorus ratio was observed in broilers offered 3% FA. Results are consistent to previous reports by El-Ratel *et al*. (2022) who observed increased levels of calcium and phosphorus in diets containing humates in broilers. According to Korsakov *et al*. (2019), low phosphorus concentrations in blood serum are associated with vitamin D deficiency, avitaminosis, hyperparathyroidism, impaired intestinal absorption and kidney diseases. Increasing FA levels corresponded with high lipase as observed in previous studies (Gao *et al*., 2017).

According to Yerramilli *et al*. (2016), SDMA is widely used as a renal biomarker for perceiving early diagnosis in renal and kidney function. SDMA is formed when methyl groups are added to the nitrogen molecules on polypeptides containing arginine during

posttranslational modification (Hall *et al*., 2016). In the present study, SDMA significantly decreased with increasing FA levels compared to the control groups. The least SDMA concentration was observed in birds fed the highest FA level (FA3), illustrating that FA did not jeopardize the birds' state of health. Usually, high SDMA levels are linked to stress, cardiac problems, and other illnesses (Thema *et al*., 2022). Nevertheless, all serum biochemical parameters obtained in the current study fell within the normal serum biochemical values for healthy animals (Manual Merk, 2012).

3.5 Conclusion

Supplementing FA in diets had a positive influence on growth attributes, wherein a linear stable increase was observed in all treatment's groups. ADG, ADFI, FCE and PUE were similar in mostly the posCON, FA2 and FA3 groups. Low concentration levels of AST/ALP and SDMA in treatment diets, indicated the importance of the hepato-protective ability of FA in reducing the possibility of liver and kidney damage. Furthermore, haematological parameters fell within the normal recommended range for normal healthy animals. Therefore, data from this present study proved that the inclusion of FA in broiler rations at 3%, promoted similar growth performance and health status as could have been the standard grower diet combined with antibiotics. Supplementing FA in drinking water can be considered a viable alternative of AGPs in broiler diets. Results obtained from this study are vital in providing low-priced feed formulation that elevate the growth performance and health status in poultry production systems. Nevertheless, it is paramount to investigate the impact of FA in meat quality attributes in broilers diets as it is an important factor for consumer acceptance.

3.6 References

Abd El-Hack, M.E., Alagawany, M., Mahrose, K.M., Arif, M., Saeed, M., Arain, M.A., Soomro, R.N., Siyal, F.A., Fazlani, S.A. & Fowler, J., 2019. Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season. Anim. Nutr. 5(1), 49─55.

Arafat, R.Y. & Khan, S.H., 2017. Evaluation of humic acid as an aflatoxin binder in broiler chickens. Ann. Anim. Sci. 17(1), 241─255.

Arif, M., Alagawany, M., Abd El-Hack, M.E., Saeed, M., Arain, M.A. & Elnesr, S.S., 2019. Humic acid as a feed additive in poultry diets. Review. Iran J. Vet. Res. 20(3), 167.

Ahamefule, F.O., Eduok, G.O., Usman, A., Amaefule, K.U., Obua, B.E. & Oguike, S.A., 2006. Blood Biochemistry and Haematology of Weaver Rabbits Fed Sundried, Ensiled and Fermented Cassava Peel Based Diets.

Akaichi, A., Jebali, A., Benlarbi, M., Mahjoub, T., Kaboudi, K., Chaouacha-Chekir, R.B., Haouas, Z. & Boudhrioua, N., 2022. Effects of humic acid and organic acids supplements on performance, meat quality, leukocyte count, and histopathological changes in spleen and liver of broiler chickens. Res. Vet. Sci. 150, 179─188.

Alagawany, M., Nasr, M., Al-Abdullatif, A., Alhotan, R.A., Azzam, M.M. & Reda, F.M., 2020. Impact of dietary cold-pressed chia oil on growth, blood chemistry, haematology, immunity and antioxidant status of growing Japanese quail. Ital. J. Anim. Sci. 19(1), 896─904.

Bahadori, Z., Esmaielzadeh, L., Karimi-Torshizi, M.A., Seidavi, A., Olivares, J., Rojas, S., Salem, A.Z.M., Khusro, A. & López, S., 2017. The effect of earthworm

(Eisenia foetida) meal with vermi-humus on growth performance, haematology, immunity, intestinal microbiota, carcass characteristics, and meat quality of broiler chickens. Livest. Sci. 202, 74-81.

Bai. H.X., Chang. Q.F., Shi. B.M. & Shan. A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing- finishing pigs. Livest. Sci. 158(13), 118─123.

Buyse, J., Jassens, K., Van Der Geyten, S., Van As, P., Decuypere, E. & Darras, V.M., 2002. Pre- and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens. Br. J. Nutr. 88, 641─653.

Chang. Q.F., Bai. H.X., Shi. B.M. & Shan. A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing-finishing pigs. Livest. Sci. 158(1-3), 118─123.

Disetlhe, A.R.P., Marume, U. & Mlambo, V., 2018. Humic acid and enzymes inclusion in canola-based diets generate different responses in growth performance, protein utilization dynamics, and hemato-biochemical parameters in broiler chickens. Poult. Sci. 97(8), 2745-2753.

El-Ratel, I.T., El Basuini, M.F., Khattab, A.A., Mekawy, A.I. & Fouda, S.F., 2022. Ameliorative impacts of sodium humate on heat-stressed laying Japanese quail (Coturnix coturnix Japonica). J. Anim Physiol Anim. Nutr.

Faye, B. & Bengoumi, M., 2018. Camel clinical biochemistry and haematology. Cham (Switzerland). Springer. 173, 216

Fuge, A.M., 2003. California avian laboratory reference ranges. California Avian Laboratory.

Gallo, M., Ferrara, L., Calogero, A., Montesano, D. & Naviglio, D., 2020. Relationships between food and diseases: What to know to ensure food safety. Food Res. Int. 137, 109414.

Gao. Y., He. J., He. Z., Li. Z., Zhang. B., Mu. Y., Lee. J. & Chu. Z., 2017. Effects of fulvic acid on growth performance and intestinal health of juvenile loach Paramisgurnus dabryanus (Sauvage*).* Fish & Shellfish Immunology. 62 47–56.

Glenn, A. & Armstrong, C.E., 2019. Physiology of red and white blood cells. Anaesth. Intensive Care Med. 20(3), 170-174.

Gudiso, X.C., Hlatini, V.A., Ncobela, C.N., Chimonyo, M. & Mafongoya, P.L., 2019. Serum metabolites and weights of internal organs of broilers fed on varying levels of Acacia angustissima leaf meal. Can. J. Anim. Sci. 99(3), 475─481.

Guti´errez-Dagnino, A., A. Luna-Gonz´alez, J. A. Fierro-Coronado, P. Alvarez-Ru´ ´ ız, M. del Carmen Flores-Miranda, S. Miranda-Saucedo, V. Medina-Beltr´an, & R. Escamilla-Monte., 2015. Effect of inulin and fulvic acid on survival, growth, immune system, and WSSV prevalence in *Litopenaeus vannamei*. Lat. Am. J. Aquat. Res. 43, 912–921.

Hall, J.A., MacLeay, J., Yerramilli, M., Obare, E., Yerramilli, M., Schiefelbein, H., Paetau-Robinson, I. & Jewell, D.E., 2016. Positive impact of nutritional interventions on serum symmetric dimethylarginine and creatinine concentrations in client-owned geriatric dogs. PLoS One. 11, 421-441.

Hall, P. & Cash, J., 2012. What is the real function of the liver 'function'tests? Ulster Med. J. 81(1), 30.

Ipek, H., Avci, M., Iriadam, M., Kaplan, O. & Denek, N., 2008., Effects of humic acid on some hematological parameters, total antioxidant capacity and laying performance in Japanese quails. Archiv fur Geflugelkunde. 72(2), 56─60.

Ismail, B.P., Senaratne-Lenagala, L., Stube, A. & Brackenridge, A., 2020. Protein demand: Review of plant and animal proteins used in alternative protein product development and production. Anim. Front. 10(4), 53-63.

Jaďuttová, I., Marcinčáková, D., Bartkovský, M., Semjon, B., Harčárová, M., Nagyová, A., Váczi, P. & Marcinčák, S., 2019. The effect of dietary humic substances on the fattening performance, carcass yield, blood biochemistry parameters and bone mineral profile of broiler chickens. Acta Vet. Brno. 88(3), 307─313

Jusadi. D., Aprilia. T., Setiawati. M., Suprayudi. M.A & Ekasari. J., 2020. Dietary supplementation of fulvic acid for growth improvement and prevention of heavy metal accumulation in Nile tilapia fed with green mussel. Egypt. J. Aquat. Res. 46(3), 95─301.

Khanyile, M., Ndou, S.P. & Chimayo, M., 2016. Influence of Acacia tortilis leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. Anim Prod Sci. 57(4), 675-682.

Korsakov, K., Simakova, I., Vasilyev, A., Lifanova, S. & Gulyaeva, L., 2019. The effect of humic acids on the natural resistance of the body of broiler chickens and the quality of their meat.

Kumanda, C., Mlambo, V. & Mnisi, C.M., 2019 "From Landfills to the Dinner Table: Red Grape Pomace Waste as a Nutraceutical for Broiler Chickens." Sustainability. 11, 19–31. doi:10.3390/su11071931

Manual, M., 2012. Haematologic reference ranges. The Merck Veterinary Manual.

Matshogo, T.B., Mnisi, C.M. & Mlambo, V., 2020. Dietary green seaweed compromises overall feed conversion efficiency but not blood parameters and meat quality and stability in broiler chickens. Agriculture. 10(11), 547.

Morales, O.Y., Navarrete, J.M., Gracia, L., Macias, L., Rivera, M. & Sanchez, F., 2011. Effect of fulvic acids on the electrolyte's physiology in vertebrates. Nucl. Instrum. Meth. 652, 838─840.

Nkukwana. T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci. 48(5), 869─884.

Oladokun, S. & Adewole, D.I., 2020. In ovo delivery of bioactive substances: an alternative to the use of antibiotic growth promoters in poultry production. A review. J. Appl. Poult. Res. 29(3), 744-763.

Pistová, V., Arpášová, H. & Hrnčár, C., 2016: The effect of the humic acid and garlic (*Allium sativum L*) on performance parameters and carcass characteristic of broiler chicken. J Cent Eur Agric. 17, 1168-1178.

Prayogi, H.S., 2011. The effect of earthworm meal supplementation in the diet on quail's growth performance in attempt to replace the usage of fish meal. Int J. Poult Sci. 10(10), 804-806.

Rajman, M., Juráni, M., Lamošová, D., Máčajová, M., Sedlačková, M., Košťál, Ľ., Ježová, D. & Výboh, P., 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 145(3), 363-371.

Rath, N.C., Huff, W.E. & Huff, G.R., 2006. Effects of humic acid on broiler chickens. Poult. Sci. 85(3), 410-414.

Swennen, Q., Janssens, G.P.J., Millet, S., Vansant, G., Decuypere, E. & Buyse, J., 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens, Endocrine functioning and intermediary metabolism. Poult Sci. 84(7), 1

Tang, C., Kong, W., Wang, H., Liu, H., Shi, L., Uyanga, V.A., Zhao, J., Wang, X., Lin, H. & Jiao, H., 2023. Effects of fulvic acids on gut barrier, microbial composition, faecal ammonia emission, and growth performance in broiler chickens. J. Appl Poult Res. 32(1), 100322.

Thema, K.K., Mnisi, C.M. & Mlambo, V., 2022. Stocking density-induced changes in growth performance, blood parameters, meat quality traits, and welfare of broiler chickens reared under semi-arid subtropical conditions. Plos one. 17(10), p.e0275811.

Vucskits, A.V., Hullár, I., Bersényi, A., Andrásofszky, E., Kulcsar, M. & Szabó, J., 2010. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J. Anim Physiol Anim Nutri. 94(6), 721─728.

Walk, C.L. & Olukosi, O.A., 2019. Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to 28 D post-hatch. Poult. Sci. 98(9), 3884-3893.

Wang, Y., Wang, R., Hao, X., Hu, Y., Guo, T., Zhang, J., Wang, W., Shi, X., An, X. & Qi, J., 2020. Growth performance, nutrient digestibility, immune responses and antioxidant status of lambs supplemented with humic acids and fermented wheat bran polysaccharides. Anim Feed Sci Technol. 269, 114644.

Wang, Y., Zhou, J., Wang, G., Cai, S., Zeng, X. & Qiao, S., 2018. Advances in low-protein diets for swine. J. Anim. Sci. Biotechnol. 9(1), 1-14.

Van Niekerk, R.F., Mnisi, C.M. & Mlambo, V., 2020. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. Br. Poult. Sci. 61(5), 566-573.

Yerramilli, M., Farace, G., Quinn, J. & Yerramilli, M., 2016. Kidney disease and the nexus of chronic kidney disease and acute kidney injury: the role of novel biomarkers as early and accurate diagnostics. Veterinary Clinics: Small Animal Practice. 46(6), 961-993.

CHAPTER 4

THE EFFECT OF FULVIC ACID ON CARCASS CHARACTERISTICS AND MEAT QUALITY PARAMATERS IN BROILER CHICKENS

Abstract

The current study was conducted to assess the effect of fulvic acid (FA) inclusion in diets on carcass characteristics and meat quality parameters on broilers. A total of 400 (Cobb 500) day-old chickens were reared in 40 pens (experimental unit), each pen carrying 10 birds, replicated 8 times. The experimental diets were formulated to be isonitrogenous and isoenergetic as follows; NegCON (T1): commercial diets without antibiotics, posCON (T2): commercial diet with antibiotics, FA1 (T3) commercial diet + 1% FA1, FA2 (T4): commercial diet + 2% FA and FA3 (T5): commercial diet with 3% FA. The feeding trial was conducted over 3 feeding phases, Starter (day 1-14); Grower (15-28) and Finisher ration (day 28-42). Chicks had *ad libitum* access to freshwater and feed throughout a period of 6 weeks. All the chickens were deprived feed for 13 hours to empty the GIT. At day 42, 20 birds per treatment were slaughtered, dressing percentages, digestive tract traits were evaluated, and breast samples (Pectoral majors) were taken to evaluate meat quality parameters. From the results, diet had a significant effect ($P < 0.05$) on all measured carcass characteristics parameters apart from the dressing percentage, chilling loss percentage and the thigh index. Chickens fed the posCON diet (1959.27 g) obtained the highest slaughter weights, followed by those fed the FA2 diet (1942.10 g), while the birds fed the negCON diet had the lowest value (1789.25 g). The heaviest gizzards and liver weights were observed in chickens fed the posCON diet (42.25 g & 49.35 g) followed by chickens offered the FA1 diet (37.68 g and 39.37 g), respectively. Chickens offered the posCON (42.61) diet had statistically similar values for lightness as with those fed the FA1 (42.62) and FA3 (42.54) diets. However, the birds fed the FA2 (41.90) diets appeared

to have darker breast muscle compared to all other treatments. Diet had no effect ($P > 0.05$) on all the SFAs parameters apart from the docosanoic (C22:00) acid. Birds fed the FA1 and FA2 diets had the highest ($P < 0.05$) concentrations of docosanoic acid (C22:00), whilst those fed the FA3 diet had the lowest. Diet had a significant effect on the following polyunsaturated fatty acids (PUFAs): eicosadienoic acid (C20:2) and 16-docosadienoic acid (C22:2). Dietary treatments had no effect on all the total fatty acids profiles from the the omega 6 fatty acid (n-6PUFA) and PUFA/SFA ratio. Findings from this study illustrate that the experimental diets cooperated with FA inclusion, revealed an increase in total PUFA/SFA ratio compared to the control diets. However, diets consisting of FA reduced the total n-3/n-6 ratio in all the FA treatments with FA2 having a ratio of 5 in the n-3PUFA, resulting in a beneficial impact on the important fatty acids needed for human and animal health maintenance. Therefore, it can be concluded that inclusion of FA up to 3% in broiler diets did not compromise internal, organ sizes, meat quality attributes and carcass characteristics.

Keywords: Carcass traits; Fatty acids; Humic substance; Meat quality; Poultry meat; Visceral organs.

4.1. Introduction

Broiler meat is the most popular and cheapest protein source globally and in the developing world because it does not have dietary or religious restriction compared to beef and pork (Haque *et al*., 2020). Apart from being cheap, broiler meat is highly nutritious with high protein content as well as the high levels of desirable polyunsaturated fatty acids that influence meat quality and acceptability (Ivanović *et al*., 2016). The nutritional composition of meat is critical and vital for consumer acceptance (Santoso *et al*., 2018). According to Mir *et al*. (2017), texture, juiciness, odour, tenderness, firmness and meat appearance are some the most

paramount meat quality attributes that influence meat acceptability. Ismail & Joo (2017) reported that texture and meat visual appearance are critical determinants of consumer purchasing decision. Therefore, the influence of production procedures on meat quality should also be considered in the broiler production industry to ensure production of safe, wholesome, and acceptable meat (Geletu *et al*., 2021).

A variety of factors, among them, breed and nutritional changes, have been observed to influence meat quality. Nutritional manipulations have been observed to significantly influence meat visual quality and nutritional composition. Currently, there has been greater efforts to replace the antibiotic growth promoters (AGPs), which have been effective in improving the feed efficiency, health, and general performance of broilers. The use conventional antibiotics is becoming problematic due to the fear of increased antimicrobial resistance and accumulation of residues in meat that may affect consumer health. In Chapter 3, the FA as an alternative to antibiotics was explored. Apart from its reported influence on growth performance in Chapter 3, FA is characterised by carboxyl and hydroxyl groups that may influence meat quality and composition in broilers as reported elsewhere (Pietrosemoli & Green, 2018; Disethle *et al*., 2019). Therefore, the study assessed the effect of FA inclusion in diets on meat quality and fatty acid profiles in broiler meat.

4.2 Materials and methods

4.2.1 Study site, source of diets and animal management

The study was carried out at Rooigrond, (Braaikuikens) as explained in Section 3.2.1. The diets and experimental design are explained in Sections 3.2.2-3.2.3., while the animal management is explained in Chapter 3 under Section 3.2.4.

4.2.2 Slaughter process

At the end of the trial (day 42), the chickens in the pens were deprived of feed for a period of 12-13 hours from 18h00 to 06h30, to empty the crop. The chickens were weighed to obtain the slaughter weight and taken to the Rooigrond Braaikuikens abattoir; a local commercial abattoir located close to the experimental houses for slaughter. At the abattoir, the chickens were placed in a movable rack facing down with their feet on the rail before being stunned. The birds were immediately exsanguinated, defeathered and eviscerated according to standard procedures for humane animal slaughter. Chickens were exsanguinated by manually severing both the carotid artery and the jugular vein with a knife and allowed to bleed for 120 seconds. After bleeding, birds were scaled in hot water at 59 degrees for 30seconds in a rotary drum scalder or plucker, followed by de-feathering and the removal of the heads and legs. The carcass were; subsequently eviscerated with the internal organs including the liver, spleen, gizzard, and intestines were removed and placed separately into small plastic bags and labelled according to their treatments.

4.2.3 Carcass traits

After slaughter, the carcasses were immediately weighed to obtain the hot carcass weight (HCW) and subsequently placed in a cold room to chill overnight at 4^{o}C. After cooling for 24 hours, the carcasses were reweighed by using an electronic weighing scale to obtain the cold carcass weight (CCW). The dressing out percentage was also determined as the proportion of HCW on slaughter weight. Breasts and thighs were then carefully removed and weighed. The proportion of HCW on slaughter weight was used to calculate the dressing percentage. Dressing percentage was calculated as follows:

$$\text{Dressing (\%)} = \text{HCW/ Slaughter weight}*100\%$$

Eight breast samples per treatment were obtained, vacuum packed and sent for analysis of fatty acids at the Food Science Division. Stellenbosch University, Western Cape, (SA).

4.2.4 Internal organs

The liver, spleen, gizzard, proventriculus and small intestine (jejunum. duodenum and ilium) were surgically removed and individually weighed while the intestinal length was also measured using a tape measure.

4.2.5. Meat quality measurements

(i) Meat pH and temperature measurements

Meat pH and temperature were measured immediately after slaughter and 24 h after slaughter on the breast and thigh muscle using a corning model 4 pH-temperature meter (Stanford *et al.,* 2003).

(ii) Meat colour

Meat colour: (*L** = Lightness. *a** = Redness and *b** = Yellowness) was determined using a Minolta colour-guide. (BYK-Gardener GmbH. Geretsried. Germany) with a 20 mm diameter measurement area, illuminant D65-daylight, and a 10º observation angle after 30 minutes blooming time. The colour meter was calibrated before measurements using the green standard. Colour recording was done on 3 freshly cut breast muscles of the chicken of each pen.

iii) Meat cooking loss

Breast muscle samples were chilled overnight at 4°C and were individually weighed (70-80 g) to obtain initial weight (w1) of the pectoralis major muscle after thawing. The samples were then placed in a small, labelled plastic bag then attached to the rack inside the water bath and

then placed inside the water and cooked to a core temperature of 80°C for 60 minutes. The boiled samples were removed from the water bath and left to cool for about 20 minutes. The samples were re-weighed to obtain the cooked weight (w2) of the pectoralis major muscle. The cooking loss was calculated based on the difference between the weight of raw meat and cooked meat using the following equation:

$$\text{Cooking loss (\%)} = (w1-w2)/w1*100\%$$

Where: w1 is the weight of raw meat. W2 is weight after cooking.

(iv) Meat tenderness

After cooking cylindrical samples of breast muscle were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using Meullenet-Owens razor shear blade (A/MORS) mounted on a Texture analyser. The reported value in Newtons (N) represented the average of the peak force measurements of each sample.

(v) Water holding capacity

The water holding capacity (WHC) of the meat measurements were done on the surface of a freshly cut slice of the pectorals major muscle (PMM) (8-16 grams) and was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure), using the filter-paper press method developed by Grau & Hamm (1957). The fresh weights and initial weights were measured using a digital scale before and after pressure application. WHC was calculated using the equation:

$$\text{WHC (\%)} = (\text{Initial weight}-\text{Weight after pressing})/\text{Initial weight}*100\%$$

(vi) Drip loss by suspension

Drip loss measurement was determined using a method adapted from Zhang *et al*. (2009). Where pieces of muscle from the pectoralis major muscle (PPM) weighing ~ 2-3 grams (wet weight. w1) were hooked and suspended using wire steel in a plastic bottle and sealed properly so that the samples did not touch the sides of the bottle. The suspended samples were stored in a cold room at 4°C for 72 hours. The meat samples were reweighed to obtain weight after drip (w2). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$\text{Drip loss (\%)} = (w1-w2)/w1*100\%$$

Where: w1 is initial weight and w2 is weight after drip.

(vii) Proximate and fatty acid analysis

Total lipid was obtained from feed material using a Soxhlet extraction method according to AOAC (2003) fat determination procedures. Total lipids from muscle samples were quantitatively extracted. According to the method of Folch *et al.* (1957), fatty acid methyl esters (FAMEs) were quantified using a Varian 430 flame ionization GC. Butylated hydroxytoluene, an antioxidant was added to the chloroform: methanol combination at a concentration of 0.001%. The fat extracts were dried under vacuum in a rotary evaporator then dried overnight in a vacuum oven at 50°C with phosphorus pentoxide as a moisture adsorbent. The total extractable fat was calculated gravimetrically and represented as percent fat (w/w) per 100 g tissue. The fat removed from feed, known as the subcutaneous fat and muscle, was frozen in a polytope (glass vial with a push-in top) and kept at –20°C until fatty acid analysis. A disposable glass Pasteur pipette was used to transfer a lipid aliquot (20 mg) from the feed (subcutaneous and muscle lipid) into a Teflon-lined screw-top test tube. 0.5 M NaOH in methanol and 14% boron trifluoride in methanol, were used to trans-esterify fatty acids to

create methyl esters (Park & Goins. 1994), using a Varian 430 flame ionization GC with a fused silica capillary column (Chrompack CPSIL 88 (100 m length. 0.25 mm ID. 0.2 m film thicknesses). (FAMEs) from the subcutaneous fat, feed and muscle were measured. An initial isothermic time was used in the analysis (40°C for 2 minutes). The temperature was then raised to 250°C at a rate of 4°C/minute. After that, there was a 10-minute isothermic period at 230°C. A Varian CP 8400 Auto sampler was used to introduce a FAMEs n-hexane (1l) onto the column. Both the injection port and detector were kept at 250°C. At 45 psi, hydrogen served as the carrier gas while nitrogen served as the makeup gas. The chromatograms were recorded using galaxy chromatography software. The retention durations of FAME peaks from samples were compared to those of Supelco standards to identify FAME samples (Supelco 37 Component Fame Mix 47885-U., Sigma-Aldrich Aston Manor. Pretoria. South Africa). All additional reagents and solvents used were purchased from Merck Chemicals and were of analytical quality (Pty Ltd. Halfway House and Johannesburg. South Africa). Individual fatty acids were measured as a percentage of the total fatty acids in the sample. Omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFAs) and the PUFA/SFA ratio (P/S) and n-6/n-3 ratio atherogenicity index were calculated.

4.2. 6 Statistical analyses

Data on carcass characteristics, meat quality parameters and fatty acid profiles were analysed using the GLM procedure of SAS (2010) with diet treatment as the only fixed effect. Separation of mean was done by using the probability of difference (PDIFF) option of SAS (2010) while contrasts SAS (2010) was used to determine the specific effects of FA inclusion in diet on different parameters.

The employed linear model is as follows:

$$Y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where Y_{ij} = the observation of the dependant variables (carcass traits. meat quality and fatty acid profiles).

μ = the overall mean (general mean) constant to all observations.

t_i = the i^{th} fixed effect of the diet level.

ε_{ij} = random error or residual effect associated with ij^{th} observation.

4.3 Results

4.3.1 Carcass characteristics

The effects of dietary inclusion of FA inclusion in diets on carcass characteristics of broilers is presented in Table 4.1. Diet had similar effect ($P < 0.05$) on all measured parameters apart from the dressing percentage, chilling loss percentage and the thigh index. Chickens fed the posCON diet (1959.27 g) obtained the highest slaughter weight and live weight, followed by those fed the FA2 diet (1942.10 g). Birds fed the negCON diet had the lowest. Similarly, the birds fed the posCON diet had the highest (1617.29 g) value for HCW followed by those fed the FA2 (1598.66g) diet whilst those fed the negCON group had the lowest (1363.75 g). Nevertheless, birds fed diets containing the FA had statistically similar HCW. The CCW was highest in broilers offered the posCON (1584.41 g) diet, followed by those fed FA1 (1559.20 g). The posCON diet also had the heaviest breasts (308.41 g) and thigh weight (118.80 g) whilst the negCON (280.41 g) had the smallest. Chickens in the posCON group consistently had the highest values for all measured parameters while those in the negCON group had the lowest.

Table 4.1. The effect of diets containing FA on carcass characteristics in broiler diets.

	[1]Dietary treatments						
[2]Parameters	negCON	posCON	FA1	FA2	FA3	[3]SEM	*P*-values
Initial weight (g)	44.70^{ab}	45.81^{b}	44.68^{ab}	45.58^{b}	44.40^{a}	1.06	0.001
Slaughter weight (g)	1789.25^{a}	1959.27^{b}	1833.31^{ab}	1942.10^{b}	1824.05^{ab}	54.50	0.001
HCW (g)	1363.75^{a}	1617.29^{c}	1597.41^{b}	1598.66^{b}	1568.37^{ab}	40.77	0.001
CCW (g)	1274.75^{a}	1584.41^{c}	1559.20^{c}	1482.95^{b}	1535.05^{bc}	46.01	0.001
Dressing (%)	83.23	90.75	83.05	81.60	86.52	3.23	NS
Chilling loss (%)	0.02	0.05	0.02	0.05	0.02	0.01	NS
Breast (g)	280.41^{a}	308.41^{b}	294.58^{ab}	302.91^{b}	290.21^{ab}	16.48	0.001
Breast **i**ndex	17.30^{a}	19.35^{c}	18.47^{b}	19.31^{c}	18.46^{b}	0.93	0.01
Thigh (g)	117.50^{b}	118.80^{b}	116.45^{ab}	112.08^{a}	116.66^{ab}	3.10	0.001
Thigh **i**ndex	7.28	7.31	7.31	7.16	7.43	0.22	NS
Wings (g)	89.16^{b}	95.83^{c}	77.29^{a}	87.91^{ab}	94.99^{bc}	3.50	0.001
Drumstick(g)	100.29^{bc}	105.54^{c}	94.79^{a}	96.25^{ab}	98.12^{b}	4.30	0.001
Quarter leg (g)	216.87^{b}	220.70^{c}	211.25^{ab}	201.87^{a}	205.00^{ab}	8.00	0.001

[a.b.c.] means significant differences are denoted by different superscripts in a row ($P < 0.05$). [3]SEM = standard error of means *P*-values are the effects of dietary FA on carcass characteristics. Significance = not significant $P < 0.05$

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.
[2]Parameters: HCW = hot carcass weight; CCW = cold carcass weight.

4.3.2 Internal organs

Table 4.2 presents the effect of FA inclusion in diets on internal organs in broiler chickens. From the table, there were no dietary effects on all parameters apart from the weights of gizzards (g), livers (g) and the spleens (g). The heaviest gizzards and liver weights were observed in chickens fed the posCON diet (42.25 g & 49.35 g) followed by chickens offered the FA1 diet (37.68 g and 39.37 g), respectively. Highest spleen weights were observed in chickens fed the FA2 (2.90 g) while birds fed the negCON (2.27 g) had the lowest value. Birds fed the FA2 and FA3 diets had statistically similar values compared to other groups. Birds fed the FA1 (22.38 g) and posCON (22.46 g) diets also had statistically comparable ileum sizes while chickens offered the posCON diet had slightly higher weights.

4.3.3 Meat quality

Table 4.3 shows the effect of experimental diets on meat quality measurements. Diet had no effect on breast initial, ultimate pH and temperature, dripping loss, cooking loss and shear force. Although diet had no effect on meat pH, the expected post-mortem decline in meat pH was evident (Figure 4.1). Nevertheless, dietary treatments had an effect (P <0.05) on WHC and the colour coordinates (lightness and yellowness). Chickens offered the posCON (42.61) diet had statistically similar values for lightness with those fed the FA1 (42.62) and FA3 (42.54) diets. However, birds fed the FA2 (41.90) diets appeared to have darker breast muscles compared to all other treatments. In terms of yellowness, birds fed the FA2 diet had the highest values followed by those fed the negCON diet. The results of WHC demonstrated that breast muscle from birds fed the FA1 (9.70 %) diet had the lowest value, showing the greatest WHC capabilities followed by those fed the FA2 (10.95 %) diet. While there was no effect of diet on shear force values, it appeared that the birds fed the posCON (6.41 N) and those fed higher level of the FA (6.38 N) diet had numerically higher shear force values compared to those fed the FA1 (5.39 N) diet.

Table 4.2. The effect of diets containing FA on internal organs in broiler chickens.

	[1]Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	SEM	*P*-values
Gizzard (g)	37.32^{b}	42.25^{c}	37.68^{b}	36.13^{ab}	32.10^{a}	2.25	0.01
Liver (g)	38.72^{a}	40.35^{b}	39.37^{ab}	38.07^{a}	39.29^{ab}	0.91	0.01
Spleen (g)	2.27^{a}	2.57^{ab}	2.47^{ab}	2.90^{b}	2.57^{ab}	0.26	0.05
Provinticulus (g)	8.14	7.01	7.46	7.71	7.59	1.44	NS
Duodenum (g)	18.04	16.62	18.10	16.66	17.44	0.61	NS
Jejunum (g)	27.58	29.11	28.32	26.59	28.51	1.40	NS
Ileum (g)	21.29^{a}	22.46^{b}	22.38^{b}	21.53^{ab}	21.66^{ab}	1.06	0.23
Colon weight (g)	3.76	3.60	2.79	2.45	1.88	1.02	NS
Caeca (g)	9.86^{a}	10.89^{ab}	9.91^{a}	11.79^{b}	11.07^{b}	1.07	0.03
Intestinal length (cm)	174.50	175.31	172.27	167.13	173.90	3.30	NS

[a.b.c.] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

SEM = standard error of means.

Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

Table 4.3. The effects of dietary treatments on meat quality traits of broiler chickens.

	[1]Dietary treatments						
[2]Parameters	negCON	posCON	FA1	FA2	FA3	[3]SEM	*P*-values
pH (45min)	6.02	6.22	6.12	6.12	6.24	0.85	NS
Temp	14.48	13.81	13.54	13.40	13.80	0.33	NS
Ultimate pH (24 h)	5.90	5.78	5.93	5.97	5.98	1.14	NS
Meat colour							
• Lightness (L*)	42.19^{ab}	42.61^{b}	42.62^{b}	41.90^{a}	42.54^{b}	0.54	0.001
• Redness (a*)	2.67	2.77	2.18	2.11	2.33	0.22	NS
• Yellowness (b*)	2.86^{ab}	2.68^{ab}	2.45^{a}	3.40^{b}	2.77^{ab}	0.30	0.03
Drip loss (%)	19.50	15.61	14.25	21.20	18.20	3.21	NS
Cooking loss (%)	27.37	27.17	26.93	27.89	27.54	1.44	NS
WHC (%)	11.32^{bb}	12.40^{c}	9.70^{a}	10.95^{ab}	12.35^{c}	1.12	0.001
Shear force (N)	5.72	6.41	5.39	6.23	6.38	0.28	NS

[a.b.c.] means significant differences are denoted by different superscripts within a row ($P < 0.05$).

SEM = standard error of means,

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = n normal commercial broiler diet including 3% of FA.

[2]Parameters: WHC = water holding capacity.

4.3.4 Individual fatty acid profiles

The effect of dietary treatments on fatty acid profiles are shown in Table 4.4. The results illustrate that diet had no effect ($P > 0.05$) on all the SFAs parameters apart from the docasanoic (C22:00) acid. Birds fed the FA1 and FA2 diets had the highest ($P < 0.05$) concentrations of docosanoic acid (C22:00), whilst those fed the FA3 diet had the lowest. The major SFAs obtained across treatments were the palmitic acid (C16:00) and stearic acid (C18:00). With regards to the MUFAs, diet had no effect on any of the fatty acids. The major MUFAs obtained across treatments were the oleic acid (C81:1) and palmitoleic acid (C16:1). However, diet had a significant effect on the following polyunsaturated fatty acids (PUFAs): icosadienoic acid (C20:2) and 16-docosadienoic acid (C22:2) although the birds fed the posCON diet had the highest icosadienoic acid concentration, the value was not statistically different from those obtained for the birds fed the FA containing diets. Birds fed the negCON diet had the lowest icosadienoic acid. Diet also significantly altered the dihomo-gamma-linolenic acid (C20:3n6) with the highest value observed in chickens fed the FA3 diet (0.25) while posCON had the lowest. Moreover, diet also influenced the eicosatrienoic acid (C20:3n3) with the posCON group having the highest, while the negCON had the lowest.

Table 4 4. The effect of diets containing FA on fatty acid composition.

	[1]Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	[2]SEM	*P*-values
SFAs							
C8:00	0.10	0.03	0.03	0.03	0.05	0.02	NS
C10:00	0.09	0.05	0.06	0.05	0.05	0.02	NS
C12:00	0.15	0.12	0.14	0.13	0.13	0.03	NS
C14:00	2.04	1.81	2.52	2.50	2.42	0.71	NS
C15:00	0.20	0.25	0.24	0.25	0.25	0.06	NS
C16:00	92.91	80.97	109.62	112.60	109.16	30.35	NS
C17:00	0.13	0.16	0.11	0.10	0.14	0.03	NS
C18:00	22.72	18.39	27.69	28.44	25.35	6.68	NS
C20:00	0.25	0.24	0.19	0.22	0.28	0.06	NS
C21:00	0.03	0.03	0.04	0.02	0.04	0.079	NS
C22:00	0.10^{a}	0.09^{a}	0.15^{b}	0.15^{b}	0.09^{a}	0.01	0.05
C23:00	0.02	0.02	0.02	0.03	0.02	0.004	NS
C24:00	0.03	0.03	0.04	0.04	0.04	0.005	NS
MUFAs							
C14:1	0.61	0.60	0.72	0.75	0.79	0.22	NS
C16:1	23.68	23.20	27.97	28.45	29.80	8.57	NS
C20:2	0.26^{a}	0.49^{b}	0.31^{ab}	0.38^{ab}	0.34^{ab}	0.06	0.05
C22:1	0.03	0.03	0.04	0.04	0.05	0.006	NS
C22:2	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.03^{a}	0.003	0.05
C24:1	0.023	0.022	0.023	0.020	0.028	0.003	NS
C18:1cis	137.18	123.26	158.71	151.35	167.30	48.55	NS
C18:2cis	48.00	57.85	58.31	62.11	60.80	16.20	NS
C18:1t	0.26	0.49	0.31	0.31	0.38	0.34	NS
PUFAs							
C18:3n6	0.61	0.60	0.72	0.75	0.79	0.22	NS
C20:3n6	0.11^{a}	0.07^{a}	0.10^{a}	0.09^{a}	0.25^{b}	0.05	0.05
C20:4n6	0.03^{a}	0.03^{a}	0.04^{a}	0.04^{a}	0.05^{a}	0.006	0.05
C20:1& C18:3n3	23.68	23.20	27.97	28.45	29.80	8.57	NS
C20:3n3	0.26^{a}	0.49^{b}	0.31^{ab}	0.38^{ab}	0.34^{ab}	0.06	0.05
C20:5n3	0.02	0.02	0.02	0.02	0.03	0.003	NS
C22:6n3	0.023	0.022	0.023	0.020	0.028	0.003	NS

[a.b.c.] means significant differences are denoted by different superscripts within a row ($P < 0.05$).

[2]SEM = standard error of means.

P-values are the effects of dietary FA on fatty acid profiles.

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1= normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA; FA3 = normal commercial broiler diet including 3% of FA.

,

4.3.5 Total fatty acids

Table 4.5 shows results of the effect of dietary tretaments on breast muscle fatty acid profiles. Dietary treatments had no effect on all the total fatty acids profiles from omega 6 fatty acid (n-6PUFA) and the PUFA/SFA ratio. Broiler chickens offered FA2 had a higher ($P < 0.05$) n-6FAs values followed by those fed the FA3 diet whilst chickens fed the negCON diet had the least omega 6 fatty acid profile. The PUFA/SFA ratio was highest in broilers fed the posCON diet followed by those fed the FA3 diet while the birds fed the FA2 diet had the lowest. Despite the lack of effect of diet on the total PUFAs, MUFAs and omega-3 FAs, an increase in all values was apparent with the increasing levels of FA in the diets, with the birds fed the FA diets having numerically higher values for the parameters when compared to the control diets.

4.4 Discussion

A diversity of factors including nutrition, sex, age and genetic, and genetics make up have been observed to alter muscle development and carcass conformation of meat producing animals (López-Pedrouso *et al*., 2019). In the current study, the results obatined on carcass traits are consistent with the results on growth performance obatined in Chapter 3 as well as the slaughter weights. The high carcass traits obatined in birds fed the posCON diet are confirmatory of the known effectiveness of antibiotics in improving growth effeciency in broilers. Nevertheless, the observed comparable values for carcass traits obtained in birds fed the FA diets also support the postulations that FA in diets may have the same effect as the conventional antibiotics on performance in broilers to a larger extend. This is consistent with findings from other studies using acids of similar nature (Disetlhe *et al*., 2019). This could be attributed to the fact that FA inclusion in broiler diets have the capacity to enhance digestion dynamics and nutrient absorption, which improve growth and animal carcass traits (Serra *et al*., 2021; Le *et al*., 2016). Moreover, the observed increase in body and carcass weights with increase in FA in the broiler diets is also consistent with findings by (Ozurk *et al*., 2012).

Table 4.5. The effect of diets containing FA on fatty acids profiles (total nutritional indices) of broiler meat.

	[1]Dietary treatments						
[2]Parameters	negCON	posCON	FA1	FA2	FA3	[3]SEM	*P*-values
∑ SFA	118.85	102.26	140.90	144.62	138.06	37.88	NS
∑ MUFA	209.96	205.58	246.25	243.25	259.42	73.12	NS
∑PUFA	137.18	123.26	158.71	151.35	167.30	48.55	NS
∑n-6PUFA	3.59^{a}	3.78^{ab}	4.22^{ab}	4.84^{c}	4.78^{b}	0.30	0.01
∑n-3 PUFA	3.94	3.68	4.54	5.00	4.93	1.05	NS
PUFA/SFA	1.15^{ab}	1.21^{b}	1.13^{ab}	1.05^{a}	1.21^{b}	1.11	0.03
PUFA/MUFA	0.65	0.60	0.64	0.62	0.64	1.03	NS
n6/n3	0.91	1.02	0.93	0.97	0.96	0.26	NS

[a.b.c.] means significant differences are denoted by different superscripts within a row ($P < 0.05$).

[3]SEM = standard error of means.

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2]∑ SFA = sum of all saturated fatty acids (C8:00, 10:00, 12:00, 14:00, 16:00, 17:00, 18:00, 20:00, 21:00, 22:00, 23:00, 24:00). ∑MUFA = sum of monosaturated fatty acids (cis MUFA, Trans MUFA). ∑n6 PUFA = sum of omega 6 fatty acids (C18:3n6, C20:3n6, C20:4n6).

∑n3 PUFA = sum of omega 3 fatty acids (C20:1& C18:3n3, C20:1& C18:3n3, C20:3n3, C20:5n3 and C22:6n3). ∑PUFA = sum of polyunsaturated fatty acids (∑N6: ∑N3).

PUFA/MUFA, n6/n3 = total omega- 3 fatty acids.

The lack of effect of the diet on dressing percentage in the current study correspondences to the findings by Biagini *et al*. (2016) who also observed no dietary effect on the dressing percentage in broilers fed diets containing FA. This is also confirmatory of the lack of the negative effects of the FA inclusion in diets of carcass characteristics of the birds, which is desirable.

According to Shang *et al*. (2018), the gastrointestinal tract (GIT) and gut microbial population play significant roles in nutrient utilisation, immune development, and disease resistance. The function of the gut is not only limited to nutrient digestion and absorption, but it also acts as the first defensive system against pathogens that may colonize and penetrate the host cells and tissues. Severance *et al*. (2015) reported that the gut is the largest immune organ found in the animal anatomy. Therefore, healthy animals consist of a vigorous gut, which digests and utilizes nutrients effectively (Yang *et al*., 2009). The results from the study showed no effect of FA inclusion in diet on intestinal parameters. The fact that all intestinal parameters were similar among all treatments, shows that FA inclusion in diets had no negative effect on intestinal structure and function, but rather may have had the same effect as the antibiotics in enhancing nutrient digestion and assimilations. Generally, humic substances such as FA have been found to be associated with significant changes in the structure and morphology of the gut (Disetlhe *et al*., 2019, Arif *et al*., 2019).

The liver is the integral organ to animal growth that deals with excessive amounts of some undesirable substance and can simply adapt to ingredient and feed additive substitutions in rations. For instance, if FA inclusion in broiler diets was excessive, the excess FA will either be deemed to be toxic then eliminated from the animal body or will be utilized to synthesize carbohydrates and fat (Zaefarian *et al*., 2019; Zhang *et al*., 2020). The results on the effect of FA inclusion on visceral organs including the gizzard, spleen, liver caeca and Ileum weights, are consistent with the findings by Abd El-Hack,

(2016) and Disetlhe *et al*. (2017), who reported that the size of gizzards, livers and intestinal weights were unaltered by dietary HA and humic substances in broiler diets or drinking water.

According to Mir *et al*. (2017), the meat functionality, taste, appearance, juiciness, and texture are the major attributes of meat quality. These attributes are predicted by meat instrumental measurements including meat pH, colour, WHC, drip loss and shear force (Kamruzzaman *et al*., 2012; Geletu *et al*., 2021). Meat pH is a meat quality parameter that has direct influence on all other meat quality parameters. Generally, a decline in pH is observed 24hour post slaughter due to post-mortem muscle biochemical activity. Thus, immediately after slaughter, broiler chicken breast muscle is expected to have a pH of 7.2, dropping to between 5.7 and 6.0 during the post-mortem period (Gonzalez-Rivas *et al*., 2020). Despite the lack of differences, the ultimate pH values for all treatments in the current study, falling between the normal setting range of 5.78 – 5.98, were within the expected range (Gonzalez-Rivas *et al*., 2020). Ismail & Joo (2017) illustrated that lower pH values (<5.7) that are near the isoelectric point of the myofibrillar protein might cause depressing WHC and meat discoloration.

The low WHC is associated with increased cooking loss and drip loss, meat tenderness and shelf life (Mir *et al*., 2017). Visual appearance (colour) is essential to consumers as it is one of the important factors that customers rely on for meat acceptance and purchasing decisions (He *et al*., 2020). Results from this study showed that the meat colour parameter for yellowness varied among all treatments. This could be attributed to the characteristic nature of the FA, which is yellow in colour that could have caused an increase in yellowness (b*) values in FA2 fed broilers while reducing the meat lightness (L*) values. Data from the present study is similar to findings of Ozturk *et al*. (2012), who observed that inclusion of 1g/kg humic substance did not influence lightness and yellowness of breast muscle meat. In contrary, Esenbuga *et al*. (2008) reported a significant influence in colorimetric

redness of broiler muscle meat between control group and humate groups. Various studies on higher concentrations of humic substance inclusion in diets showed that HA improved the visual meat appearance in consideration in colorimetric redness and yellowness (Disetlhe *et al.*, 2019; Semjon *et al.*, 2020; Hudak *et al.*, 2021 & Akaichi *et al.*, 2022). Meat lightness (*L**) and redness (*a**) typically increase with increasing weight and age at slaughter (Teixeira *et al.*, 2005). Generally, the results of the study showed that inclusion of FA in the diets did not negatively alter the meat colour traits as observed in other studies (Disetlhe *et al.*, 2019; Semjon *et al.*, 2020; Hudak *et al.*, 2021 and Akaichi *et al.*, 2022).

The ability to retain water describes WHC, which is a crucial parameter for consumer visual meat acceptability, which has a great impact on the amount of water lost during processing, storage, and transportation (Bertram *et al.*, 2003; Sekali *et al.*, 2020). Dietary treatments had no influence on drip loss, cooking loss and meat tenderness. This is consistent with the observations by Bai *et al.* (2013). Although the chickens offered the FA1 (9.70 %) diet had the least water loss, it appears the differences among the treatments was subtle. Nevertheless, the broilers fed the posCON diet showed the least capacity to retain water. Data from the current study is in line with similar observations made by Hudák *et al.* (2021) who reported that humic substances have some effect in meat quality attributes.

Results from this study showed that inclusion of FA did not alter most of the fatty acid parameters as indicated by no differences in all the saturated fatty acids with the exception of the docasanoic fatty acid (C22:00). Experimental diets that were supplemented with FA had higher values of saturated fatty acids (SFAs) compared to the control group. High levels of unsaturated fatty acids, especially, myristic (C14:00), palmitic (C16:00) and stearic (C18:00) acids are undesirable as they are known for their hypercholesterolemia properties, which often lead to obesity, heart attack, abnormal blood

lipid profiles and insulin resistance in humans (Gilani *et al*., 2018; Wang *et al*., 2020). With regards to PUFAs, the results from the current study show that broiler chickens offered FA appeared to desirably increase the proportion of eicosadienoic (C20:2) and docasadienoic (C22:2) fatty acids. According to Ahmed *et al*. (2015), n-3 and n-6 (PUFAs) play a crucial role in human nutrition as they produce thromboxanes, eicosanoids and leucotriens, which are critical in the control of the cardiovascular system and immune functions. The breast muscle meat of broilers offered the FA3 diet had the highest proportions in eicosatrienoic (C20: 3n6) (0.25) and arachidonic (C20: 4n6) (0.05) fatty acid.

The observed increase in total MUFA and PUFAs in broiler meat with the inclusion of FA in the diets, compared to the control, is in line with results showed by (Mao *et al*., 2019). Meat containing a lower PUFA/SFA ratio of less than 0.4 and higher n-6/n-3 ratio (>5) is undesirable as it can lead to high cholesterol levels (Disetlhe *et al*., 2019; Wang *et al*., 2019). In general, the results from this study illustrated that inclusion of FA in diets may result in an increase in the PUFA/SFA ratio compared to control diets. However, diets consisting of FA appeared to reduce the total n-3/n-6 ratio. Therefore, the inclusion of FA can desirably affect some important fatty acid needed for the benefit of human and animal health.

4.5 Conclusion

Findings from this study demonstrates that inclusion of FA in drinking water as a natural feed additive in broiler diets can have positive effects on the carcass characteristics and meat quality attributes in terms of WHC, meat colour coordinates, drum, and breast muscle weights. Inclusion of FA as a dietary supplementation in broiler diet has a potential effect of increasing the quantity of PUFAs, n-6 PUFA, n-3 PUFA fatty acids and the PUFA/SFA ratio, which is significant in assessing the nutritional value of meat. Therefore, inclusion of FA administered up to 3% does not compromise

the carcass and meat quality attributes of broiler chickens. Growth performances are closely correlates to bone development. Therefore, it is of importance to investigate the effect of FA inclusion in broiler diets on bone development.

4.6. References

Abd El-Hack, M.E., 2016. Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks. Indian J. Anim. Sci. 86(9), 1073─1078.

Ahmed, S.T., Islam, M.M., Bostami, A.B.M.R., Mun, H.S., Kim, Y.J. & Yang, C.J., 2015. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate *(Punica granatum L.*) by-products. Food Chem. 188, 481–488.

Akaichi, A., Jebali, A., Benlarbi, M., Mahjoub, T., Kaboudi, K., Chaouacha-Chekir, R.B., Haouas, Z. & Boudhrioua, N., 2022. Effects of humic acid and organic acids supplements on performance, meat quality, leukocyte count, and histopathological changes in spleen and liver of broiler chickens. Res. Vet. Sci. 150, 179─188.

Arif, M., Alagawany, M., Abd El-Hack, M.E., Saeed, M., Arain, M.A. & Elnesr, S.S., 2019. Humic acid as a feed additive in poultry diets: A review. Iran. J. Vet. Res. 20(3), 167.

Bai, H.X., Chang, Q.F., Shi, B.M. & Shan, A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing- finishing pigs. Livest. Sci. 158, 118─123.

Bertram, H.C., Andersen, H.J. Karlsson, A.H., Horn, P., Hedegaard, J., Nørgaard, L. & Engelsen, S.B., 2003. Prediction of technological quality (cooking loss and Napole Yield) of pork based on fresh meat characteristics. Meat Sci. 65, 707–712.

Biagini, D., Gasco, L., Rosato, R., Peiretti, P.G., Gai, F., Lazzaroni, C., Montoneri, C. & Ginepro, M., 2016. Compost-sourced substances (SBO) as feedstuff additives in rabbit production. Anim. Nutr. Sci. Technol. 214, 66─76.

Disetlhe, A.R., Marume, U., Mlambo, V. & Hugo, A., 2019. Effects of dietary humic acid and enzymes on meat quality and fatty acid profiles of broiler chickens fed canola-based diets. Asian-Australas. J. Anim. Sci. 32(5), 711.

Esenbuga, N., Macit, M., Karaoglu, M., Aksu, M.I. & Bilgin, O.C., 2008. Effects of dietary humate supplementation to broilers on performance, slaughter, carcass and meat colour. J. Sci. Food Agric. 88(7), 1201-1207.

Geletu, U.S., Usmael, M.A., Mummed, Y.Y. & Ibrahim, A.M., 2021. Quality of cattle meat and its compositional constituents. Vet. Med. Int. 1─9.

Gilani, A., Pandey, V., Garcia, V., Agostinucci, K., Singh, S.P., Schragenheim, J., Bellner, L., Falck, J.R., Paudyal, M.P., Capdevila, J.H. & Abraham, N.G., 2018. High-fat diet-induced obesity and insulin resistance in CYP4a14−/− mice is mediated by 20-HETE. Am. J. Physiol. Integr. 315(5), 934─944.

Gonzalez-Rivas, P.A., Chauhan, S.S., Ha, M., Fegan, N., Dunshea, F.R. & Warner, R.D., 2020. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Sci. 162, 108025.

Haque, M.H., Sarker, S., Islam, M.S., Islam, M.A., Karim, M.R., Kayesh, M.E.H., Shiddiky, M.J. & Anwer, M.S., 2020. Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. Biology, 9(11),411.

He, J., Evans, N.M., Liu, H. & Shao, S., 2020. A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. Comprehensive Reviews. Food Sci. food Saf. 19(5), 2639-2656.

Hudk, M., Semjon, B., Marcinčáková, D., Bujňák, L., Naď, P., Koréneková, B., Nagy, J., Bartkovský, M. & Marcinčák, S., 2021. Effect of broilers chicken diet supplementation with natural and acidified humic substances on quality of produced breast meat. Animals. 11(4), 1087.

Ismail, I. & Joo, S.T., 2017. Poultry meat quality in relation to muscle growth and muscle fiber characteristics. Korean J. Food sci. Anim. Res. 37(6), 873.

Ivanovic, S., Pavlovic, I. & Pisinov, B., 2016. The quality of goat meat and its impact on human health. Biotechnol Anim Husb. 32(2), 111─122.

Kamruzzaman, M., ElMasry, G., Sun, D.W. & Allen, P., 2012. Prediction of some quality attributes of lamb meat using near-infrared hyperspectral imaging and multivariate analysis. Analytica Chimica Acta. 714, 57─67.

Li, X.L., He. W.L., Wang. Z.B. & Xu. T.S., 2016. The effects of Chinese herbal mixture on performance. egg quality and blood biochemical parameters of laying hens. J. Anim. Physiol. Anim. Nutr. DOI:10.1111/jpn.12473

López-pedrouso, M., Cantalapiedra, J., Munekata, P.E.S., Barba, F.J., Lorenzo, J.M. & Franco, D., 2019. Carcass characteristics, meat quality and nutritional profile of pheasant, quail and Guinea fowl. In: more than beef, pork and chicken the production, processing, and quality traits of other sources of meat for human diet. Springer international publishing, Berlin, Germany. 269–311.

Mao, Y., 2019. Modulation of the growth performance, meat composition, oxidative status, and immunity of broilers by dietary fulvic acids. Poult. Sci. 98(10), 4509-4513.

Mir, N.A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54, 2997─3009.

Ozturk, E., Ocak, N., Turan, A., Erener, G., Altop, A. & Cankaya, S., 2012. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. J. Sci. Food. Agric. 92, 59–65.

Park, P.W. & Goins. R.E., 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59, 1262 – 1266.

Pietrosemoli, S. & Green, J.T., 2018. Pasture systems for pigs. In Achieving sustainable production of pig meat. Burleigh Dodds Science Publishing. 3, 167-218.

Santos, L. & Ramos, F., 2018. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. Int. J. Antimicrob. Agents. 52, 135-143.

Sekali, M., Mlambo, V., Marume, U. & Mathuthu, M., 2020. Replacement of soybean meal with heat-treated canola meal in finishing diets of meat master lambs: physiological and meat quality responses. Animals. 10(10), 1735.

Semjon, B., Marcinčáková, D., Koréneková, B., Bartkovský, M., Nagy, J., Turek, P. & Marcinčák, S., 2020. Multiple factorial analysis of physicochemical and organoleptic properties of breast and thigh meat of broilers fed a diet supplemented with humic substances. Poult. Sci. 99(3), 1750─1760.

Serra, V., Salvatori, G. & Pastorelli, G., 2021. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. Animals. 11(2), 401.

Severance, E.G., Prandovszky, E., Castiglione, J. & Yolken, R.H., 2015. Gastroenterology issues in schizophrenia: why the gut matters. Current psychiatry reports, 17, 1-10.

Shang, Y., Kumar, S., Oakley, B. & Kim, W.K., 2018. Chicken gut microbiota: Importance and detection technology. Front. Vet. Sci. 5, 254.

Teixeira, A., Batista, S.M.G., Delfa, R. & Cadavez, V., 2005. Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. Meat Sci. 71, 530–536.

Wang, L., Xu, F., Song, Z., Han, D., Zhang, J., Chen, L. & Na, L., 2020. A high fat diet with a high C18: 0/C16: 0 ratio induced worse metabolic and transcriptomic profiles in C57BL/6 mice. Lipids in health and disease. 19. 1─13.

Wang, Q., Chen, YJ. & Yoo, J.S., 2019. Effects of supplemental humic substances on growth performance, blood characteristics and meat quality in finishing pigs. Livest Sci. 117, 270─4.

Yang, Y., Iji, P. A. & Choct, M., 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World Poult. Sci. J. 65, 97─114.

Zaefarian, F., Abdollahi, M.R., Cowieson, A.& Ravindran, V., 2019. Avian liver: the forgotten organ. Animals. 9(2), 63.

Zhang, B., Zhang, X., Schilling, M.W., Tabler, G.T., Peebles, E.D. & Zhai, W., 2020. Effects of broiler genetic strain and dietary amino acid reduction on (part I) growth performance and internal organ development. Poult. Sci. 99(6), 3266-3279.

CHAPTER 5

THE EFFECT OF FULVIC ACID ON BONE DEVELOPMENT AND BROILER WELFARE

Abstract

The current study was conducted to evaluate the effect of fulvic acid (FA) inclusion in diets on carcass characteristics and meat quality parameters on broilers. A total of 400 (Cobb 500) day old chickens were reared in 40 pens (experimental unit), each pen carrying 10 birds replicated 8 times. The experimental diets were formulated to be isonitrogenous and isoenergetic as follows; NegCON (T1): commercial diets without antibiotics, posCON (T2): commercial diet with antibiotics, FA1 (T3) commercial diet + 1% FA1, FA2 (T4): commercial diet + 2% FA and FA3 (T5): commercial diet with 3% FA. The feeding trial was conducted over 3 feeding phases, Starter (day 1-14); Grower (15-28) and Finisher ration (day 28-42). Chicks had *ad libitum* access to freshwater and feed throughout a period of 6 weeks. At day 35, one bird per pen, per treatments was randomly selected for broiler welfare measurements. Each bird was individually placed in a plastic storage tub, a bucket of 32℃ tepid water was poured into the tub of the bird(s) to a depth of 30 mm. At the end of the experiment, tibial samples were collected, de-flashed, weighed and examined visually to assess rickets prevalence. Mineral content was obtained using the ICP Spectrometer (Perkin-Elmer, 1982) Nex Ion 300Q. Results showed that diet had no effect on latency to lie, feather and gait scoring test. The highest latency to lie was observed in broilers fed the positive control diet (416.21 seconds) with the highest standing persistence observed in broilers fed FA2 (462.28 seconds) followed by FA3 (435.36 seconds). Diet had no effect on tibia weight, tibia length and tibia diameter proximal end. However, diet had a significant influence on tibia weight and tibia diameter distal end. Birds offered the posCON (0.60 g) diet had the highest tibia weight, whilst chickens fed the FA2 (0.50 g) diet had the lowest. Diet showed no dietary influence on all micro and macro minerals apart from phosphorus, iron and molybdenum. Serum calcium levels were higher in all the diets containing FA compared to

the control. FA inclusion in diets also increased calcium levels and ash concentration with increment levels. From the results, it can be concluded that increasing the levels of FA in drinking water improved the broiler welfare in chickens.

Keywords: Gait; Latency-to-lie; Poultry health; Rickets incidence; Tibia biomechanics; Tibia gross mineralization.

5.1 Introduction

The broiler industry has made significant progress in improving the economics, genetics, management, development of vaccines, nutrition, and availability of chicken as a cost-effective source of protein (Weimer *et al*., 2020). Moreover, the use of antibiotics has resulted in significant enhancement of growth, feed efficiency and immunocompetency in broilers. Nevertheless, the rapid broiler growth rates have been associated with some negative effects on welfare, health, and general production in broilers (Bessei, 2006). Rapid growth rate in broilers has been observed to cause skeletal deformities, lameness, and metabolic illnesses such as sudden death syndrome and peritoneal fluid excess, which all increase mortality rates (Wideman, 2016; Hartcher & Lum, 2020). Apart from the associated welfare issues, the use of conventional antibiotics has been causing increased anxiety among consumers because of the accumulation of residues in meat and increased antimicrobial resistance, which may affect human health (Weimer *et al*., 2018). This, therefore, calls for the exploration of alternatives to antibiotics, which have the effects of improving growth rates, welfare and survival rates in broilers.

The inclusion of some organic acids in diets have shown great potential as replacements for the conventional antibiotics particularly with regards to enhancement of bone development. For example, supplementation of broiler diets with humic substances was reported to prevent bone resorption while

stimulating bone formation, leading to an increase in bone mass matrix (Bezuglova & Klimenko, 2022). Moreover, humates were reported to positively influence mineral repartition towards bone development, ensuring clinically strong legs and welfare of broiler (Yan *et al*., 2016). According to Steenfeldt *et al*. (2018) and Kolakshyapati *et al*. (2019), leg health and broiler metabolism are of major concern to geneticists, veterinarians, nutritionists, researchers, and broiler producers. Assimilation of minerals during digestion can be improved by organic acids and humic substances Nkukwana *et al*. (2014) and increase mineralization degree in the bone matrix (Jad'uttová *et al*., 2019). Normally, lack of calcium and phosphorus in diets could result in poor bone development and cause blood hypocalcaemia that can cause decreased mineralization and bone strength (Jasielska and Grzybowska-Chlebowczyk, 2021).

FA is one of the humic substances that can be included in broiler diets with potential beneficial effects on welfare in broilers (Domínguez-Negrete *et al*., 2019). While there is a vast body of information on the possible inclusion of HA in broiler diets as alternatives to the conventional diets, nothing is known on the effects of FA on bone development and welfare in broilers. Therefore, the study investigated the effects of FA inclusion in diets on bone development and welfare in broiler chickens.

5.2 Materials and methods

5.2.1 Study site, source of diets and animal management

The description of the study site, diet formulation and experimental design was provided earlier on in Chapter 3 under Sections 3.2.1-3.2.3 whilst the slaughter procedure is described in the previous Chapter under Section 4.2.2.

One chicken was randomly chosen from a pen per treatment at 35 days old. The birds were exposed to the latency to lie (LTL) test. According to Berg & Sanotra (2003), the test is conducted on the basis that broiler chickens tend to avoid contact with water with their bodies as long as possible. Each bird was individually placed in a plastic storage tub, and a bucket of 32°C tepid water was then poured into the tub of the bird(s) to a depth of 30 mm. According to the theory, it is stated that the better the leg health of the broiler, the longer it would stand up to avoid body contact with the water. The time it takes for the bird to sit down was recorded. The test was stopped if the chicken remained standing after 10 minutes because the legs were regarded sturdy and healthy.

5.2.3 Gait scoring

Gait scoring is known as the ability to walk, by showing little variation overtime and showing good leg health status (Perpetuini *et al*., 2022). One bird per treatment was randomly chosen to observe the leg health by their gesture and locomotion. This was done to increase the likelihood of observing chickens across a range of walking abilities. A 5-point rubric for gait scoring chickens was used to access the chickens' walking abilities. According to Karcher *et al*. (2013), a score of 0 was assigned to broilers that walked without any obstacles. Chickens deemed to walk almost perfectly were assigned between 1 and 2 (moderate gait). Chickens deemed to have a slight limp were assigned a score of 3. Chickens that were reluctant to walk were scored 4, while those that could not walk at all were assigned a score of 5 (poor gait).

5.2.4 Feather scoring

Feather scoring was performed at day 35. One bird per treatment was randomly chosen and then feather scored. Four body parts including the neck, wings, chest and back were given a score from 1 to 3 scoring system adapted from (Bright *et al*., 2016 and Schwarzer *et al*., 2022).

5.2.5 Tibia gross lesion analysis and tibia linear parameters

At exactly day 42 of the experiment, the chickens were sent for necropsy to obtain bone samples for analysis of bone development. 6 right tibiae were surgically removed from each treatment, with all the flesh meticulously removed including cartilage caps. Thereafter, the muscle around the tibia was carefully removed, a very sharp scalpel was used to dissect the proximal end and expose the growth plates. The epiphyseal plates of the tibia bones were visually assessed for gross lesions and growth plate parameter measurements taken. The left tibiae were also obtained and weighed to obtain the tibia weight (TW), after which the following tibia linear measurements were determined using a Toshiba-Rotanode X-ray (Toshiba Electron Tubes and Devices. Tochigi. Japan) connected to the point-of-care (CR140) system for digital imaging: Tibia diameter proximal end (TDPE), tibia diameter mid-section (TDM), tibia length (TL) and tibia diameter distal end (TDDE).

5.2.6. Bone ash and mineral content

For the purpose of determining the laboratory dry matter (DM), the tibiae were placed into pre-weighed crucibles and heated to 105°C for a duration of 12 hours. The difference between the initial sample weight and the dry weight was used to calculate the moisture content, which was then used to measure the weight loss. The tibiae were broken into small pieces then placed into a muffle furnace at 600°C for 24 hours to determine the amount of organic matter (OM). Samples where later cooled down in a desiccator for 40- 60 minutes before being weighed to determine ash percentage. Ash was used to represent the residue and organic matter (OM) content served as a proxy for weight loss.

The following formula was employed as a percentage expression for tibia ash.

$$\text{Tibia bone ash \%} = \frac{Tibia\ ash\ weight}{tibia\ dry\ weight} \times 100$$

Mineral content was determined at the Animal Health laboratory to the procedures of the Agri-Laboratory Association of Southern Africa's criteria (Agri LASA, 1998). The bone samples were ashed for 12 hours in a muffle furnace. The ash was weighed and digested in a Microwave Reaction System Model 3000 with 1 mL of 55 % nitric acid, therefore, samples evaporated to dryness on a slightly hot plate. Samples were later removed and allowed to cool and put into volumetric flasks (100 mL), which was later topped up with distilled water and permitted the sediment to settle for 24 hours. Samples were decanted to McCartney bottles using Whatman filter papers into 14/15 ml centrifuge tubes without disturbing the sediment once 24 hours had passed. The concentration of minerals was determined using an ICP mass spectrometer. (Perkinelmer, NexION 300Q). Microminerals were measured in mg/kg DM, while macrominerals were measured in g/100 g DM.

5.3 Statistical analysis

Data on latency to lie, tibia parameters and mineral content were analysed using the GLM procedure of (SAS, 2010) with dietary treatment as the only fixed effect. Mean separation was compare using the probability of difference option of (SAS, 2010). The level of significance was set at $P < 0.05$, for all statistical tests.

The employed linear model is as follows:

$$Y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where Y_{ij} = the observation of the dependant variables (bone development).

μ = the overall mean (general mean) constant to all observations.

t_i = the i^{th} fixed effect of the diet level.

ε_{ij} = random error or residual effect associated with ij^{th} observation.

5.4 Results

5.4.1 Latency to lie test, gait and feather scores

The effects of dietary FA inclusion in broiler diet on broiler welfare indicators are shown in Table 5.1. From the table, diet lacked significance effect on all the welfare measurements (latency to lie, gait and feather score) of the broiler chickens. Broilers fed the posCON diet had the highest tendency to lie as reflected by the lowest time before they lay down (416.21 seconds). Nevertheless, chickens fed the FA2 and FA3 diets appeared to have higher standing persistence with birds fed the FA2 diet having numerically higher standing periods (462.28 seconds) followed by those fed the FA3 diet (435.36 seconds). Gait score ranged from 1.62 to 2.00 and feather score ranged from 1.25 to 1.75.

5.4.2 Tibial growth plate analysis

The results on the tibia histomorphometry of the birds are presented in Table 5.3. The results revealed no effect of diet on all the tibial growth plate parameters (hypertrophic zone, proliferative zone and growth plate). Nevertheless, the birds fed the posCON and FA3 diets appeared to marginally have higher values for the proliferative zone and the total growth plate area.

Table 5.1. The effect of dietary FA inclusion on welfare indicators in broiler diets.

	[1]Dietary treatments						
[2]Parameters	negCON	posCON	FA1	FA2	FA3	[3]SEM	*P*-value
LTL (seconds)	423.10	416.21	403.93	462.28	435.36	83.42	NS
Gait Score	1.87	2.00	2.00	1.75	1.62	0.14	NS
Feather Score	1.25	1.62	1.75	1.25	1.62	0.19	NS

[3]SEM = standard error of the means.

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2]Parameters: LTL = latency to lie.

NS = no significance

Table 5.2. The effect of dietary FA inclusion on tibia gross lesion analysis in broilers.

	[1]Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	[2]SEM	*P*-value
Hypertrophic zone (mm)	4.33	5.33	4.86	5.66	4.66	0.93	NS
Proliferative zone (mm)	0.90	1.00	0.93	0.83	1.00	0.09	NS
Growth plate(mm)	5.23	6.33	5.80	5.50	6.66	0.92	NS

[2]SEM = standard error of the means.

NS = no significance

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

5.4.3 Tibia biomechanical parameters

The results of the effect of FA inclusion in tibia linear parameters in broiler diets are shown in Table 5.3. Diet had no effect on tibia weight, tibia length and tibia diameter proximal end. However, diet had a similar influence ($P < 0.05$) on tibia weight and tibia diameter distal end. Birds offered the posCON (0.60 g) diet had the highest tibia weight, whilst chickens fed the FA2 (0.50 g) diet had the lowest. Values for posCON, FA1 and FA3 were not statistically different in terms of tibial diameter distal end measurements with the highest value observed in chickens fed the FA3 (1.80 g) diet followed by those offered the posCON (1.77 g) diet with FA3 having the least values.

5.4.4 Bone ash and mineral content

Tibia bone ash and mineral content results are demonstrated in Table 5.4. From the results, there was no effect of diet ($P < 0.05$) on all minerals apart from phosphorus, iron and molybdenum. Serum calcium levels were higher in all the diets containing FA compared to the control. Highest calcium levels were observed in birds fed the FA3 diet (5.20 mg/l) and the lowest levels in broilers offered posCON (3.99 mg/l). Phosphorus levels were also higher in chickens offered FA3, while chickens offered the negCON diet contained the least phosphorus levels. The highest iron content was observed in chickens fed the FA3 (322.51 mg/l) diet while the negCON diet had the lowest (272.41 mg/l). Ash percentage significantly increased in dietary FA supplementation groups. The highest ash percentage was recorded in chickens offered the FA3 diet (97.92%) followed by chickens fed the FA diet (97.52%). Generally, an increase in bone ash and mineral content was observed in dietary treatments that included FA.

Table 5.3. The effect of dietary FA inclusion on tibia biomechanics in broiler diets.

	[1] Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	[2]SEM	*P*- value
Tibia weight (g)	23.79	24.63	21.82	22.63	24.15	2.11	NS
Diameter weight (g)	0.57[b]	0.60[c]	0.53[b]	0.50[a]	0.56[b]	0.04	0.03
Tibia length (cm)	10.50	10.53	10.46	10.10	10.26	0.16	NS
Tibia proximal (cm)	1.70	1.83	1.66	1.56	1.80	0.10	NS
Tibia distal (cm)	1.65[b]	1.80[b]	1.66[b]	1.53[a]	1.77[b]	0.10	0.05

[a.b c.] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

[2]SEM = standard error of the means.

NS = no significance.

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

Table 5.4. Bone ash, micro and macro mineral content in broiler bones supplemented with FA.

	[1]Dietary treatments						
Parameters	negCON	posCON	FA1	FA2	FA3	[2]SEM	*P*-value
Calcium (mg/l)	4.96[b]	3.99[a]	4.97[b]	4.89[b]	5.20[c]	0.40	0.001
Phosphorus (mg/l)	1.25	1.44	1.31	1.37	1.44	0.09	NS
Ca:P	3.97	2.77	3.79	3.57	3.61	0.41	NS
Copper (mg/l)	11.55	14.46	6.38	3.59	8.99	2.80	NS
Cobalt (mg/l)	2.62	3.35	3.44	3.65	4.99	0.23	NS
Iodine (mg/l)	0.21	0.18	0.24	0.22	0.24	0.04	NS
Manganese (mg/l)	93.80	75.52	63.83	78.38	78.45	23.15	NS
Zinc (mg/l)	270.34	107.07	101.05	93.91	105.64	79.86	NS
Iron (mg/l)	272.41[a]	294.44[b]	281.37[b]	320.36[c]	322.51[c]	58.21	0.03
Sulphur (mg/l)	1081.43	1335.80	1575.12	848.94	1664.71	220.48	NS
Molybdenum (mg/l)	16.48[c]	6.27[a]	9.39[b]	8.49[b]	16.48[c]	2.58	0.03
Ash (%)	96.92[ab]	97.16[b]	93.85[a]	97.52[b]	97.92[b]	1.47	0.06

[a.b.c,] means significant differences are denoted by different superscripts in a row ($P < 0.05$).

[1]Diet: negCON = negative control containing a standard antibiotic-free commercial broiler diet; posCON = positive control containing a standard commercial broiler diet combined with antibiotics; FA1 = normal commercial broiler diet including 1% of FA; FA2 = normal commercial broiler diet including 2% of FA. FA3 = normal commercial broiler diet including 3% of FA.

[2]SEM = standard error of the means.

NS = no significance.

5.5 Discussion

This study intended to assess the effect of FA inclusion in broiler diet on broiler welfare and bone development. The results of the welfare measurement indices demonstrated no effect of FA inclusion on all the welfare measurements. This is contrary to previous studies by Disetlhe *et al*. (2017), who observed differences in relative measurements caused by HA, a humic substance similar to FA. According to Weeks *et al*. (2002), the LTL test can either be conducted in groups or individually (Berg & Santora, 2003). In this current study, the LTL was conducted individually. Data from this current study is in line with results of Weimer *et al*. (2020), who reported that the LTL test would provide a quantitative grit of leg health that was sensitive enough to scale stress and pain experienced by broilers, which had bigger body weights. The presumption was that birds with weaker bones are expected to sit sooner as they might experience more leg pain quicker compared to chicken with stronger bones. Numerically, chickens fed the posCON (1959.27 g) diet had lower standing persistency followed by chickens offered FA1 the (1942.10 g) diet, this might be due to the higher body weights allowing them to sit within minutes. Moreover, chickens offered the diets containing higher levels of FA had longer standing persistency compared to the control groups. This could be attributed to the humic substances such as fulvic, humic and ulmic acid capabilities in influencing the cell wall absorption and accelerated mineral transfer from the blood to the bone and the cells, enhancing stronger leg health (Yildiz *et al*., 2013; Jaďuttová *et al*., 2019). Data from the current study is also consistent with the findings of Disetlhe *et al*. (2017), who observed higher standing persistency in birds fed diets containing 1.5% potassium humate.

Gait score systems are the most often used instruments for assessing the walking ability and asymmetry in chickens (Sassi *et al*., 2016). Gait score definitions should be descriptive enough to allow different users to interpret and use them in a similar manner for the results of their analyses to be meaningful and to allow comparisons to be made across assessments or studies (Makagon *et al*.,

2015). In the present study, it was discovered that the 3-point gait score system categories correlated with gait structural measurements and were linked to both strong intra-rater agreement. Therefore, results from the current study, falling within a range of 1.62 to 2.00, were equivalent to best gait scoring, attributing to strong healthy legs of broilers fed diets containing FA as a feed additive. Previous studies on FA supplement effect on feather scoring is limited. However, results from this study showed positive feedback on chickens fed FA inclusion, which had moderate feather scoring as the visual plumage assessment score. The results fell within a range of 1.25-1.62. Data from the current study is in line with observation of (Schwarzer *et al*., 2022).

From the results, it appeared that broiler chickens fed the FA1 and FA2 diets resulted in a slight decrease in tibia linear measurement. Nevertheless, high values were obtained in birds fed the FA3 diet. According to Jad'uttová *et al*. (2019), high values obtained on birds fed the FA3 diets could be due to the effects of FA in enhancing intestinal permeability, subsequently increasing the absorption and assimilation of calcium and phosphorus for bone development. Humic substances have previously been reported to increase mineral uptake in broiler bone mineralization matrix (Disetlhe *et al*., 2017; Angeles *et al*., 2022). Studies by Nkukwana *et al*. (2014) and Yan *et al*. (2006), reported that depression in linear biochemical parameters can be attributed to low available phosphorus and increased calcium concentration levels without phytase supplementation. It has been revealed that the ability of the bone structure of an immature growing bone to withstand weights is genetically associated and further influenced by whether the chickens were given access to feed *ad libitum* (Williams *et al*., 2004). In general, when a chick gets older, its phosphorus needs to decrease. Furthermore, a few studies have concentrated on phosphorus requirements at three or four weeks of age to correspond with increases in broiler feed intake when faecal excretion is higher due to poor utilization (Yan *et al*., 2001). However, this observation does not appear to be associated with any severe incidence of rickets as previously reported by (Pelicia *et al*., 2012). The organic material

in the bone mass of broiler tibial bones was converted normally, resulting in healthy tibial without any signs of rickets. This finding conflicts with those of Disetlhe *et al*. (2017), who reported least calcium and phosphorus levels supplemented with 1.5% humates, observing a transition of the proliferate zones in the tibia zones, which is connected to the incidence of rickets.

Phosphorus and calcium are micro elements which plays a crucial role in bone development and energy metabolism (Ciosek *et al*., 2021). Data on the current study shows that inclusion of FA was linked to an increase in calcium. However, both the phosphorus and calcium concentration in birds fed diets containing FA increased with increasing levels of FA in diets. This data is in line with Ozturk *et al*. (2012), who also observed similar results of increased levels of plasma phosphorus concentrations in broiler diets supplemented with 1.5 g/kg humic substance. In contrast to the increased levels of plasma phosphorus and calcium in FA diets, Rath *et al*. (2006) showed a trend for calcium and phosphorus content to be reduced by HA. Additionally, Yildiz *et al*. (2013) also observed no influence of humic substances on bone development and mineral content.

According to Jaďuttová1*et al*. (2019), humate substances consist of a significant number of carboxylic acid groups with chelating properties of metals and could have caused a reduction of calcium and phosphorus concentration in blood serum, which can be allied to improve mineral uptake in broiler bone mineralization (Jad'uttová *et al*., 2019). The results of the current study also concur with findings by Yildz *et al*. (2013) and Angeles *et al*. (2022), who observed that broilers offered humate substances demonstrated an increase in calcium and ash concentration in tibia bone as well as an increases in copper, iron, and calcium content in broiler meat concentration (Angeles *et al*., 2022). For most chicken rations, the micromineral calcium generally has a higher accessibility than phosphorus, but phosphorus accessibility varies significantly depending on the source (Williams *et al*., 2000; Waldenstedt, 2006). Because of this, when calcium is also restricting, phytase

supplementation has no effect on phosphorus deficiency (Yan *et al*., 2006). The lack of significant influence of FA on phosphorus does not agree with results presented from previous studies in broiler supplementation of HS that increased both calcium and phosphorus mineral content (Disetlhe *et al*., 2017, Domínguez-Negrete *et al*., 2019). This can be due to the reduction in calcium and available phosphorus intake in conjunction with reduced feed intake in broilers offered FA (Domínguez-Negrete *et al*., 2019).

5.6 Conclusion

From the results, it can be concluded that increasing levels of FA in drinking water improved the broiler welfare in chickens. FA inclusion in diets also increased calcium levels and ash concentration with increment levels. Although there was a slight depression at the tibial biomechanical measurements in birds fed diets containing FA, this did not have any effect on the tibial gross lesion incidences as no incidence of rickets was observed in the leg health. Therefore, it is evident that FA inclusion in diets can desirably influence bone development, leg health and general welfare in broilers.

5.7 References

Agri Laboratory Association of Southern Africa (AgriLASA). 1998. Feed and Plant Analysis Methods. AgriLASA. Pretoria. South Africa.

Angeles, M.L., Gómez-Rosales, S., López-Garcia, Y.R. & Montoya-Franco, A., 2022. Growth performance and tibia mineralization of broiler chickens supplemented with a liquid extract of humic substances. Braz. J. Poult. Sci. 24.

Berg, C. & Sanotra, G.S., 2003. Can a modified latency-to-lie test be used to validate gait-scoring results in commercial broiler flocks. Anim. Welf. 12(4), 655-659.

Bessei, W., 2006. Welfare of broilers: a review. World's Poult. Sci. J. 62(3), 455-466.

Bezuglova, O. & Klimenko, A., 2022. Application of humic substances in agricultural industry. Agronomy. 12(3), 584.

Bright, A., Gill, R. & Willings, T.H., 2016. Tree cover and injurious feather-pecking in commercial flocks of free-range laying hens: a follow up. Anim. Welf. 25(1), 1-5.

Ciosek, Ż., Kot, K., Kosik-Bogacka, D., Łanocha-Arendarczyk, N. & Rotter, I., 2021. The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue. Biomolecules. 11(4), 506.

Disetlhe ARP, Marume U, Mlambo V. & Dinev, I., 2017. Humic acid and enzymes in canola-based broiler diets: Effects on bone development, intestinal histomorphology and immune development. S. Afr. J Anim. Sci. 47, 914-922.

Domínguez-Negrete, A., Gómez-Rosales, S., Angeles, M.D.L., López-Hernández, L.H., Reis-de Souza, T.C., López-García, Y., Zavala-Franco, A. & Téllez-Isaias, G.,

2019. Effect of the addition of humic substances as growth promoter in broiler chickens under two feeding regimens. Animals. 9(12), 1101.

Hartcher, K.M. & Lum, H.K., 2020. Genetic selection of broilers and welfare consequences: a review. World's Poult. Sci. J. 76(1), 154-167.

Jaďuttová, I., Marcinčáková, D., Bartkovský, M., Semjon, B., Harčárová, M., Nagyová, A., Váczi, P. & Marcinčák, S., 2019. The effect of dietary humic substances on the fattening performance, carcass yield, blood biochemistry parameters and bone mineral profile of broiler chickens. Acta Vet. Brno. 88(3), 307-313.

Jasielska, M. & Grzybowska-Chlebowczyk, U., 2021. Hypocalcaemia and Vitamin D deficiency in children with inflammatory bowel diseases and lactose intolerance. Nutrients. 13(8), 2583.

Karcher, D.M., Makagon, M.M., Fraley, G.S., Fraley, S.M. & Lilburn, M.S., 2013. Influence of raised plastic floors compared with pine shaving litter on environment and Pekin duck condition. J. Poult. Sci. 92(3), 583-590.

Kolakshyapati, M., Flavel, R.J., Sibanda, T.Z., Schneider, D., Welch, M.C. & Ruhnke, I., 2019. Various bone parameters are positively correlated with hen body weight while range access has no beneficial effect on tibia health of free-range layers. J. Poult. Sci. 98(12), 6241-6250.

Makagon, M.M., Woolley, R. & Karcher, D.M., 2015. Assessing the waddle: an evaluation of a 3-point gait score system for ducks. Poult. Sci. 94(8), 1729-1734.

Nkukwana, T.T., Muchenje, V., Masika, P.J., Hoffman, L.C. & Dzama, K., 2014. The effect of Moringa oleifera leaf meal supplementation on tibia strength, morphology and inorganic content of broiler chickens. S. Afr. J. Anim. Sci. 44(3), 28-239.

Ozturk, E., Ocak, N., Turan, A., Erener, G., Altop, A & Cankaya, S., 2012. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. J. Sci. Food Agric. 92, 59-65.

Pelicia, K., Aparecido Jr, I.M., Garcia, E.A., Molino, A.B., Santos, G.C., Berto, D.A., Vieira Filho, J.A., Murakami, E.S.M., Montenegro, A.T. & Silva, A.M., 2012. Evaluation of a radiographic method to detect tibial dyschondroplasia lesions in broilers. Braz. J. Poult. Sci. 14, 129-135.

Perpetuini, D., Russo, E.F., Cardone, D., Palmieri, R., Filippini, C., Tritto, M., Pellicano, F., De Santis, G.P., Pellegrino, R., Calabrò, R.S. & Filoni, S., 2022. Psychophysiological Assessment of Children with Cerebral Palsy during Robotic-Assisted Gait Training through Infrared Imaging. Public Health. Int. J of Environ. Res. 19(22), 15224.

Rath, N.C., Huff, W.E. & Huff, G.R., 2006. Effects of humic acid on broiler chickens. Poult. Sci. 85(3), 410-414.

Sassi, B. N., Averós, X. & Estevez, I., 2016. Technology and poultry welfare. Animals. 6(10), 62.

Schwarzer, A., Rauch, E., Erhard, M., Reese, S., Schmidt, P., Bergmann, S., Plattner, C., Kaesberg, A. & Louton, H., 2022. Individual plumage and integument scoring of laying hens on commercial farms: Correlation with severe feather pecking and prognosis by visual scoring on flock level. Poult. Sci. 101(10), 102093.

Steenfeldt, S., Lübeck, M. & Engberg, R., 2018. Alternative protein sources for organic poultry. In Proceedings of the XV's European Poultry Conference. Croatian Branch of the World's Poultry Science Association. 39-51

Waldenstedt, L., 2006. Nutritional factors of importance for optimal leg health in broilers: A review. Anim. Feed Sci. Technol. 126, 291-307.

Weimer, S.L., Mauromoustakos, A., Karcher, D.M. & Erasmus, M.A., 2020. Differences in performance, body conformation, and welfare of conventional and slow-growing broiler chickens raised at 2 stocking densities. J. Poult. Sci. 99(9), 4398-4407.

Weimer, S.L., Wideman, R.F., Scanes, C.G., Mauromoustakos, A., Christensen, K.D. & Vizzier-Thaxton, Y., 2018. Animal well-being and behaviour. J. Poult. Sci. 97, 3381-338.

Weimer, S.L., Wideman, R.F., Scanes, C.G., Mauromoustakos, A., Christensen, K.D. & Vizzier-Thaxton, Y., 2020. Broiler stress responses to light intensity, flooring type, and leg weakness as assessed by heterophil-to-lymphocyte ratios, serum corticosterone, infrared thermography, and latency to lie. J. Poult. Sci. 99(7), 3301-3311.

Weeks, C.A., Knowles, T.G., Gordon, R.G., Kerr A.E., Peyton S.T. & Tillbrook, N.T., 2002. New method for objectively assessing lameness in broiler chickens. Vet. Rec. 151, 762─764.

Wideman Jr, R.F., 2016. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. J. Poult. Sci. 95(2), 325-344.

Williams, B., Waddington, D., Murray, D.H. & Farquharson, C., 2004. Bone strength during growth: Influence of growth rate on cortical porosity and mineralization. Calc. Tiss. Int. 74, 236-245.

Yıldız, G., Köksal, B.H. & Sızmaz, Ö., 2013. Influence of dietary boric acid and liquid humate inclusion on bone characteristics, growth performance and carcass traits in broiler chickens. Arch Geflügelk 77, 260-26.

Yan, F., 2016. The effects of dietary probiotic inclusion on skeletal health of poultry and its possible mechanisms.

Yan, F., Kersey, J.H. & Waldroup, P.W., 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci. 80, 455-459.

Yan, F., Kersey, J.H., Fritts, C.A. & Waldroup, P.W., 2006. Effect of phytase supplementation on the calcium requirement of broiler chicks. Int. Poult. Sci. 5 (2), 112-120.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

The conflict between animals and humans for food and feed has increased due to the development of both human populations and livestock. The fastest way to meet the continent of Africa's growing demand for animal protein is probably through poultry production. This is because of their rapid reproduction, short generation interval and effective food processing into high quality protein (Nkukwana, 2018). The high poultry productivity, broilers, has been achieved through genetic improvement and the use of AGPs. Nevertheless, the use of conventional antibiotics has caused some anxiety among consumers due to the associated risks on consumer health. Hence the need to explore alternatives to conventional antibiotics. Previous studies that have proposed to substitute humic substances for part of AGPs showed that this feed additive can potentially promote improved poultry performance and meat quality traits without jeopardizing the health status and well-being of the chickens (Schmidt *et al*., 2019).

In this experiment a study was conducted for a period of 42 days to investigate the impact of FA in broiler diets on growth performance, blood parameters, physiological characteristics, and meat quality parameters on Cobb 500 broiler chickens. FA was included in water in place of AGPs at 1% (FA1,) 2% (FA2) and 3% (FA3). The effect of dietary FA inclusion in diets on growth traits, blood parameters and protein utilization efficiency of broilers was assessed in Chapter 3. The results showed that inclusion levels of FA in broiler diets had no negative effects on growth performance and protein utilisation efficiency, particularly in the finisher phase, as highlighted by the similarities in performance among the dietary treatments. Nevertheless, the inclusion of FA above 2% appeared to depress the daily feed intake in the last two feeding phases, which could have compromised the

performance of broilers. With regards to haematology, the inclusion levels of FA up to 3% had no effect on haematology parameters apart from the platelet count. However, the inclusion of FA in diets significantly influenced the following serum biochemical parameters: SDMA & ALK/ALP, whereby lower values were observed in FA supplemented diets. This indicates that FA did not affect the liver and kidney functions. Generally, all values for serum biochemistry and haematology observed in this study fell within the normal setting range for normal and healthy broilers.

In Chapter 4, the effect dietary FA inclusion in broiler diets on carcass characteristics and meat quality parameters was assessed. The results of the study showed that diet significantly affected all measured carcass parameters apart from the dressing percentage, chilling loss percentage and the thigh index. The HCW was the highest in broilers fed the posCON diet followed by in FA2 and FA1. The breast muscle and shank weights were the lowest in the negCON diets, implying that FA can be used as a potential supplement without affecting carcass traits and external organs. The results on meat quality parameters also demonstrated a lack of significant effect on diet on breast initial, ultimate pH and temperature, dripping loss, cooking loss and shear force. However, diet altered the WHC and 2 colour coordinates (yellowness and lightness). With regard to meat colour, broiler muscle fed the posCON, FA1 and FA3 groups had the highest colour coordinates for lightness, while broilers fed the FA2 diet had the lowest. Results on WHC illustrated that breast muscles from broilers fed the FA1 group had the lowest value showing the greatest WHC capabilities followed by breast muscle broilers from the FA2 diet. With regards to fatty acid profiles, highest values for PUFAs, n-3 fatty acids and n-6 fatty acids were observed in all the FA containing diets particularly in the FA2 and FA3 groups. FA inclusion increased the PUFA/SFA ratio whilst at the same time, reducing the n-6/n-3 ratios, which are essential tools in assessing the nutritional value of the meat.

In Chapter 5, the effect of FA inclusion in diets on bone development and welfare measures was assessed. The results showed that diet had no effect on all the welfare measurements. Nevertheless, broilers fed the FA2 and FA3 diets appeared to have the highest standing persistence, indicating that the inclusion of FA in two experimental diets led to the optimum bone strength qualities. Diet had no effect on tibia length and tibia diameter proximal end. However, diet had a significant influence on tibia weight and tibia diameter distal end. Birds offered the posCON diet had the highest tibia weight, whilst chickens fed the FA2 diet had the lowest. Diet showed no dietary influence on all micro and macro minerals apart from phosphorus, iron and molybdenum. Serum calcium levels were higher in all the diets containing FA compared to the control group. Overall, FA was shown to have great potential as an alternative for antibiotics in broiler diets. Collectively, findings from this study may be useful in developing low-cost feed formulas that will benefit customers and enhance future chicken farming systems for growth performance, health status, carcass features, meat quality, and bone development.

6.2 Conclusion and recommendations

The study showed that inclusion of FA in broiler diets did not compromise the nutrient utilization efficiency, broiler performance, health status, meat quality traits, internal organs carcass traits and bone development of broiler diets. The inclusion FA in broiler diets represents a sustainable, cost-effective, safe and environmentally friendly way of providing high quality and naturally produced meat and meat by products. Therefore, FA can be recommended to small scale and commercial farmers as a natural feed additive, safely administered at 3%, with significant improvement in broiler performance, health status, meat quality, bone development and broiler welfare in broilers.

6.3 Future prospects

While the findings from the current study revealed that adding FA in broiler diets encouraged comparable performance, in terms of growth, haematology, serum biochemistry, carcass characteristics and meat quality traits, as with the antibiotics diet in unchallenged broilers, inclusion levels higher than 3% may negatively affect broiler health and growth performance. Therefore, possible research can be designed to investigate the administration of FA in broiler diets on performance traits and immune development in challenged birds. The challenge can be in the form of heat stress, feed deprivation and infection. This may provide valuable information on how to use FA in cases of disease outbreaks or some discomforts that may affect the welfare of birds.

6.4 References

Nkukwana, T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci. 48(5), 869-884.

Schmidt, H.P., Hagemann, N., Draper, K. & Kammann, C., 2019. The use of biochar in animal feeding. Peer Rev. 7, 7373.

7. LIST OF APPENDICES

7.1 Ethical certificate

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za

Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 29/07/2021, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Effect of fulvic acid inclusion in broiler diets on growth performance, physiological and meat quality parameters

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof U Marume

Student: Keamogetswe C Mokubung

Ethics number:

N	W	U	-	0	0	8	1	5	-	2	1	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2023/08/10 **Risk Category:** 2
Expiry date: 2024/08/09
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*

7.2. Language editing certificate

SCHOOL OF BIOLOGICAL SCIENCES

Tel: +27 18 389 2289 **Fax:** 018 389 2134

Cell: +27 72 626 8446 **E-mail:** Oziniel.Ruzvidzo@nwu.ac.za

Date: 11th November 2023

To Whom It May Concern,

REF: Language Editing and Proof-reading of Dissertations/Theses

Dear Sir or Madam,

This serves to confirm that I have proof-read and edited the **MSc Dissertation** of **KC Mokubung** (Student number: **29206898**; orcid.org/**0000-0001-5847-4098**) entitled: **Effect of fulvic acid inclusion in broiler diets on growth performance, physiological and meat quality parameters**. The candidate then later corrected all the identified language and technical errors to the satisfaction of the supervisor. Thus, the document presented here is of sufficient and acceptable academic standards.

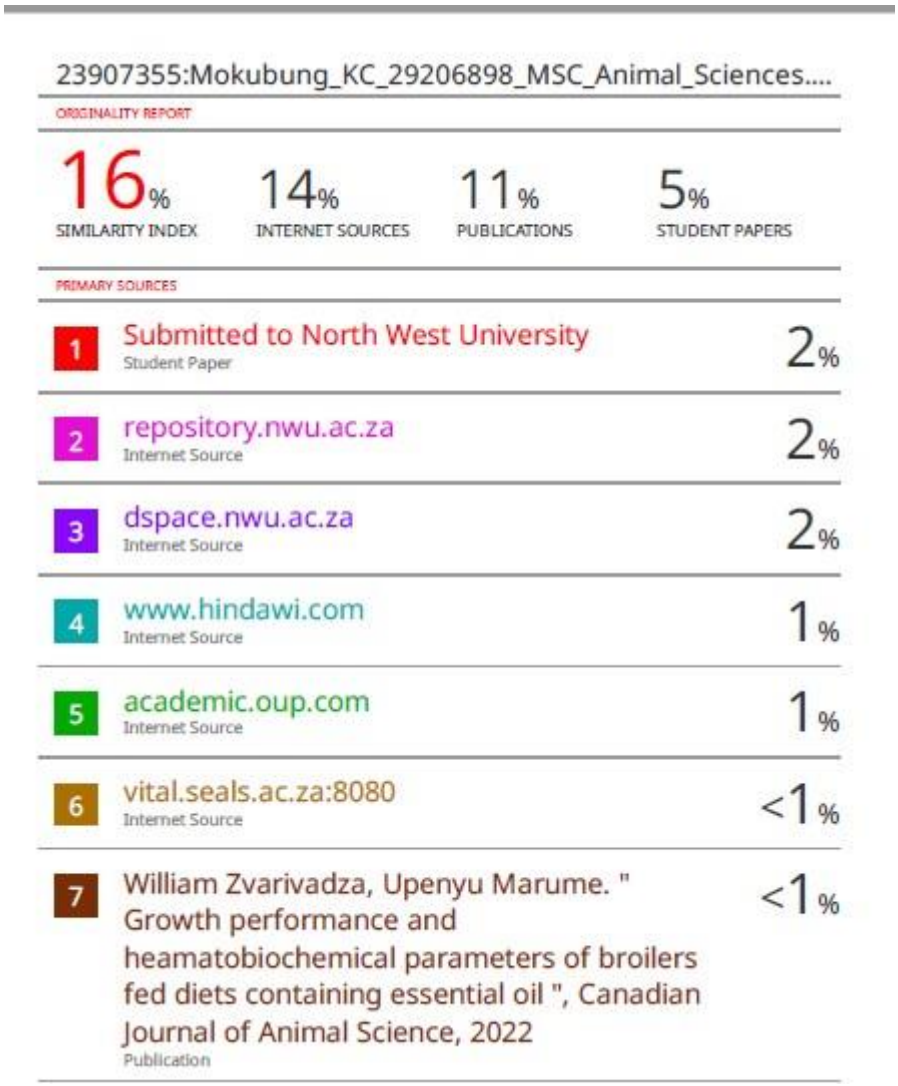
Editor

Prof. O Ruzvidzo

Supervisor

Prof. U Marume

7.3. Turnitin report



23907355:Mokubung_KC_29206898_MSC_Animal_Sciences....

ORIGINALITY REPORT

16%	14%	11%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to North West University Student Paper	2%
2	repository.nwu.ac.za Internet Source	2%
3	dspace.nwu.ac.za Internet Source	2%
4	www.hindawi.com Internet Source	1%
5	academic.oup.com Internet Source	1%
6	vital.seals.ac.za:8080 Internet Source	<1%
7	William Zvarivadza, Upenyu Marume. " Growth performance and heamatobiochemical parameters of broilers fed diets containing essential oil ", Canadian Journal of Animal Science, 2022 Publication	<1%