INFLUENCES OF DIFFERENT PHYTOBIOTIC SUPPLEMENTATIONS ON GROWTH PERFORMANCE, HEALTH PERFORMANCE AND TELOMERE LENGTH EXPRESSION OF BROILER CHICKENS

By

NAFEESA BINTI ABU KASSIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2022

IPTSM 2022 12

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

INFLUENCES OF DIFFERENT PHYTOBIOTIC SUPPLEMENTATIONS ON GROWTH PERFORMANCE, HEALTH PERFORMANCE AND TELOMERE LENGTH EXPRESSION OF BROILER CHICKENS

By

NAFEESA BINTI ABU KASSIM

November 2022

Chairman : Eric Lim Teik Chung, PhD
Institute : Tropical Agriculture and Food Security

Antibiotic resistance in poultry is detrimental to human health mainly due to the transmission of harmful antibiotic residuals from food-producing animals to consumers. Owing to public pressure, antibiotics for instance avoparcin, chloramphenicol, nitrofurans, teicoplanin, vancomysin and norfloxacin are banned in Malaysia. It is critically important to discover a safer alternative replacer for antibiotics such as phytobiotic as natural compounds that possess antibiotic properties due to their pharmacological active compound that may improve growth performance, improve microbial properties, and immunological response in poultry. The primary isssue here is a gap of knowladege on phytobiotic utilization in enhancing poultry growth and health performance. Therefore, this study intends to determine the effect of three different phytobiotic supplementations on growth performance, health performance, telomere length expression of broiler chickens.

A total of 300 day-old male Ross 308 was randomly assigned to six different dietary treatments. There were five replications of each dietary treatment, with ten broilers in each replication. The dietary treatments include T1(negative control) commercial feed without antibiotic, T2(positive control) commercial feed added with 100mg/kg oxytetracycline, T3(25mg/kg), T4(50mg/kg), T5(70mg/kg), and T6(100mg/kg) were commercial feed supplemented with respective concentration of powdered *Yucca shidigera* saponin for experiment A in both starter and finisher diets. Throughout 42 days of rearing, birds were weighed weekly for the determination of feed conversion ratio, while the mortality and abnormal clinical sign were recorded daily. A total of 60 broilers were selected for starter and finisher phase sampling, one from each replicate in each dietary treatment at random. Samples were slaughter for leucocyte profiling analysis, organ measurement, blood biomarkers, and telomere length analysis. The same experimental design was conducted for another two more studies using dried *Brachiaria*

decumbens grass as experiment B and saponins extract from *Brachiaria decumbens* grass as experiment C.

As for the result, T6(100mg/kg) broilers supplemented with *Y. schidigera* saponins, experiment (A); T3(25mg/kg) broilers supplemented of *B. decumbens*, experiment (B); and T6(100mg/kg) broilers supplemented *B. decumbens* saponins extract, experiment (C) elucidated significantly better ($P<0.05$) growth performance, higher white blood cell count, higher blood biomarkers, and longer telomere length expression during the finisher phase as compared to the other treatments. Those treatments showed a superior growth performance with the highest body weight and body weight gain leading to the best FCR. However, there were no notable clinical signs observed with a low mortality rate among all treatments. In addition, there was no significant difference ($P>0.05$) observed in the organ morphometric among different treatments. On the other hand, T6(100mg/kg) from experiments A and C, whereas T3(25mg/kg) from experiment B showed better expression of total white blood cell count, and lower heterophil and lymphocyte ratio indicating a better health performance. This was supported by the higher concentration of immunoglobulin A, immunoglobulin G, immunoglobulin M and antibody-related cytokines; interleukin-4 and interleukin-7. Besides, the telomere length analysis of broilers from experiments A, B, and C respectively exhibited longer telomere length expression as compared to the other treatments.

In summary, commercial broilers supplemented with 100 mg/kg of *Y. schidigera* saponins, 25 mg/kg of *B. decumbens* grass, and 100 mg/kg B. *decumbens* saponins extract demonstrated better results of growth performance, higher WBC total count, higher blood biomarkers, and better telomere length expression. 100 mg/kg of *B. decumbens* saponins extract was the best candidate comparing to all treatments in each experiment and this shows pure *B. decumbens* saponins extract supplementation was strongly advised be the best option as a secure natural additive that may enhanced the health and production of broilers. This can be used in the broiler industry as an antibiotic replacer, which ultimately prevents antibiotic resistance as well as increases the food safety of broiler products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGARUH SUPLEMEN FITOBIOTIK YANG PELBAGAI TERHADAP PRESTASI KESIHATAN, BIOMARKER DARAH DAN UNGKAPAN PANJANG TELOMER AYAM PEDAGING

Oleh

NAFEESA BINTI ABU KASSIM

November 2022

Penasihat : Eric Lim Teik Chung, PhD
Institut : Pertanian Tropika dan Sekuriti Makanan

Ketahanan antibiotic dalam ungas adalah berbahaya untuk kesihatan manusia akibat sisa antibiotic tersebut berpindah daripada sumber makanan berasaskan haiwan kepada pengguna. Oleh kerana tekanan dari awam, antibiotik seperti avoparcin, chloramphenicol, nitrofurans, teicoplanin, vancomysin dan norfloxacin telah diharamkan di Malaysia. Ini adalah sangat penting untuk menemukan bahan alternatif yang selamat bagi menggantikan antibiotik seperti fitobiotik yang dianggap sebagai sebatian semula jadi yang berpotensi tinggi dalam memiliki sifat antibiotik kerana sebatian farmakologi aktif yang boleh meningkatkan prestasi pertumbuhan, memperbaiki sifat mikrob, dan meningkatkan tindak balas imunologi dalam ayam. Masalah utamanya adalah, kekuranagn ilmu dalam penggunaan fitobiotik dalam meningkatkan prestasi pertumbuhan unggas dan prestasi kesihatannya. Oleh itu, kajian ini berhasrat untuk melihat kesan tiga diet fitobiotik tambahan yang berbeza terhadap prestasi pertumbuhan, prestasi kesihatan, dan ungkapan panjang telomer ayam pedaging.

Sejumlah 300 ekor ayam jantan Ross 308 berusia sehari telah diletakkan secara rawak dalam enam rawatan pemakanan yang berbeza. Setiap rawatan ini terdiri daripada lima replikat dengan 10 ekor ayam pedaging pada setiap replikat. Rawatan pemakanan ini terdiri daripada T1 (kawalan negative) makanan komersial tanpa antibiotik dan T2 (kawalan positif) makanan komersial ditambah dengan 100 mg/kg oxytetracycline T3(25mg/kg), T4(50mg/kg), T5(70mg/kg) dan T6(100mg/kg) makanan komersial yang ditambah dengan serbuk saponin *Yucca schidigera* untuk eksperimen A untuk kedua-dua fasa diet pemula dan penyudah. Sepanjang 42 hari pemeliharaan, berat ayam akan ditimbang secara mingguan untuk menentukan kadar nisbah penukaran makanan, manakala kadar kematian dan tanda-tanda klinikal yang tidak normal direkodkan setiap hari. Sejumlah 60 ekor ayam pedaging telak dipilih secara rawak di fasa pemula dan penyudah untuk pensampelan, seekor dari setiap replikat dari setiap rawatan pemakanan. Sampel disembelih bagi menganalisa profil leukosit, pengukuran organ, biomarker

darah, serta menganalisa ungkapan panjang telomer. Bentuk eksperimen yang sama telah dijalankan bagi dua lagi kajian menggunakan daun *Brachiaria decumbens* sebagai eksperimen B dan ekstrak saponin daripada rumput *Brachiaria decumbens* sebagai eksperimen C.

Hasil dapatannya, ayam pedaging T6 (100 mg/kg) yang ditambah dengan saponin *Y. Schidigera* (A); ayam pedaging T3(25mg/kg) yang ditambah dengan daun *B. decumbens* (B); dan ayam pedaging T6 (100mg/kg) yang ditambah dengan ekstrak saponin *B. decumbens* (C) menunjukkan prestasi jauh lebih baik ($P < 0.05$) prestasi pertumbuhan, kiraan sel darah putih lebih tinggi, biomarker darah lebih tinggi dan ungkapan panjang telomer yang lebih panjang di fasa penyudah berbanding dengan rawatan yang lain. Rawatan tersebut menunjukkan prestasi pertumbuhan yang unggul dengan berat badan tertinggi dan pertambahan berat badan ini yang membawa kepada FCR yang terbaik. Walaupun demikian, tiada sebarang tanda-tanda klinikal yang ketara dan kadar kematian juga amatlah rendah di setiap eksperimen.

Tambahan pula, tiada perubahan yang ketara ($P > 0.05$) yang diperhatikan pada ukuran organ antara rawatan. Selain itu, T6(100 mg/kg) daripada eksperimen A dan C, manakala T3 (25 mg/kg) daripada eksperimen B menunjukkan ekspresi yang lebih baik bagi jumlah kiraan sel darah putih, dan nisbah heterofil dan limfosit yang lebih rendah menunjukkan prestasi kesihatan yang lebih bagus. Ini disokong oleh hasil pemerhatian kepekatan immunoglobulin A, immunoglobulin G, immunoglobulin M dan sitokin berkaitan antibodi; interlukin-4 dan interlukin-7 yang lebih tinggi. Selain daripada itu, analisis ungkapan telomer T6(100mg/kg), T3(25mg/kg), and T6(100mg/kg) pada experiment A, B, dan C menunjukkan ungkapan yang lebih panjang berbanding dengan rawatan yang lain di dalam eksperimen masing-masing.

Kesimpulannya, ayam pedaging komersial yang diberikan tambahan 100 mg/kg serbuk saponin *Y. schidigera*, 25 mg/kg daun *B. decumbens*, and 100 mg/kg ekstrak saponin *B. decumbens* menunjukkan prestasi pertumbuhan yang lebih baik, jumlah kiraan WBC yang lebih tinggi, biomarker darah yang lebih tinggi, dan ungkapan telomer yang lebih baik. Dibandingkan dengan rawatan yang terbaik di setiap experiment, suplemen 100mg/kg ekstrak saponin *B. decumbens* adalah calon terbaik berbanding kepada semua rawatan di setiap eksperimen. Oleh itu, ekstrak saponin *B. decumbens* tulen sangat dicadangkan sebagai bahan tambahan semula jadi yang selamat dan mampu untuk meningkatkan kesihatan serta pengeluaran ayam pedaging. Ini boleh digunakan dalam industri ayam pedaging sebagai pengganti antibiotik, yang akhirnya mampu menghalang isu ketahanan antibiotik serta meningkatkan keselamatan makanan produk ayam pedaging.

ACKNOWLEDGEMENTS

Alhamdulillah ala kulli hal,

Greatest thanks to my supervisor, Dr Eric Lim Teik Chung, for your patience, guidance, and support. I have greatly benefited from your professionalism, wisdom, and your expertise in meticulous editing. I am very grateful that you are so supportive, constructive and you continued to have faith in me over the years. You not only taught me on my research field but a valuable lesson to be a matured and professional adult. You are the best supervisor I ever had, and I will always be proud to be your mentee.

Thank you to my committee members, Dr Mamat Hamidi bin Kamalludin, Prof Loh Teck Chwen and Prof Faez Firdaus Jesse bin Abdullah. Your encouraging words and taught bring me up being more confident and critical thinkers. Dr Mamat, thank you for your time and you always lending me your ears listening to all my genetics and extractions problems. You are very helpful and supportive in resolving all the problems.

Thank you to my parents, Mr Abu Kassim bin Yaacob and Mrs. Patimah binti Sulaiman, all my family members and my life partner for your endless support. You have always stood behind me and support me every single time. Thank you to my teammates, lab mates, my lab staff and my master's friends who make everything easier for me throughout my Master journey.

May Allah bless all of you.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science .The members of the Supervisory Committee were as follows:

Eric Lim Teik Chung, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Faez Firdaus Jesse bin Abdullah, PhD
Professor, Gs, Ts.
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Loh Teck Chwen, PhD
Professor
Faculty of Agricuture
Universiti Putra Malaysia
(Member)

Mamat Hamidi bin Kamalludin, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 May 2023

Declaration by the Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Nafeesa binti Abu Kassim

Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Eric Lim Teik Chung

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Faez Firdaus Jesse bin Abdullah

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Loh Teck Chwen

Signature: _____
Name of Member of Supervisory Committee: Dr. Mamat Hamidi bin Kamalludin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BWG	Body weight gain
CAT	Catalase
CF	Crude fibre
CORT	Corticosterone
CP	Crude protein
DM	Dry matter
DVS	Department of Veterinary Service
EE	Ether extract
EMA	European Medicines Agency
FCR	Feed conversion ratio
FeC_{13}	Ferum chloride
FI	Feed intake
GAPDH	Glyceraldehyde-3-phosphate
H/L	Heterophil to lymphocyte ratio
HRP	Horseradish peroxidase
HSF1	Heat Shock Factor 1
IB	Infectious bronchitis
IBD	Infectious bursal disease
IBDV	Infectious bursa disease virus
Ig	Immunoglobulin
IL	Interleukin
IMR	Institute of Medical Research
MeOH	Aqueous methanol

MG	Mycoplasma gallisepticum
MOH	Ministry of Health
NaOH	Sodium hydroxide
ND	Newcastle disease
NTC	No-templet Control
OD	Optical density
QY	Yucca schidigera + Quillaja saponaria
ROS	Reactive oxygen species
Rt-PCR	Real time polymerase chain reaction
SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity
TELO2	Telomere maintenance gene 2
TERRA	Telomeric repeat-containing RNA
TRF1	Telomeric repeat transcriptional factor 1
TWBC	Total white blood cell
VFA	Volatile fatty acid
WBC	White blood cell

CHAPTER 1

INTRODUCTION

1.1 General introduction

Globally, the poultry industry is a fast-growing industry in the agriculture livestock sub-sector. The demand for broiler meat is intensely increasing as the human population is growing exponentially. Moreover, broiler requires only a short production cycle as compared to the other meat sources, and therefore, it has become favourable in developing countries (Arshad et al., 2017). In Malaysia, the broiler is an agricultural livestock essential sector as the broiler industry provides a primary option of cheap, highly nutritive (Naji et al., 2013), good quality meat, and has no issues regarding multi-ethnic and multi-religions (Abdurofi et al., 2017). The broiler population in Malaysia is increasing annually and is it reach up to 207 million birds collectively: chicken and duck in 2020 (DVS, 2021). The self-sufficiency level in Malaysia for broiler is 104.53% while 116.21% for eggs (DVS, 2021). Poultry meat was recorded the highest per capita consumption, PCC of 57.8 kilogrammes per annum (DVS, 2021).

Despite the industry growth and monetary benefits, the broiler industry faces numerous challenges in order to fulfil the needs of demand, which include poor management, high feed production cost, disease outbreak, the uncertainty of government policy, and technology advancement pressure (Alghirani et al., 2021). Moreover, in the tropical region, the nature of high humidity and high temperature triggered thermal stress in broiler may also hinder optimal health and growth (Kpomasse et al., 2021). This defying challenge definitely will harm the meat supply consequent in large economic losses, especially on commercial broiler farms (Alghirani et al., 2021). Perceiving these concerns, the broiler industry frequently practised vaccination, medical treatments, and the inclusion of feed additives as a prophylactic preventive measure to optimise broiler production and health. The high demand for poultry meat is forcing the poultry subsector to include in-feed antibiotics to promote growth and health.

Antibiotic is one feed additive that is predominantly used in the livestock industry (Nisha, 2008). Antibiotic works in controlling infectious pathologies and successfully preventing the negative effect of various avian diseases (Bermudez, 2003). Antibiotics have the properties of immunostimulant, antimicrobial, and are also excellent in enhancing meat yield (Zulkifli et al., 2000), health (Ziaie et al., 2011), and feed efficiencies (Engberg et al., 2000) in poultry. The discovery of antibiotics was a thriving and inspiring hope, however, scientific evidence proved that misuse of antibiotics can threaten food safety by causing antibiotic resistance in consumers (Paganini, 2005; Nisha, 2008). Antibiotic residuals were found in human food and the environment (Carvalho & Santos, 2016; Ronquillo & Hernandez, 2017). For instance, several antibiotics such as penicillin, tetracycline, macrolide, aminoglycoside, and amphenicol were reported and have been detected in human foods (Diarra & Malouin, 2014). The feeding of antibiotics to food-producing livestock is one of the two major sources of antibiotic transmission that cause the emergence of resistant bacteria in hospitals and human communities (Abadi et al., 2019). Antibiotic resistance is detrimental to humans

as it may cause death due to infectious pathogen intolerance toward the infected hose. Antibiotic resistance may also increase the morbidity and mortality of humans as the infection can be more severe and harder to treat (Ahmad & Khan, 2019).

Therefore, with this strong awareness, antibiotic has become a global concern and the usage of antibiotic was started to be banned (Sarmah et al., 2006). Antibiotic additive in animal feed such as bambermycin and avilamycin was banned in European Union countries in 2006 (Ferket, 2004). European Medicines Agency (EMA) also has raised serious concerns about the increased risk to humans from the wide use of colistin in livestock (Mayor, 2016). On the contrary, harmful antibiotics are still widely used in animal feed in Malaysia, and the government is still making tremendous efforts towards banning the use of antibiotics in livestock feed due to its adverse effects. In 2017, avoparcin, chloramphenicol, nitrofurans, teicoplanin, vancomycin, and norfloxacin are banned in livestock and aquaculture product in Malaysia. (MOH, 2017). In the following years, awareness among the public and professionals on the effects of overuse and misuse of antibiotics in humans and the food chain on global health has triggered the ban of colistin in animal feed in Malaysia (MOH, 2019). Moving forward, four more high resistance rate antibiotics have been banned, including erythromycin, enrofloxacin, tylosin, and fosfomycin (IMR, 2020). Hence it is critical to explore and find an antibiotic replacer in order to control infectious diseases and most importantly to promote broiler growth and health. Here, a knowledge gap about the use of phytobiotics to improve poultry development and health performance is the main problem. In poultry, there are a few trials to replace antibiotics with better alternatives, which include organic acid, probiotic, prebiotic, synobiotic, vitamins and minerals, herbal drugs, antimicrobial peptides, as well as plant extract or also known as phytobiotic (Yadav et al., 2016).

In recent years, phytobiotic draws world attention as it derived from herbs and natural bases (Dhama et al., 2015). These derivative plant-based products originated from leaves, roots, tubers, fruits from herbs, and spices. Phytobiotic is a promising alternative to replace antibiotics due to their high content of pharmacologically active compounds and may act as a natural growth promoter in broiler production (Mountzouris et al., 2009; Grashorn, 2010). For instance, *Yucca shidigera* is an herbaceous dessert shrub that originated in Southwestern United States America, which is rich with phytobiotic namely glycol and steroidal saponin (Khaskheli et al., 2020). Commercial saponins extract from *Y. schidigera* is now commonly used in the temperate commercial broiler industry as it is capable of enhancing broiler growth and health (Ranjbar et al., 2014; Sahoo et al., 2015). This is attributed to the steroidal saponins present in the plant, which contribute to the emulsification of oil fats, promotion of broiler digestion, and the absorption of vitamins and minerals leading to positive effects in poultry (Alghirani et al., 2021). Furthermore, saponins were reported to have pharmacological properties such as anti-inflammatory, immunostimulant, hypocholesterolaemic (Francis et al., 2002; Guclu-Ustundag & Mazza, 2007) antifungal, cytotoxic activities, antimicrobial, anticarcinogenic, and antioxidant (Francis et al., 2002; Guclu-Ustundag & Mazza, 2007). On top of food safety, saponins can also be used as a growth enhancer for the broiler industry which will eventually strengthen the food security of the country. Essentially, even though saponins are categorised as an anti-nutritional factor that can disrupt the chemical structure of fats and may obstruct the absorption of fats, they could also be used effectively if supplemented at an optimum level to replace antibiotics owing to the positive benefits mentioned earlier.

However, the availability and cost price could be the limiting factors in applying *Y. schidigera* saponins extract in Malaysia's poultry industry. Moreover, the effect of *Y. schidigera* extracted saponins on broiler reared in tropical conditions is still poorly understood. Therefore, this is an opportunity to explore the benefits of novel saponins extracted from local plants that are available abundantly in Malaysia. For an instant, *Brachiaria decumbens* or also known as signal grass has high concentrations of saponins and is vastly planted in more than 8% of the grassland in Malaysia (Riet-Correa et al. 2011; Chung et al., 2018). *B. decumbens* is originated from the highlands of Central and Eastern Africa and is now widespread in the tropics and sub-tropics such as South America, Australia, Indonesia, Vanuatu as well as Malaysia due to its adaptation to a wide range of soil types and environments (Low, 2015). The steroidal saponins presence in *B. decumbens* is identical to *Y. schidigera*, which could be developed as a potential antibiotic replacer for the poultry industry in the country. Even though the feeding of *B. decumbens* has deleterious effects on small ruminants especially sheep due to the activation of rumen microbes toward saponins (Assumaidaee & Mustapha 2012; Muniandy et al., 2020), in-feed of *B. decumbens* and its saponins to broiler supposedly have no issues with rumen microbes since broiler is a monogastric animal. The vast availability of *B. decumbens* in Malaysia and correspond to previous studies on steroidal saponins benefits from *Y. schidigera* creates an interlink question. Do *B. decumbens* and its saponins have the same potential as growth and health enhancer toward commercial broilers? On the other hands there is a gap of knowledge regarding utilisation and the effects of different phytobiotics on the health performance, white blood cell profile, blood biomarkers, and telomere length expression of broilers reared under tropical conditions.

1.2 Problem statement

1. The use of commercial *Y. shidigera* and other locally available phytobiotics as an antibiotic replacement for the poultry industry in the tropics requires further investigation.

1.3 Hypotheses

It is hypothesised that there will be different health responses in commercial broilers supplemented with different types and levels of phytobiotics as compared to the control groups.

1. H_o: Commercial broilers supplemented with novel whole grass *B. decumbens* and *B. decumbens* saponins extract will have the same effects as commercial *Y. schidigera* saponins extract.
2. H_i: Commercial broilers supplemented with *Y. schidigera* saponins extract, whole grass *B. decumbens*, and *B. decumbens* saponins extract will demonstrate a better health performance with different patterns of blood parameters and telomere length expression.

1.4 Objectives

The main aim of this study was to elucidate the growth and health performance of commercial broiler supplemented with three different phytobiotic sources, which include commercial *Y. schidigera* saponins extract, whole grass *B. decumbens*, and *B. decumbens* saponins extract at different concentrations. The specific objectives were:

1. To determine the growth performance of commercial broilers supplemented with different types and levels of phytobiotics; commercial *Y. schidigera* saponins extract, whole grass *B. decumbens*, and *B. decumbens* saponins extract.
2. To evaluate the effects of different types and levels of phytobiotics supplementation on the health performance including clinical signs, mortality rate, organ morphometric, white blood cell profile, immunoglobulins, and cytokines of commercial broilers chickens.
3. To determine the telomere length of commercial broilers supplemented with different types and levels of phytobiotics.

REFERENCES

Abadi, A. T. B., Rizvanov, A. A., Haertlé, T., & Blatt, N. L. (2019). World Health Organization report: current crisis of antibiotic resistance. *BioNanoScience*, *9*(4), 778-788.

Abdel-Moneim, A. M. E., Shehata, A. M., Khidr, R. E., Paswan, V. K., Ibrahim, N. S., El-Ghoul, A. A., ... & Ebeid, T. A. (2021). Nutritional manipulation to combat heat stress in poultry–A comprehensive review. *Journal of Thermal Biology*, *98*, 102915.

Abdurofi, I., Ismail, M. M., Kamal, H. A. W., & Gabdo, B. H. (2017). Economic analysis of broiler production in Peninsular Malaysia. *International Food Research Journal*, *24*(2), 761.

Afrose, S., Hossain, M. S., Maki, T., & Tsujii, H. (2010). Hypocholesterolemic response to Karaya saponin and Rhodobacter capsulatus in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *23*(6), 733-741.

Ahmad, M., & Khan, A. U. (2019). Global economic impact of antibiotic resistance: A review. *Journal of global antimicrobial resistance*, *19*, 313-316.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., & De Smet, S. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of animal science and biotechnology*, *7*(1), 1-14.

Alfaro, D. M., Silva, A. V. F., Borges, S. A., Maiorka, F. A., Vargas, S., & Santin, E. (2007). Use of Yucca schidigera extract in broiler diets and its effects on performance results obtained with different coccidiosis control methods. *Journal of Applied Poultry Research*, *16*(2), 248-254.

Alghirani, M. M., Chung, E. L. T., Sabri, D. S. M., Tahir, M. N. J. M., Kassim, N. A., Kamalludin, M. H., ... & Loh, T. C. (2021). Can Yucca schidigera Be Used to Enhance the Growth Performance, Nutrient Digestibility, Gut Histomorphology, Cecal Microflora, Carcass Characteristic, and Meat Quality of Commercial Broilers Raised under Tropical Conditions? *Animals*, *11*(8), 2276.

Alkhalf, A., Alhaj, M. & Al-Homidan, I. (2010). Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. *Saudi J. Biol. Sci*., 17: 219-225.

Alp, M. Ü. J. D. A. T., Midilli, M., Kocabağlı, N., Yılmaz, H., Turan, N. U. R. İ., Gargılı, A., & Acar, N. Ü. K. E. T. (2012). The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. *Journal of Applied Poultry Research*, *21*(3), 630-636.

Al-Sagan, A. A., Khalil, S., Hussein, E. O., & Attia, Y. A. (2020). Effects of fennel seed powder supplementation on growth performance, carcass characteristics, meat quality, and economic efficiency of broilers under thermoneutral and chronic heat stress conditions. *Animals*, *10*(2), 206.

Altan, Ö. Z. G. E., Pabuçcuoğlu, A., Altan, A., Konyalioğlu, S., & Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science*, *44*(4), 545-550.

Altan, Ö., Altan, A. L. I., Cabuk, M., & Bayraktar, H. (2000). Effects of heat stress on some blood parameters in broilers. *Turkish Journal of Veterinary & Animal Sciences*, *24*(2), 145-148.

Angelier, F., Weimerskirch, H., Barbraud, C., & Chastel, O. (2019). Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Functional Ecology*, *33*(6), 1076-1087.

Anitha, A., Thanseem, I., Vasu, M. M., Viswambharan, V., & Poovathinal, S. A. (2019). Telomeres in neurological disorders. *Advances in Clinical Chemistry*, *90*, 81-132.

Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., & Dabur, R. (2009). Natural products–antifungal agents derived from plants. *Journal of Asian Natural Products Research*, *11*(7), 621-638.

Arora, B., Bhadauria, P., Tripathi, D., & Sharma, A. (2012). Sapindus emarginatus: phytochemistry & various biological activities. *Indo Global Journal of Pharmaceutical Sciences*, *2*(3), 250-257.

Arshad, M. S., Anjum, F. M., Khan, M. I., Saeed, F., Imran, A., Sohaib, M., ... & Hussain, S. (2017). Manipulation of natural antioxidants in feed to enhance the oxidative stability and quality of broiler breast meat and nuggets. *Journal of Food Processing and Preservation*, *41*(1), e12849.

Ashraf, M. F., Abd Aziz, M., Stanslas, J., Ismail, I., & Abdul Kadir, M. (2013). Assessment of antioxidant and cytotoxicity activities of saponin and crude extracts of Chlorophytum borivilianum. *The Scientific World Journal*, *2013*.1-20.

Assumaidaee, A. A. M., & Mustapha, N. M. (2012). Toxicity of signal grass (Brachiaria decumbens): a review article. *Journal of Advanced Medical Research*, *2*(2012), 18-39.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426-436.

Badmus, K. A., Idrus, Z., Meng, G. Y., Sazili, A. Q., & Mamat-Hamidi, K. (2021). Telomere length and regulatory genes as novel stress biomarkers and their diversities in broiler chickens (gallus gallus domesticus) subjected to corticosterone feeding. *Animals*, *11*(10), 2759.

Bafundo, K. W., Männer, K., & Duerr, I. (2021). The combination of quillaja and yucca saponins in broilers: effects on performance, nutrient digestibility and ileal morphometrics. *British Poultry Science*, *62*(4), 589-595.

Barnes, P. J. (2019). Pulmonary diseases and ageing. *Biochemistry and Cell Biology of Ageing: Part II Clinical Science*, 45-74.

Basit, M. A., Kadir, A. A, Loh, T. C., Aziz, S. A., Salleh, A., Kaka, U., & Banke Idris, S. (2020). Effects of inclusion of different doses of Persicaria odorata leaf meal (POLM) in broiler chicken feed on biochemical and haematological blood indicators and liver histomorphological changes. *Animals*, *10*(7), 1209.

Begum, M., Hossain, M. M., & Kim, I. H. (2015). Effects of caprylic acid and Yucca schidigera extract on growth performance, relative organ weight, breast meat quality, haematological characteristics and caecal microbial shedding in mixed sex Ross 308 broiler chickens. *Veterinarni Medicina*, *60*(11).

Benamirouche, K., Baazize-Ammi, D., Hezil, N., Djezzar, R., Niar, A., & Guetarni, D. (2020). Effect of probiotics and Yucca schidigera extract supplementation on broiler meat quality. *Acta Scientiarum. Animal Sciences*, *42*.

Benedict, A. A., Hersh, R. T., & Larson, C. (1963). The temporal synthesis of chicken antibodies: The effect of salt on the precipitin reaction. *The Journal of Immunology*, *91*(6), 795-802.

Bera, I., Tyagi, P. K., Mir, N. A., Begum, J., Dev, K., Tyagi, P. K., ... & Mandal, A. B. (2019). Effect of dietary saponin rich soapnut (Sapindus mukorossi) shell powder on growth performance, immunity, serum biochemistry and gut health of broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, *103*(6), 1800-1809.

Bermudez, A.J., (2003). Principles of Disease Prevention: diagnosis and control. *Disease of Poultry*, 3-60

Bhardwaj, J., Chaudhary, N., Seo, H. J., Kim, M. Y., Shin, T. S., & Kim, J. D. (2014). Immunomodulatory effect of tea saponin in immune T-cells and T-lymphoma cells via regulation of Th1, Th2 immune response and MAPK/ERK2 signaling pathway. *Immunopharmacology and Immunotoxicology*, *36*(3), 202-210.

Botsoglou, N.A., Florou-Paneri, E., Christaki, E., Fletouris, D. J. & Spais B.A. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science*, 43:223-230.

Brum, K. B., Haraguchi, M., Lemos, R. A., Riet-Correa, F., & Fioravanti, M. C. S. (2007). Crystal-associated cholangiopathy in sheep grazing Brachiaria decumbens containing the saponin protodioscin. *Pesquisa Veterinária Brasileira*, *27*, 39-42.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223-253.

Cabuk, M., Alcicek, A., Bozkurt, M., Akkan, S. (2004). Effect of Yucca schidigera and Natural Zeolite on Broiler Performance. *International Journal Poultry Science*, 3(10): 651-654.

Carisch, L., Stirn, M., Hatt, J. M., Federer, K., Hofmann-Lehmann, R., & Riond, B. (2019). White blood cell count in birds: evaluation of a commercially available method. *BMC veterinary research*, *15*(1), 1-7.

Carvalho, I. T., & Santos, L. (2016). Antibiotics in the aquatic environments: a review of the European scenario. *Environment international*, 94, 736-757.

Castro, M. B., Santos Jr, H. L., Mustafa, V. S., Gracindo, C. V., Moscardini, A. C. R., Louvandini, H., ... & Riet-Correa, F. (2011). Brachiaria spp. poisoning in sheep in Brazil: experimental and epidemiological findings. *Poisoning by Plants, Mycotoxins and Related Toxins*, *1*, 110-117.

Chaieb, I. (2010). Saponins as insecticides: A review. *Tunis J Plant Prot*. 5:39-5.

Chakma, S. (2015). Epidemiology of infectious bursal disease in broiler birds of three districts in Bangladesh. *Asian Journal of Medical and Biological Research*, *1*(1), 59-64.

Chand, N., Naz, S., Khan, A., Khan, S., & Khan, R. U. (2014). Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. *International Journal of Biometeorology*, *58*(10), 2153-2157.

Chang, A., et al. "Performance of ross 308 broilers fed different levels of energy and balanced protein under moderate heat stress." *26th Annual Australian Poultry Science Symposium, Sydney New South wales*. 2015.

Chaudhary, S. K., Rokade, J. J., Aderao, G. N., Singh, A., Gopi, M., Mishra, A., & Raje, K. (2018). Saponin in poultry and monogastric animals: A review. *Int. J. Curr. Microbiol. Appl. Sci*, *7*(7), 3218-3225.

Cheeke, P. R. (2000). Actual and potential applications of Yucca schidigera and Quillaja saponaria saponins in human and animal nutrition. In Saponins in food, *Feedstuffs and medicinal plants* (pp. 241-254).

Cheeke, P. R. (2009). Application of saponin as feed additives in poultry production. In: Selle P, editor. Proceedings of 20th Annual Australian Poultry Science Symposium. Sydney (Australia): *World's Poultry Science Association, Australian branch*. p. 50-55.

Cheok, C. Y., Salman, H. A. K., & Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16-40.

Cheville, N. F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken. *The American journal of pathology*, 51 (4), 527

Chuang, W. Y., Liu, C. L., Tsai, C. F., Lin, W. C., Chang, S. C., Shih, H. D., ... & Lee, T. T. (2020). Evaluation of waste mushroom compost as a feed supplement and its effects on the fat metabolism and antioxidant capacity of broilers. *Animals*, *10*(3), 445.

Chung, E. L. T., Alghirani, M. M., Kamalludin, M. H., Nayan, N., Jesse, F. F. A., Wei, O. T. A., ... & Loh, T. C. (2021). Do different vaccination regimes affect the growth performance, immune status, carcase characteristics and meat quality of broilers?. *British poultry science*, *62*(1), 32-37.

Chung, E. L. T., Nayan, N., Kamalludin, M. H., Alghirani, M. M., Jesse, F. F. A., Kassim, N. A., ... & Loh, T. C. (2020). The effects of alkaline water and rainwater on the production and health performance of commercial broilers under tropical conditions. *The Thai Journal of Veterinary Medicine*, *50*(1), 65-73.

Chung, E. L. T., Predith, M., Nobilly, F., Samsudin, A. A., Jesse, F. F. A., & Loh, T. C. (2018). Can treatment of Brachiaria decumbens (signal grass) improve its utilisation in the diet in small ruminants?—a review. *Tropical Animal Health and Production*, 50(8), 1727-1732.

Clavijo, V., & Flórez, M. J. V. (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. *Poultry science*, *97*(3), 1006-1021.

Cook, B. G., Pengelly, B. C., Brown, S. D., Donnelly, J. L., Eagles, D. A., Franco, M. A., ... & Schultze-Kraft, R. (2005). Tropical Forages: an interactive selection tool. *Tropical Forages: an interactive selection tool.*

Cruz, C., Driemeier, D., Pires, V. S., Colodel, E. M., Taketa, A. T., & Schenkel, E. P. (2000). Isolation of steroidal sapogenins implicated in experimentally induced cholangiopathy of sheep grazing Brachiaria decumbens in Brazil. *Veterinary and Human Toxicology*, *42*(3), 142-145.

Cusanelli, E., Romero, C. A. P., & Chartrand, P. (2013). Telomeric noncoding RNA TERRA is induced by telomere shortening to nucleate telomerase molecules at short telomeres. *Molecular cell*, *51*(6), 780-791.

Davinelli, S., Trichopoulou, A., Corbi, G., De Vivo, I., & Scapagnini, G. (2019). The potential nutrigeroprotective role of Mediterranean diet and its functional components on telomere length dynamics. *Ageing research reviews*, *49*, 1-10.

De Oliveira, O. C., De Oliveira, I. P., Alves, B. J. R., Urquiaga, S., & Boddey, R. M. (2004). Chemical and biological indicators of decline/degradation of Brachiaria pastures in the Brazilian Cerrado. *Agriculture, Ecosystems & Environment*, *103*(2), 289-300.

Delezie, E., Swennen, Q., Buyse, J. & Decuypere, E. (2007). The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. *Poultry Science*. 86:1414–1423

Department of Veterinary Services (3 January 2023) Perangkaan ternakan 2020/2021, Retrieved from: https://www.dvs.gov.my/dvs/resources/user_1/2021/BPSPV/Perangkaan%202020.2021/Final_Combine_Buku_Perangkaan_Ternakan_2020_.pdf

Dhama, K., Latheef, S. K., Saminathan, M., Samad, H. A., Karthik, K., Tiwari, R., Khan, R. U., Alagawany, M., Farag, M. R., Gazi, M. A., Laudadio, V., & Tufarelli, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production – A review. *International Journal of Pharmacology* 11: 152-176.

Diarra, M. S., & Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontiers in microbiology*, *5*, 282.

Dinarello, C. A. (2007). Historical insights into cytokines. *European Journal of Immunology*, *37*(S1), S34-S45.

Dong, X. F., Gao, W. W., Tong, J. M., Jia, H. Q., Sa, R. N., & Zhang, Q. (2007). Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. *Poultry science*, *86*(9), 1955-1959.

Engberg, R. M., Hedemann, M. S., Leser, T. D., & Jensen, B. B. (2000). Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poultry Science*, *79*(9), 1311-1319.

Etriwati, D., Ratih, E., Handharyani & Setiyaningsih, S. (2017). Pathology and immunohistochemistry study of Newcastle disease field case in chicken in Indonesia. *Vet. World*, 10:1066–1071.

Factor-Litvak, P., Susser, E., & Aviv, A. (2017). Environmental exposures, telomere length at birth, and disease susceptibility in later life. *JAMA pediatrics*, *171*(12), 1143-1144.

Fallah, R., Kiani, A., & Azarfar, A. (2013). A review of the role of five kinds of alternatives to in-feed antibiotics in broiler production. *Journal of Veterinary Medicine and Animal Health*, *5*(11), 317-321.

Fassbinder-Orth, C. A., & Karasov, W. H. (2006). Effects of feed restriction and realimentation on digestive and immune function in the leghorn chick. *Poultry Science*, *85*(8), 1449-1456.

Feighner, S.D.; Dashkevicz, M.P. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Appl. Environ. Microbiol. 1987, 53, 331–336.

Ferdous, M. F., Arefin, M. S., Rahman, M. M., Ripon, M. M. R., Rashid, M. H., Sultana, M. R., ... & Rafiq, K. (2019). Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. *Journal of Advanced Veterinary and Animal Research*, *6*(3), 409.

Ferket, P. R. (2004). Alternatives to antibiotics in poultry production: responses, practical experience and recommendations. In Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Annual Symposium: re-imagining the feed industry, Lexington, Kentucky, USA, 23-26 May 2004 (pp. 57-67). Alltech UK.

Ferket,P. R., (2011) Alternatives to antibiotics in poultry production: responses, practical experience and recommendations, 65-72.

Fleck, J. D., Schwambach, J., Almeida, M. E., Yendo, A. C., de Costa, F., Gosmann, G., & Fett-Neto, A. G. (2009). Immunoadjuvant saponin production in seedlings and micropropagated plants of Quillaja brasiliensis. *In Vitro Cellular & Developmental Biology-Plant*, *45*(6), 715-720. DOI:10.1016/S0169-409X(98)00013-1.

Fouedjou, R. T., Teponno, R. B., Quassinti, L., Bramucci, M., Petrelli, D., Vitali, L. A., ... & Barboni, L. (2014). Steroidal saponins from the leaves of Cordyline fruticosa (L.) A. Chev. and their cytotoxic and antimicrobial activity. *Phytochemistry Letters*, *7*, 62-68.

Francis, G., Kerem, Z., Makkar, H. P. S. & Becker, K. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition*. 88: 587–605.

Gallo, M., Vitulano, M., Andolfi, A., DellaGreca, M., Conte, E., Ciaravolo, M., & Naviglio, D. (2017). Rapid Solid-Liquid Dynamic Extraction (RSLDE): A new rapid and greener method for extracting two steviol glycosides (stevioside and rebaudioside A) from stevia leaves. *Plant Foods for Human Nutrition*, *72*(2), 141-148.

Garcia-Vaquero, M., Rajauria, G., & Tiwari, B. (2020). Conventional extraction techniques: Solvent extraction. In *Sustainable seaweed technologies* (pp. 171-189). Elsevier.

Gheisar, M. M., & Kim, I. H. (2018). Phytobiotics in poultry and swine nutrition–a review. *Italian Journal of Animal Science*, *17*(1), 92-99.

Glick, B. (1977): The bursa of Fabricius and immunoglobulin synthesis. International Review of Cytology 48, 345–402.

Godstime, C. O., Felix, O. E., Augustina, O. J., & Christopher, O. E. (2014). Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens–a review. *J Pharm Chem Biol Sci*, *2*(2), 77-85.

Gomar, M.S., Driemeier, D., Colodel, E.M. and Gimeno, E.J. (2005). Lectin histochemistry of foam cells in tissues of cattle grazing Brachiaria spp. *Journal of Veterinary Medicine*, 52, 18-21.

González-Recio, O., Gianola, D., Long, N., Weigel, K. A., Rosa, G. J., & Avendano, S. (2008). Nonparametric methods for incorporating genomic information into genetic evaluations: an application to mortality in broilers. *Genetics*, *178*(4), 2305-2313.

Gous, R. M., & Morris, T. R. (2005). Nutritional interventions in alleviating the effects of high temperatures in broiler production. *World's Poultry Science Journal*, *61*(3), 463-475.

Graczyk, S., Pliszczak-Król, A., Kotoński, B., Wilczek, J., & Chmielak, Z. (2003). Examinations of hematological and metabolic changes mechanisms of acute stress in turkeys. *Electron J Pol Agric Univ*, *Volume 6 issue 1. 1-8*

Grashorn, M. A. (2010). Use of phytobiotics in broiler nutrition–an alternative to infeed antibiotics. *Animal Feed Sci Journal*, 19(3), 338-347.

Güçlü-Üstündağ, Ö., & Mazza, G. (2007). Saponins: properties, applications and processing. *Critical reviews in food science and nutrition*, *47*(3), 231-258.

Guenni, O., Marín, D., & Baruch, Z. (2002). Responses to drought of five Brachiaria species. I. Biomass production, leaf growth, root distribution, water use and forage quality. *Plant and soil*, *243*(2), 229-241.

Han, L. K., Xu, B. J., Kimura, Y., Zheng, Y. N., & Okuda, H. (2000). Platycodi radix affects lipid metabolism in mice with high fat diet–induced obesity. *The Journal of nutrition*, *130*(11), 2760-2764.

Hamdy, A. M. (2020). Climatic change and chicken immunity. *Climate Change Impacts on Agriculture and Food Security in Egypt: Land and Water Resources—Smart Farming—Livestock, Fishery, and Aquaculture*, 499-521.

Haraguchi, M. (2003). Furostanol glicosídicos nas folhas de Brachiaria decumbens. Poços de Caldas, MG, 2003. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE QUÍMICA, 26., 2003, Poços de Caldas, MG, Anais... Poços de Caldas, Sociedade Brasileira de Química, 2003, 66.

Hassan, H. M., Samy, A., Youssef, A. W., & Mohamed, M. A. (2018). Research Article Using Different Feed Additives as Alternative to Antibiotic Growth Promoter to Improve Growth Performance and Carcass Traits of Broilers. *International Journal of Poultry Science*, *17*, 255-261.

Hayflick, L. (1997). Mortality and immortality at the cellular level. A review. *Biochemistry-New York-English Translation of Biokhimiya*, *62*(11), 1180-1190.

Heng, M. Y., Tan, S. N., Yong, J. W. H., & Ong, E. S. (2013). Emerging green technologies for the chemical standardization of botanicals and herbal preparations. *TrAC Trends in Analytical Chemistry*, *50*, 1-10.

Hidanah, S., Sabdoningrum, E. K., Wahjuni, R. S., & Chusniati, S. (2018). Effects of meniran (Phyllanthus niruri L.) administration on leukocyte profile of broiler chickens infected with Mycoplasma gallisepticum. *Veterinary World*, *11*(6), 834.

Hirschmann, R., (2021). Poultry consumption per capita in Malaysia from 2006 to 2020, with a forecast for 2025 (in kilograms). 1

Hou, L., Savage, S. A, Blaser, M. J., Perez-Perez, G., Hoxha, M., Dioni, L., Pegoraro, V., Dong, L. M., Zantonski, W., Lissowska, J., Chow, W. H., Baccerelli, A. (2009). Telomere length in pheripheral leucocyte DNA and gastric cancer risk. Cancer Epidemiol Biomarker Prev. 18 (11), 3103-3109.

Hussain, Z., Khan, M. Z., Saleemi, M. K., Khan, A., & Rafique, S. (2016). Clinicopathological effects of prolonged intoxication of aflatoxin B1 in broiler chicken. *Pak Vet J*, *36*(4), 477-481.

Husson, O., Charpentier, H., Razanamparany, C., Moussa, N., Michellon, R., Naudin, K., Razafintsalama, H., Rakotoarinivo, C., Rakotondramanana; Séguy, L. (2008). *Brachiaria* sp., *B. ruziziensis*, *B. brizantha*, *B. decumbens*, *B. humidicola*. CIRAD, Manuel pratique du semis direct à Madagascar, Vol. III, Chap. 3, par. 4.1

Institute of Medical Research [IMR] (2020). *Malaysian One Health Antimicrobial Resistance.* https://www.imr.gov.my/MyOHAR/index.php/site/archive_rpt

Iser, M., Martinez, Y., Ni, H., Jiang, H., Valdivie Navarro, M., Wu, X., ... & Fang, J. (2016). The effects of agave fourcroydes powder as a dietary supplement on growth performance, gut morphology, concentration of IgG, and hematology parameters in broiler rabbits. *BioMed Research International*, *2016*.

Kamboh, A. A., Arain, M. A., Mughal, M. J., Zaman, A., Arain, Z. M., & Soomro, A. H. (2015). Flavonoids: Health promoting phytochemicals for animal production-a review. *J. Anim. Health Prod*, *3*(1), 6-13.

Kamboh, A. A., Khan, M. A., Kaka, U., Awad, E. A., Memon, A. M., Saeed, M., ... & Kumar, C. (2018). Effect of dietary supplementation of phytochemicals on immunity and haematology of growing broiler chickens. *Italian Journal of Animal Science*, *17*(4), 1038-1043.

Kang, S. Y., Ko, Y. H., Moon, Y. S., Sohn, S. H., & Jang, I. S. (2011). Effects of the combined stress induced by stocking density and feed restriction on hematological and cytokine parameters as stress indicators in laying hens. *Asian-Australasian Journal of Animal Sciences*, *24*(3), 414-420.

Keles, H., A. F. Fidan, I. H. Cigerci, I. Kucukkurt, e. Karadas, and Y. Dundar. (2010). Increased DNA damage and oxidative stress in chickens with natural Marek's disease. *Vet. Immunol. Immunopathol*. 133:51–58

Khaskheli, A. A., Khaskheli, M. I., Khaskheli, A. J., & Ali, A. (2020). International Journal of Veterinary Science. Int J Vet Sci, 9(3), 458-461.

Khenenou, T., Bougherara, M., Melizi, M., & Lamraoui, R. (2017). Histomorphological study of the bursae of fabricius of broiler chickens during Gumboro disease in Algeria area. *Glob. Vet*, *18*(2), 132-136.

Kidd, M. T. 2004. Nutritional modulation of immune function in broilers. *Poultry Science*. 83:650–657

Kikusato, M. (2021). Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. *Animal Bioscience*, *34*(3), 345.

Killeen, G. F., Madigan, C. A., Connolly, C. R., Walsh, G. A., Clark, C., Hynes, M. J., ... & Power, R. F. (1998). Antimicrobial saponins of Yucca schidigera and the implications of their in vitro properties for their in vivo impact. *Journal of Agricultural and Food Chemistry*, *46*(8), 3178-3186.

Kim, S. Y., & Velando, A. (2015). Antioxidants safeguard telomeres in bold chicks. *Biology Letters*, *11*(5), 20150211.

Kittipatarin, C., & Khaled, A. R. (2007). Interlinking interleukin-7. *Cytokine*, *39*(1), DOI: 75-83.

Kpomasse, C. C., Oke, O. E., Houndonougbo, F. M., & Tona, K. (2021). Broiler production challenges in the tropics: A review. *Veterinary Medicine and Science*, *7*(3), 831-842.

Krams, I., Vrublevska, J., Cirule, D., Kivleniece, I., Krama, T., Rantala, M. J., ... & Hõrak, P. (2012). Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (Parus major). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *161*(4), 422-428.

Lee, M. H., Lee, S. H., Kim, Y. J., Ko, Y. H., Jang, I. S., Moon, Y. S., ... & Sohn, S. H. (2008). Effect of dietary antioxidant supplementation on telomere length and egg quality in laying hens. *Korean Journal of Poultry Science*, *35*(3), 267-274.

Li, S. P., Zhao, X. J., & Wang, J. Y. (2009). Synergy of Astragalus polysaccharides and probiotics (Lactobacillus and Bacillus cereus) on immunity and intestinal microbiota in chicks. *Poultry Science*, *88*(3), 519-525.

Lian, F., Wang, J., Huang, X., Wu, Y., Cao, Y., Tan, X., ... & Gao, X. (2015). Effect of vegetable consumption on the association between peripheral leucocyte telomere length and hypertension: a case–control study. *BMJ open*, *5*(11), e009305.

Lilliehook, I., H. Wall, R. Tauson, & H. Tvedten. (2008). "Differential leukocyte counts determined in chicken blood using the Cell-Dyn 3500." *Veterinary Clinical Pathology* 33, 133-138.

Lima, F. G. D., Haraguchi, M., Pifster, J. A., Guimarães, V. Y., Andrade, D. F., Ribeiro, C. S., ... & Fioravanti, M. C. S. (2012). Weather and plant age affect the levels of steroidal saponin and Pithomyces chartarum spores in Brachiaria grass. 1-20.

Liu, J. P., Chen, S. M., Cong, Y. S., Nicholls, C., Zhou, S. F., Tao, Z. Z., & Li, H. (2010). Regulation of telomerase activity by apparently opposing elements. *Ageing research reviews*, *9*(3), 245-256.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, *134*(12), 3479S-3485S.

Liu, T. T., Liu, X. T., Chen, Q. X., & Shi, Y. (2020). Lipase inhibitors for obesity: A review. *Biomedicine & Pharmacotherapy*, *128*, 110314.

Liu, Z. Y., Wang, X. L., Ou, S. Q., Hou, D. X., & He, J. H. (2020). Sanguinarine modulate gut microbiome and intestinal morphology to enhance growth performance in broilers. *PloS one*, *15*(6), e0234920.

Lorenzi, H. (2000). Plantas daninhas do Brasil: terrestres, aquaticas, parasitas, toxic e medicinais, theird ed. *Plantarum, Nova Odessa*, 608

Low, S. G. (2015). Signal grass (Brachiaria decumbens) toxicity in grazing ruminants. *Agriculture*, 5(4), 971-990.

Low, C. X., Tan, L. T. H., Ab Mutalib, N. S., Pusparajah, P., Goh, B. H., Chan, K. G., ... & Lee, L. H. (2021). Unveiling the impact of antibiotics and alternative methods for animal husbandry: A review. *Antibiotics*, *10*(5), 578.

Ludlow, A. T., Gratidao, L., Ludlow, L. W., Spangenburg, E. E., & Roth, S. M. (2017). Acute exercise activates p38 MAPK and increases the expression of telomere-protective genes in cardiac muscle. *Experimental physiology*, *102*(4), 397-410.

Ma, X., Bi, S., Wang, Y., Chi, X., & Hu, S. (2019). Combined adjuvant effect of ginseng stem-leaf saponins and selenium on immune responses to a live bivalent vaccine of Newcastle disease virus and infectious bronchitis virus in chickens. *Poultry science*, *98*(9), 3548-3556.

Mack, L. A., Felver-Gant, J. N., Dennis, R. L., & Cheng, H. W. (2013). Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science*, *92*(2), 285-294.

Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). Plant secondary metabolites, *Humana Press* ,Vol. 393, pp. 1-122.

Manjaniq, A., Wihandoyo & Dono, N. D. (2017). The effect of dietary violet Roselle flower and Moringa leaf meal supplementation on blood profile of broiler chickens. The 7th International Seminar on Tropical Animal Production. Contribution of Livestock Production on Food Sovereignty in Tropical Countries. 12-14 September 2017, Yogyakarta Indonesia

Marelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules*, 21(10), 1100-1404.

Martel, J., Ojcius, D. M., Ko, Y. F., & Young, J. D. (2020). Phytochemicals as prebiotics and biological stress inducers. *Trends in biochemical sciences*, *45*(6), 462-471.

Mashaly, M. M., Hendricks 3rd, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O., & Patterson, P. H. (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science*, *83*(6), 889-894.

Matsuura, H. (2001). Saponins in garlic as modifiers of the risk of cardiovascular disease. *The Journal of nutrition*, *131*(3), 1000S-1005S.

Mayor, S. (2016). European drug agency recommends limiting colistin use in animals to cut resistance in patients. *BMJ Journal*. 353.

Mert-Turk, F. (2006) Saponins versus plant fungal pathogens. *J Cell Mol Biol.* 5:13-17.

Miah, M. Y., Rahman, M. S., Islam, M. K., & Monir, M. M. (2004). Effects of saponin and L-carnitine on the performance and reproductive fitness of male broiler. *Int J Poult Sci*, *3*(8), 530-533.

Ministry of Health Malaysia,MOH. (2019). Joint press statement Ministry of Health Malaysia and Ministry of Agriculture and Agro-based Industry: National Antibiotic Awareness Week Campaign 2019. Available online at: https://www.moh.gov.my/index.php/database_stores/attach_download/657/1259

Moghimipour, E., & Handali, S. (2015). Saponin: properties, methods of evaluation and applications. *Annual Research & Review in Biology*, 207-220.

Mohammed, L. S., Sallam, E. A., Edris, S. N., Khalifa, O. A., Soliman, M. M., & Shehata, S. F. (2021). Growth performance, economic efficiency, meat quality, and gene expression in two broiler breeds fed different levels of tomato pomace. *Veterinary Research Communications*, *45*(4), 381-397.

Mondet, J., Hussein, K., & Mossuz, P. (2015). Circulating cytokine levels as markers of inflammation in Philadelphia negative myeloproliferative neoplasms: diagnostic and prognostic interest. *Mediators of Inflammation*, *2015*.

Mountzouris, K. C., Paraskevas, V., & Fegeros, K., (2009). Phytogenic compounds in broiler nutrition. In: T. Steiner (Editor). *Phytogenies in Animal Nutrition*. Nottingham University Press, Nottingham, ISBN 978-1-904761-71-6

Munhoz, L. S., Vargas, G. D. Á., Fischer, G., Lima, M. D., Esteves, P. A., & Hübner, S. D. O. (2014). Avian IgY antibodies: characteristics and applications in immunodiagnostic. *Ciência Rural*, *44*, 153-160.

Muniandy, K. V., Chung, E. L. T., Jaapar, M. S., Hamdan, M. H. M., Salleh, A., & Jesse, F. F. A. (2020). Filling the gap of Brachiaria decumbens (signal grass) research on clinico-pathology and haemato-biochemistry in small ruminants: A review. *Toxicon*, *174*, 26-31.

Murugaiyah, V. (2008). *Phytochemical, Pharmacological and Pharmacokinetic Stuches of Phyllathus niruri Linn, Lignans As Potential Antihyperuricemic Agent* (Doctoral dissertation, Tesis S2, Universiti Sains Malaysia, Malaysia).

Naidoo, V., McGaw, L. J., Bisschop, S. P. R., Duncan, N., & Eloff, J. N. (2008). The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. *Veterinary parasitology*, *153*(3-4), 214-219.

Naji, T. A., Amadou, I., Abbas, S., Zhao, R. Y., Shi, Y. H., & Le, G. W. (2013). Phytosterol supplementation improves antioxidant enzymes status and broiler meat quality. *Pakistan Journal of Food Sciences*, *23*(4), 163-171.

Nakatani, N. (2000). Phenolic antioxidants from herbs and spices. *Biofactors*, *13*(1-4), 141-146.

National Pharmaceutical Regulatory Agency. Ministry of Health Malaysia. List of Registered Veterinary Products. (2017). Available from: http://www.npra.moh.gov.my/images/reg.info/Veterinary/2017/LIST_OF_REGISTERED_VETERINARY_PRODUCTS_2017.pdf .

Ndomou, S. C. H., Djikeng, F. T., Teboukeu, G. B., Doungue, H. T., Foffe, H. A. K., Tiwo, C. T., & Womeni, H. M. (2021). Nutritional value, phytochemical content, and antioxidant activity of three phytobiotic plants from west Cameroon. *Journal of Agriculture and Food Research*, *3*, 100105.

Nguku, S., Musimba, N., Njarui, D., Mwobobia, R., & Kaindi, E. (2016). Effects of Acid Scarification on germination of the genus Brachiaria grass Cultivars. *International J Sci Res Innov Technol*, *3*, 45-50.

Nisha, A. R. (2008). Antibiotic residues-a global health hazard. *Veterinary world*, *1*(12), 375.

Noordin, M.M., Salam Abdullah, A. and Rajion, M.A. (1989). Experimental Brachiaria decumbens toxicity in cattle. *Veterinary Research Communications*, 13, 491–494.

Oakenfull, D. (1981). Saponins in food—a review. *Food chemistry*, *7*(1), 19-40.

OCAKOĞLU, G., & Ercan, I. (2013). Traditional and modern morphometrics. *Turkiye Klinikleri Journal of Biostatistics*, *5*(1).

O'Callaghan, N. J., & Fenech, M. (2011). A quantitative PCR method for measuring absolute telomere length. *Biological procedures online*, *13*(1), 1-10.

Olas, B., Wachowicz, B., Stochmal, A., & Oleszek, W. (2005). Inhibition of blood platelet adhesion and secretion by different phenolics from Yucca schidigera Roezl. bark. *Nutrition*, *21*(2), 199-206.

Orakpoghenor, O., Oladele, S. B., Abdu, P. A., Markus, T. P., Andamin, A. D., & Esievo, K. A. N. (2021). Comparative Pathological Changes Induced by Very Virulent Infectious Bursal Disease Virus Infection in Inoculated, Sentinel Pigeons and Chickens. *Open Veterinary Science*, *2*(1), 55-64.

Osuntokun, O. T., Yusuf-Babatunde, A. M., Ige, O. O., & Odufuwa, A. E. (2016). Phytochemical screening and evaluation of antioxidant and proximate properties of Morinda lucida ethanolic extract. *Journal of Advances in Medical and Pharmaceutical Sciences*, *11*(2), 1-11.

Paganini, F. (2005). Alternatives to drugs in poultry feed and their impact on food safety. In 11th European Symposium on the Quality of Eggs and Egg Products. Doorwerth: Schering-Plough Animal Health Corporation.

Paul, W. E. (2015). History of Interleukin-4. *Cytokine*, *75*(1), 3-7.

Piacente, S., Montoro, P., Oleszek, W., & Pizza, C. (2004). Yucca schidigera Bark: Phenolic Constituents and Antioxidant Activity. *Journal of natural products*, *67*(5), 882-885.

Piacente, S., Pizza, C., & Oleszek, W. (2005). Saponins and phenolics of Yucca schidigera Roezl: Chemistry and bioactivity. *Phytochemistry Reviews*, *4*(2), 177-190.

Procópio, S. O., da Silva, A. A., Ferreira, E. A., da Silva, A. F., & Galon, L. (2015). Weed management. In *Sugarcane* (pp. 133-159). Academic Press.

Qu, Z., Tang, J., Sablani, S. S., Ross, C. F., Sankaran, S., & Shah, D. H. (2021). Quality changes in chicken livers during cooking. *Poultry Science*, *100*(9), 101316.

Rafie, N., Golpour Hamedani, S., Barak, F., Safavi, S. M., & Miraghajani, M. (2017). Dietary patterns, food groups and telomere length: a systematic review of current studies. *European journal of clinical nutrition*, *71*(2), 151-158.

Rajput, Z. I., Hu, S. H., Xiao, C. W., & Arijo, A. G. (2007). Adjuvant effects of saponins on animal immune responses. *Journal of Zhejiang University Science B*, *8*(3), 153-161.

Ramzan, M., Ashraf, M., Hashmi, H. A., Iqbal, Z., & Anjum, A. A. (2015). Evaluation of diclofenac sodium toxicity at different concentrations in relation to time using broiler chicken model. *Journal of Animal & Plant Sciences*, *25*(2).

Rani, R., Golbar, H. M., & Rauf, S. M. A. (2020). Gross anatomy and morphometry of selected visceral organs of broiler chicken at different age groups. *Asian Journal of Medical and Biological Research*, *6*(3), 555-563.

Ranjbar, Z., Shariatmadari, F., & Karimi, T. M. A. (2014). Effect of different levels of yucca extract and antibiotic on growth performance, immune response and blood factors of broiler chickens. *Iran J Med Aromat Plant* 30:675–681.

Rashid, M. H., Xue, C., Islam, M. R., Islam, M. T., & Cao, Y. (2013). A longitudinal study on the incidence of mortality of infectious diseases of commercial layer birds in Bangladesh. *Preventive veterinary medicine*, *109*(3-4), 354-358.

Reichardt, C., & Welton, T. (2010). Solvents and solvent effects in organic chemistry. *John Wiley & Sons*. 73-78

Reichert, S., Bize, P., Arrivé, M., Zahn, S., Massemin, S., & Criscuolo, F. (2014). Experimental increase in telomere length leads to faster feather regeneration. *Experimental Gerontology*, *52*, 36-38.

Richter, T., and C. Proctor. (2007). The role of intracellular peroxide levels on the development and maintenance of telomere-dependent senescence. exp. *Gerontol*. 42:1043–1052

Riet-Correa, B., Castro, M. B., Lemos, R. A., Riet-Correa, G., Mustafa, V., & Riet-Correa, F. (2011). Brachiaria spp. poisoning of ruminants in Brazil. *Pesquisa Veterinária Brasileira*, 31, 183-192.

Rokade, J. J., Bhanja, S. K., Shinde, A. S., Mukesh, K., & Mandal, A. B. (2016). Evaluation of mannan-oligosaccharides (MOS) in broiler chicken during hot humid summer using zoo technical, molecular and physio-biochemical tools. *Indian Journal of Animal Sciences*, *86*(4), 460-467.

Ronquillo, M. G., & Hernandez, J. C. A. (2017). Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. *Food control*, *72*, 255-267.

Sahoo, S. P., Kaur, D., Sethi, A. P. S., Chandrahas, Saini, A.L. and Chandra, M. 2015. Effect of dietary supplementation of Yucca schidigera extract on the performance and litter quality of broilers in winter season. *Animal Nutrition and Feed Technology*. 16: 475-484.

Sarmah, A. K., Meyer, M. T., & Boxall, A. B. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, *65*(5), 725-759.

Sasidharan, S., Jothy, S. L., Vijayarathna, S., Kavitha, N., Oon, C. E., Chen, Y., ... & Kanwar, J. R. (2018). Conventional and non-conventional approach towards the extraction of bioorganic phase. In *Bioorganic Phase in Natural Food: An Overview*, 41-57

Satterlee, D. G., Aquilera-Quintana, I. A., Munn, B. J., Karutmann B.A., 1989. Vitamin C amelioration of the adrenal stress response in broiler chickens being preparedfor slaughter. *Comparative Biochemistry and Physiol-ogy Part A* 94, 569-574.

Scanes, G. C., (2015). Blood. In C. G. Scanes (Ed.), Sturkie's Avian Physisology (Sixth Edition). *London, UK: Academic Press*, 167-185.

Schreiber, G. H., Schreiber, R. D., Angus, W., Thompson, A., & Lotze, M. T. (2003). The cytokine handbook. In *Interferon-g*, 567-602.

Serrano Jr, A. E. (2013). Effects of Quillaja saponins on growth, feed efficiency, digestive enzyme activities and metabolism of common carp (C yprinus carpio L). *Aquaculture Nutrition*, *19*(4), 468-474.

Setha, B., Gaspersz, F. F., Idris, A. P. S., Rahman, S., & Mailoa, M. N. (2013). Potential of seaweed Padina sp. as a source of antioxidant. *International Journal of Scientific and Technology Research*, *2*(6), 221-224.

Shalev, I., Moffitt, T. E., Sugden, K., Williams, B., Houts, R. M., Danese, A., ... & Caspi, A. (2013). Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: a longitudinal study. *Molecular psychiatry*, *18*(5), 576-581.

Sharaf, R., Khan, A., Khan, M. Z., Hussain, I., Abbas, R. Z., Gul, S. T., Mahmood, F., & Saleemi, M. K. (2013). Arsenic induced toxicity in broiler chicks and its amelioration with ascorbic acid: Clinical, hematological and pathological study. *Pak Vet J*, 33(3): 277-281

Shin, D., Shin, J., & Lee, K. W. (2019). Effects of inflammation and depression on telomere length in young adults in the United States. *Journal of Clinical Medicine*, *8*(5), 711.

Simmoson-Boyce, J., Tinto, W. F. (2007). Steroidal saponin and sapogenins from the Agavaceae family. *Nat. Prod. Commun*. 2, 99-114.

Şimşek N, Karadeniz A, Karaca T (2007): Effects of the Spirulina platensis and Panax ginseng oral supplementation on peripheral blood cells in rats. Revue Med Vet, 158, 483-488.

Singh, J., Sethi, A.P.S., Sikka, S.S., Chatli, M.K. and Kumar, P. (2014). Effect of cinnamon (Cinnamomum cassia) powder as a phytobiotic growth promoter in commercial broiler chickens. *Animal Nutrition and Feed Technology*, 14: 471-479.

Singh, M., & T O'Hagan, D. (2003). Recent advances in veterinary vaccine adjuvants. *International journal for parasitology*, *33*(5-6), 469-478.

Skhirtladze, A., Perrone, A., Montoro, P., Benidze, M., Kemertelidze, E., Pizza, C., & Piacente, S. (2011). Steroidal saponins from Yucca gloriosa L. rhizomes: LC–MS profiling, isolation and quantitative determination. *Phytochemistry*, *72*(1), 126-135.

Sohn, S. H., Cho, E. J., Jang, I. S., & Moon, Y. S. (2013). The effects of dietary supplementation of vitamin C and E on the growth performance and the stress response in broiler chickens. *Korean Journal of Poultry Science*, *40*(1), 31-40.

Sohn, S. H., Jang, I. S., Moon, Y. S., Kim, Y. J., Lee, S. H., Ko, Y. H., ... & Kang, H. K. (2008). Effect of dietary siberian ginseng and eucommia on broiler performance, serum biochemical profiles and telomere length. *Korean Journal of Poultry Science*, *35*(3), 283-290.

Sohn, S. H., Subramani, V. K., Moon, Y. S., & Jang, I. S. (2012). Telomeric DNA quantity, DNA damage, and heat shock protein gene expression as physiological stress markers in chickens. *Poultry Science*, *91*(4), 829-836.

Sohn, S. H., & Subramani, V. K. (2014). Dynamics of telomere length in the chicken. *World's Poultry Science Journal*, *70*(4), 721-736.

Sparg, S., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of ethnopharmacology*, *94*(2-3), 219-243.

Su, J. L., Shi, B. L., Zhang, P. F., Sun, D. S., Li, T. Y., & Yan, S. M. (2016). Effects of yucca extract on feed efficiency, immune and antioxidative functions in broilers. *Brazilian Archives of Biology and Technology*, *59*.

Sugiharto, S. (2020). The potentials of two underutilized acidic fruits (Averrhoa bilimbi L. and Phyllanthus acidus L.) as phytobiotics for broiler chickens. *Journal of Advanced Veterinary Research*, *10*(3), 179-185.

Suhaimi, D., Sharif, S., Normah, M. A., Nadia, M. N., & Syahidah, H. W. (2017). Estimating relative feed value of local Brachiaria decumbens. *Malaysian Journal of Veterinary Research*, *8*(2), 78-82.

Sun, Qi, Ling Shi, Jennifer Prescott, Stephanie E. Chiuve, Frank B. Hu, Immaculata De Vivo, Meir J. Stampfer, Paul W. Franks, JoAnn E. Manson, and Kathryn M. Rexrode. "Healthy lifestyle and leukocyte telomere length in US women." *PloS one* 7, no. 5 (2012): e38374.

Takeuchi, T. M., Pereira, C. G., Braga, M. E. M., Maróstica, M. R., Leal, P. F., & Meireles, M. A. A. (2009). Low-pressure solvent extraction (solid–liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants. *Extracting bioactive compounds for food products: theory and applications*, 140-142.

Thaxton, J. P., Dozier, W. A., Branton, S. L, Morgan, G. W., Miles, D. W., Roush, W. B., Lott, B. D., & Vizzier-Thaxton, Y. (2006). Stocking density and physiological adaptive response of broilers. *Poultry Science*. 85:819–824.

Tiainen, A. M., Männistö, S., Blomstedt, P. A., Moltchanova, E., Perälä, M. M., Kaartinen, N. E., ... & Eriksson, J. G. (2012). Leukocyte telomere length and its relation to food and nutrient intake in an elderly population. *European journal of clinical nutrition*, *66*(12), 1290-1294.

Timofeev, N. P. (2021). Phytobiotics in world practice: plant species and active substances, efficiency and limitations, perspectives. *Journal: Agricultural Science Euro-North-East*, (6), 804-825.

Utkualp, N., & Ercan, I. (2015). Anthropometric measurements usage in medical sciences. *BioMed research international*.

Vincken, J. P., Heng, L., de Groot, A., & Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry*, *68*(3), 275-297.

Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in biochemical sciences*, *27*(7), 339-344.

Wald, C.H. R. (2003). Gewurze und Co - eine Ubersicht. *Lohmann Inform*. No. 3,7-11.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B. and Newbold, C.J. 2002. Natural products as manipulators of rumen ferementation. *Asian-Australasian Journal of Animal Sciences*. 15: 1458-1468.

Wallach, M. (2010). Role of antibody in immunity and control of chicken coccidiosis. *Trends in Parasitology*, *26*(8), 382-387.

Wang, Y., Fang, M. Y., Jiang, F., & Peng, H. J. (2004). Effects of arsenic trioxide, ginseng saponin and beta-elemene on telomere-telomerase system in K562 cell line. *Zhongguo shi yan xue ye xue za zhi*, *12*(3), 315-320.

Wang, Y., McAllister, T. A., Yanke, L. J., Xu, Z. J., Cheeke, P. R., & Cheng, K. J. (2000). In vitro effects of steroidal saponins from Yucca schidigera extract on rumen microbial protein synthesis and ruminal fermentation. *Journal of the Science of Food and Agriculture*, *80*(14), 2114-2122.

Wei, A., & Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils. *Journal of agricultural and food chemistry*, *55*(5), 1737-1742.

Windisch W., Rohrer E., Schedle K., (2009). Phytogenic feed additives to young piglets and poultry: mechanisms and application. In: T. Steiner (Editor). Phytogenies in Animal Nutrition. Nottingham University Press, Nottingham,

Yadav, A. S., Kolluri, G., Gopi, M., Karthik, K., & Singh, Y. (2016). Exploring alternatives to antibiotics as health promoting agents in poultry-a review. *J Exp Biol*, *4*(3s), 368-383.

Yannopoulos, D., Zviman, M., Castro, V., Kolandaivelu, A., Ranjan, R., Wilson, R. F., & Halperin, H. R. (2009). Intra–cardiopulmonary resuscitation hypothermia with and without volume loading in an ischemic model of cardiac arrest. *Circulation*, *120*(14), 1426-1435.

Yener, Y., Yalcin, S., & Colpan, I. (2021). Effects of dietary supplementation of red ginseng root powder on performance, immune system, caecal microbial population and some blood parameters in broilers. *ANKARA UNIVERSITESI VETERINER FAKULTESI DERGISI*, *68*(2).

Youssef, I. M., Männer, K., & Zentek, J. (2021). Effect of essential oils or saponins alone or in combination on productive performance, intestinal morphology and digestive enzymes' activity of broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, *105*(1), 99-107.

Yu, J., Chen, Y., Zhai, L., Zhang, L., Xu, Y., Wang, S., & Hu, S. (2015). Antioxidative effect of ginseng stem-leaf saponins on oxidative stress induced by cyclophosphamide in chickens. *Poultry Science*, *94*(5), 927-933.

Yusuf, H. U., & Aliyu-Paiko, M. (2020). Evaluation of cashew nut meal as phytobiotics in diet of broiler chickens and effects on feed efficiency, growth performance, blood metabolic and antioxidant profiles.

Zhai, L., Li, Y., Wang, W., Wang, Y., & Hu, S. (2011). Effect of oral administration of ginseng stem-and-leaf saponins (GSLS) on the immune responses to Newcastle disease vaccine in chickens. *Vaccine*, *29*(31), 5007-5014.

Zhang, Y. N., Wang, J., Qi, B., Wu, S. G., Chen, H. R., Luo, H. Y., ... & Qi, G. H. (2017). Evaluation of mango saponin in broilers: effects on growth performance, carcass characteristics, meat quality and plasma biochemical indices. *Asian-Australasian Journal of Animal Sciences*, *30*(8), 1143.

Ziaie, H., Bashtani, M., Torshizi, M. K., Naeeimipour, H., Farhangfar, H., & Zeinali, A. (2011). Effect of antibiotic and its alternatives on morphometric characteristics, mineral content and bone strength of tibia in Ross broiler chickens. *Global Veterinaria*, *7*(4), 315-322.

Zimmerman, P. H., A. C. Lindberg, S. J. Pope, e. Glen, J. e. Bolhuis, and C. J. Nicol. 2006. The effect of stocking density, flock size and modified management on laying hen behavior and welfare in a non-cage system. *Appl. Anim. Behav. Sci*. 101:111–124.

Zulkifli, I., Abdullah, N., Azrin, N. M., & Ho, Y. W. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. *British poultry science*, *41*(5), 593-597.