EFFECTS OF SUPPLEMENTING SAPONINS FROM *Brachiaria decumbens* Stapf ON BROILER GROWTH AND PRODUCTION PERFORMANCES

By

ALGHIRANI MOHAMED M

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

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DEDICATION

To my late lovely mother

To my late father, Haj Mustafa Alghirani and my late brother Rajab, who were honourable men and had deceased during my Phd study.

To my brothers, my sisters, My Dearest Wife, My Cute Children and my friends especially My Best Friends Faisel Al-Atshan and Basem Ertimi for their care, great source of motivation, inspiration, encouragement and endless support, as well as I would like to dedicate this work to that who taught, motivated and helped me throughout my study My Supervisor Dr. Eric Lim Teik Chung.

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Chairman : Eric Lim Teik Chung, PhD
Faculty : Agriculture

Although using antibiotics as a growth promoter leads to improve the poultry industry, it is also considered as one of contributing factors that affect human health through antimicrobial resistance. For that reason, using in-feed antibiotics as a promoter has been banned in developed countries including Malaysia due to their harmful effects on humans. Hence, finding alternatives of using antibiotics as growth promotion and improving poultry health care has become necessary. Phytobiotics (herbs and their natural plant extracts) are one of the alternatives that have been used as feed additives in the poultry industry due to their several benefits including improving the digestibility and feed intake leading to enhance the growth performance, as well as playing a substantial role in poultrys' health in terms of its antimicrobial activity, immune stimulant, antioxidant, gut microflora manipulation, nutrigenomics effect, anti-stress properties, and cholesterol-lowering effect.

Therefore, this study aims to determine the effect of three different phytobiotics supplementation in enhancing the production performance of commercial broilers reared under tropical environments. A total of 300 day-old male Ross 308 were randomly assigned to six different dietary treatments with 5 replicates consisting of 10 broilers per replicate. The dietary treatments consist of T1: commercial feed without antibiotic (negative control) and T2: commercial feed added with 100mg/kg oxytetracycline (positive control). T3, T4, T5, and T6 were fed with commercial feed supplemented with 25mg/kg, 50mg/kg, 75mg/kg, and 100mg/kg of powdered *Yucca Shidigera* (A) respectively in both starter and finisher diets. Throughout the 42-day feeding trial, body weight and feed intake were recorded weekly for each replicate to calculate the body weight gain and FCR. A total of 10 broilers were randomly selected and slaughtered from each treatment during the starter and finisher phases to measure nutrient digestibility, gut histomorphology, cecal microflora population, carcass characteristics, meat quality, as well as blood biomarkers and biochemistry. The same experimental design was conducted for another two more studies using dried *Brachiaria decumbens* leaves (B) and *B. decumbens* saponins extract (C).

The results showed significant differences ($p<0.05$) in the growth performance, nutrient digestibility, gut histomorphology, cecal microflora, carcass characteristics, meat quality, blood biomarkers and lipid profile in studies A, B, and C during the starter and finisher phases. T6 broilers supplemented with 100 mg/kg of *Y. Schidigera* saponins (A), T3 broilers supplemented with 25 mg/kg of *B. decumbens* (B), and T6 broilers supplemented with 100 mg/kg *B. decumbens* saponins extract (C) showed a superior growth performance with the highest body weight and body weight gain leading to the best FCR. The apparent ileal digestibility of fiber, protein, and ether extract was the highest, while the digestibility of dry matter and ash were the lowest as compared to the other treatments. Longer villi height and shorter crypt depth with different cecal microbial populations were also observed. Besides, T6, T3, and T6 broilers from experiments A, B, and C respectively exhibited the highest carcass values with superior meat quality. However, those same treatments showed lower acute phase proteins, corticosterone, heat shock protein, and serum lipid profile concentrations in contrast to the other broiler treatments.

In conclusion, commercial broilers supplemented with 100 mg/kg of *Y. Schidigera* saponins, 25 mg/kg of *B. decumbens*, and 100 mg/kg *B. decumbens* saponins extract demonstrated better results of growth performance, nutrient digestibility, gut histomorphology, cecal microflora, carcass characteristics, meat quality, and blood parameters under tropical conditions. However, based on the overall findings, 100 mg/kg of *B. decumbens* saponins extract was the best saponins concentration, which can be used as a potential additive to reduce or replace the use of antibiotics in broiler feeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PENAMBAHAN SAPONINS DARI *Brachiaria deceumbens* Stapf TERHADAP PRESTPERTUMBUHAN DAN PRODUKSI AYAM PEDAGING

Oleh

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Penggunaan antibiotik sebagai penggalak pertumbuhan membawa kepada penambahbaikan dalam industri poultri. Namun begitu, ianya juga merupakan salah satu factor penyumbang yang boleh menjejaskan kesihatan manusia melalui rintangan antimikrob. Justeru, penggunaan antibiotik dalam makanan ternakan sebagai penggalak telah diharamkan di negara-negara membangun termasuk Malaysia kerana kesan memudaratkan terhadap manusia. Oleh sebab itu, penemuan alternatif penggunaan antibiotik sebagai penggalak pertumbuhan dan penambahbaikan kesihatan poltri menjadi satu keperluan. Fitobiotik (herba dan ekstrak semulajadi tumbuhan) adalah salah satu alternatif yang telah digunakan sebagai bahan tambahan makanan dalam industri poltri atas beberapa manfaat termasuk meningkatkan kebolehcernaan dan pengambilan makanan yang membawa kepada peningkatan prestasi tumbesaran, serta memainkan peranan penting dalam kesihatan ayam dari segi aktiviti mikrob, perangsang imun, manipulasi mikroflora perut, kesan nitrogenomik, sifat anti-tekanan, dan kesan pengurangan kolesterol.

Oleh itu, kajian ini bertujuan untuk menentukan kesan tiga penambahan fitibiotik berbeza dalam meningkatkan prestasi pengeluaran ayam pedaging komersial yang diternak di bawah persekitaran tropika. Sebanyak 300 anak ayam jantan Ross 308 berumur sehari telah ditempatkan secara rawak kepada 6 rawatan pemakanan yang berbeza dengan 5 replika yang terdiri daripada 10 ekor ayam pedaging setiap replika. Rawatan pemakanan terdiri daripada T1: makanan komersial tanpa antibiotik (kawalan negatif) dan T2: makanan komersial ditambah dengan 100mg/kg oxytetracylin (kawalan positif), T3, T4, T5 dan T6 masing-masing diberi makan makanan komersial ditambah dengan 25mg/kg, 50mg/kg, 75mg/kg dan 100mg/kg *Yucca Shidigera* (A) dalam kedua-dua diet pemula dan penggemuk. Sepanjang 42 hari ujian pemakanan, berat badan dan pengambilan makanan direkod secara mingguan bagi setiap replika untuk mengira kenaikan berat badan dan nisbah penukaran makanan (FCR). Sebanyak 10 ayam pedaging dari setiap rawatan dipilih secara rawak dan disembelih pada fasa pemula dan penggemuk untuk menyukat kebolehcernaan nutrisi, histomorfologi perut, populasi

mikroflora cecal, karakteristik karkas, kualiti daging serta biokimia dan biopenanda darah. Reka bentuk eksperimen yang sama dijalankan untuk dua lagi kajian menggunakan daun *Brachiaria decumbens* kering (B) dan ekstrak saponin *B. decumbens* (C).

Keputusan menunjukkan perbezaan signifikan ($p<0.05$) pada prestasi tumbesaran, kebolehcernaan nutrisi, histomorfologi perut, mikroflora cecal, karakteristik karkas, kualiti daging, biopenanda darah dan profil lipid di kajian A, B dan C semasa fasa pemula dan penyudah. Ayam pedaging T6 ditambah dengan 100mg/kg saponin *Y. Schidigera* (A), ayam pedaging T3 ditambah dengan 25mg/kg *B. decumbens* (B) dan ayam pedaging T6 ditambah dengan 100mg/kg ekstrak saponin *B. decumbens* (C) menunjukkan prestasi tumbesaran yang unggul dengan berat badan dan kenaikan berat bdan tertinggi menjurus kepada FCR terbaik. Kebolehcernaan ileal yang jelas bagi serat, protein dan ekstrak eter adalah yang tertinggi manakala kebolehcernaan bahan kering dan abu adalah yang paling rendah berbanding rawatan yang lain. Vili usus yang lebih tinggi dan kedalaman lurah yang lebih pendek serta populasi mickrob cecal yang berbeza juga ditemukan. Selain itu, ayam pedaging T6, T3 dan T6 dari eksperimen A, B dan C masing-masing menunjukkan nilai karkas yang tertinggi termasuk kualiti daging yang unggul. Tambahan lagi, rawatan yang sama menunjukkan protein, kortikosteron, protein kejutan haba dan kepekatan profil lipid serum fasa akut berbeza dengan rawatan ayam pedaging yang lain.

Kesimpulannya, ayam pedaging komersial dengan penambahan 100mg/kg *Y. Schidigera*, 25mg/kg *B. decumbens* dan 100mg/kg ekstrak saponin *B. decumbens* menunjukkan keputusan prestasi tumbesaran, kebolehcernaan nutrisi, histomorfologi perut, mikroflora cecal, karakteristik karkas, kualiti daging dan parameter darah yang lebih baik di bawah kondisi tropika. Akan tetapi, berdasarkan penemuan keseluruhan, 100mg/kg ekstrak saponin *B. decumbens* merupakan tahap saponin yang terbaik, yang boleh digunakan sebagai bahan tambahan berpotensi untuk mengurangkan atau menggantikan penggunaan antibiotik dalam makanan ayam pedaging.

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LIST OF ABBREVIATIONS

%	percentage
µg	micro gram
µm	*micrometer*
a*	redness
AGP	alpha-1-acid glycoprotein
AID	apparent ileal digestibility
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	one-way analysis of variance
AST	aspartate aminotransferase
ATP	adenosine triphosphate
b*	yellowness
B. decumbens	*brachiaria decumbens*
BW	body weight
BWG	body weight gains
C. crepidioides	*crassocephalum crepidioides*
C. innocuum	*clostridium innocuum*
C. jejuni	*campylobacter jejuni*
C. sporogenes	*clostridium sporogenes*
Ca	calcium
CAL	calibrator
CF	crude fibre
CFU	colony-forming units
ChT	chestnut tannins

Cm	centimeter
CP	ceruloplasmin
CP	crude protein
CRD	completely randomized design
CTRL	control L
d	day
DM	dry matter
E. avium	*enterococcus avium*
E. coli	*escherichia coli*
E. faecalis	*enterococcus faecalis*
EDTA	ethelenedimethyl tetra acetic acid
EE	ether extract
ELISA	enzyme linked immune absorbent assay
F. varium	*fusobacterium varium*
FAO	Food and Agriculture Organization
FCR	feed conversion ratio
Fe	ferrous
FI	feed intake
g	gram
GGT	gamma-glutamyl transferase
GHz	gigahertz
GSLS	ginseng stem-leaf
h	hour
H:L	heterophil lymphocyte ratio
H^+/K^+ ATPase	the gastric hydrogen potassium ATPase

H_2O_2	hydrogen peroxide
H2SO4	sulfuric acid
HDL	high-density lipoprotein cholesterol
HMGCoA	3-hydroxy-3-methylglutaryl coenzyme A
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
HSP	heat shock protein
HSP-70	heat shock protein 70
IACUC	Institutional Animal Care and Use Committee
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
IQ	isoquinoline alkaloids
IU	international unit
kcal	kilo calories
Kg	kilogram
Kg/ha	kilogramme per hectare
L	liliter
L*	lightness
L. acidophilus	*lactobacillus acidophilus*
L. plantarum	*lactobacillus plantarum*
L. javanica	lippia javanica
LD	drip loos
LDL	low-density lipoprotein cholesterol
LE	licorice extract
M	Molar

MDA	Serum Malondialdehyde
ME	metabolizable energy
MeOH	methanol
Mg	magnesium
mg	milligram
mg/L	milligrams per liter
MICs	minimum inhibitory concentrations
min	minutes
mL	milliliter
mm	millimeter
mmol/L	millimoles per liter
MS	mango saponin
MT	metric tons
ND	Newcastle Disease
NH3	ammonia molecule
NLF *E. coli*	non-lactose fermenting *E. coli*.
^{o}C	degrees centigrade
OD	optical density
P	phosphorus
P. multocida	*pasteurella multocida*
POLM	*Persicaria odorata* leaf meal
ppm	parts per million
PSE-like	pale, soft, and exudative
Q. saponaria	*quillaja saponaria*
S. aureus	*staphylococcus aureus*

S. enteritidis	*salmonella enteritidis*
S. typhimurium	*salmonella typhimurium*
SAA	serum amyloid A
SAS	Statistical Analysis System
SEM	standard error of mean
SSP	soapnut shell powder
TAC	total antioxidant capacity
TG	triacylglycerol and triglycerides
TiO_2	titanium dioxide
USDA	United States Department of Agriculture
VLDL	very low-density lipoprotein cholesterol
W1	initial weight
WHC	water holding capacity
Y. schidigera	*yucca schidigera*
Zn	zinc
µl	micro liter
µmol/L	micromoles per liter

CHAPTER 1

INTRODUCTION

1.1 General introduction

Poultry contributes significantly to nutrition and food security, providing protein, essential micro-nutrients, and energy to humans in short production cycles at an inexpensive cost. Thus, chicken meat is considered an essential source of animal protein in human diets due to its nutrient-dense composition, which makes it an integral part of healthy and balanced diets, as well as it is appreciated by many cultural culinary traditions. The poultry industry also contributes to poverty alleviation by assisting household income and market participation (Mottet and Tempio, 2017). Therefore, the poultry industry is rapidly growing particularly in developing countries (Bahri, 2019). Based on statistics, there is over 23 billion poultry around the world (Mottet and Tempio, 2017). Comparatively, the human population is now at 7 billion and growing at a rate of 1.4% yearly, and it is expected to reach 9.7 billion by 2050 (Jenkins *et al.*, 2020). Hence, there is approximately three poultry for each person, which is five times more than five decades ago (FAOSTAT, 2016). This growth has led to an increase in meat and egg production (Barbut, 2016). In 2016, the global poultry meat production was 116 million metric tons (MT), which increased to 120.5 MT in 2017 and 122.5 MT in 2018, representing a 1.6% increase from 2017 to 2018 (Bahri, 2019). From the total, 20 MT of poultry meat are produced by USA, 18 MT by China, and Brazil with EU around 13 MT yearly. The Asia regions will determine the primary growth in the future. According to the OECD/FAO (2014), poultry meat production in South and East Asia would increase to around 1.8 MT annually by 2025. For example, Malaysia today is trending to develop commercial poultry production largely into big enterprises via the vertically integrated system. Its broiler production has increased from 471.56 million birds in 2007 to more than 720.11 million birds in 2013, and its broiler meat production was 1,520,000 tons in 2015 (Baluch *et al.*, 2017). Consequently, Malaysia is self-sufficient in poultry meat production, which is increasing at an exponential rate in line with domestic demand growth predictions (USDA, 2014).

A broiler took around four months to reach a kilogram of body weight in the early 1900s, with a feed conversion ratio (FCR) of 5.0, whereas it now takes fewer than 50 days to reach the body weight of 2.6 kg with an FCR of 1.9 (Boyd, 2001). This advancement has also contributed by the improvement of husbandry practices and the use of pharmaceutical drugs such as antibiotics which reduce the mortality rate to 4% as compared to 20% in the initial 1900s. As a result, all these have assisted the poultry industry to be more cost-effective and inexpensive compared to other meat industries. Antibiotics were discovered in the 1920s and have since been successfully used to treat infections in the poultry industry (Gadde *et al.*, 2017). They are, on the other hand, commonly used in a small amount in poultry diets for growth promotion to enhance feed efficiency and disease prevention, resulting in improved growth performance (Giamarellos-Bourboulis and Scaglione, 2010). The United States Food and Drug Administration approved the use of antibiotics at sub-therapeutic levels in animal feeds in the 1950s (Buchanan *et al.*, 2008). Antibiotics as growth promoters in poultry have significantly increased feed conversion efficiency and growth performance by 3 to 5%, respectively in the poultry industry (Dahiya *et al.*, 2006; Pirgozliev *et al.*, 2008).

Although using antibiotics leads to improving the poultry industry as an important resource for nutrition and food security for humans, it is also considered as one of the contributing factors that affect human health through antimicrobial resistance. The excessive usage of these substances as a growth promoter may cause drug toxicity as well as the development of antibiotic-resistant bacteria (Gheisar and Kim, 2018). The development of antibiotic-resistant pathogens owing to the contamination of antibiotic residues in poultry products such as meat and egg is the biggest threat of the poultry industry on consumer's health (Rahmani and Speer, 2005; Giamarellos-Bourboulis and Scaglione, 2010; World Health Organization, 2012). Several studies confirmed that there is a link between the practice of using sub-dose antibiotics and the development of antimicrobial resistance amongst the gut microflora in poultry (Medeiros *et al.*, 2011; Cosby *et al.*, 2015). Numerous studies have found that farms that use antibiotic growth promoters had more resistant bacteria in the intestinal floras of farm workers and farm animals than farms that do not use antibiotic growth promoters (Marshall and Levy, 2011). Furthermore, there are different views on whether or not antibiotic resistance genes can be transferred from animals to humans (M'ikanatha *et al.*, 2010).

For that reason, using in-feed antibiotics at sub-therapeutic levels in animal nutrition as a promoter has been banned in developed countries due to their harmful effects on humans (Gheisar and Kim, 2018). For instance, the United Kingdom started to ban the use of tetracycline and penicillin in the 1970s. Then, Sweden and Denmark banned the application of all growth-promoting antibiotics in 1986 and 1999, respectively (Buchanan *et al.*, 2008). In January 2006, the European Union banned the use of antibiotics as growth promoters on precautionary grounds, especially in egg and meat-producing animals (Windisch *et al.*, 2008). In addition, in 2005, the United States prohibited the use of enrofloxacin, and then halted the manufacturing of animal drugs to stop the use of antibiotics for growth promotion; instead, it is solely used for treatment (FAO, 2014). Besides, in 2015, the state of California strictly banned the use of medically significant antimicrobials in animal diets either for growth enhancement or preventing diseases (Gheisar and Kim, 2018). Similarly, the Malaysia Animal Feed Act 2009 has stated that antibiotics (group A) and maximum residue limits for the allowed antibiotics (group B) are banned. The Malaysian Department of Veterinary Services established an organic chicken production guideline in 2014, stating that no antibiotics or medicinal substances may be used in the diets to stimulate growth or production (Coller, 2019). In 2019, the Malaysian government banned the use of colistin in animal feed, which is widely used as a growth promoter in the poultry industry, because it is considered the last line of defense for severe infection and is a common life-saving last resort medicine, particularly when patients are infected by superbugs (Coller, 2019). Moving forward, the Department of Veterinary Services Malaysia has banned the use of six antibiotics in animal feed, including erythromycin, enrofloxacin, tetracycline, ceftiofur, tylosin, and fosfomycin, as of August 31st, 2020.

Hence, finding alternatives to utilizing antibiotics for growth promotion and improving poultry health care has become necessary, since removing antibiotics may raise morbidity and mortality while decreasing broiler performance, resulting in increasing chicken production costs (Pirgozliev *et al.*, 2008). According to Buchanan *et al.* (2008), broiler producers could lose up to $12 billion over the next five years if no effective alternatives to antibiotics are identified. Thus, discovering effective and health-safe alternatives to antibiotics has gained attention, particularly in the poultry industry (Baurhoo *et al.*, 2007; Yang *et al.*, 2009). Prebiotics, probiotics, phytobiotics, enzymes, symbiotics, toxin binders, organic minerals, organic acids, oligosaccharides, and other feed additives have all been tried as better alternatives (Hashemi and Davoodi, 2011). These alternatives have been demonstrated to effectively enhance poultry performance while requiring little therapeutic inter vention in veterinary medicine. They do not create deleterious changes to the gut normal flora, absorbed from the gut into edible tissue, cause drug resistance at actual use level or cause a rise in environmental pollution, as well as not toxic to the birds or their human handlers (Yadav *et al.*, 2016).

Phytobiotics or natural plant extracts are one example of growth promoters, that have been used for several purposes in the poultry industry including improving digestibility and feed intake, thus leading to the advancement in growth performance (Windisch and Kroismayr, 2007). This natural plant extract also plays a substantial role in poultrys' health in terms of its antimicrobial activity, immune stimulant, antioxidant, gut microflora manipulation, nutrigenomics effect, anti-stress properties, digestibility enhancer, cholesterol-lowering effect, and also considered as an environmentally friendly pesticide and insecticide (Fallah *et al.*, 2013; Dhama *et al.*, 2014; Gopi *et al.*, 2014; Karangiya *et al.*, 2016).

Approximately 50,000 out of 422,000 types of flowering plants are used for medicinal purposes around the world (El Aziz *et al.*, 2019). For example, Malaysia has an abundant amount of grass such as *Brachiaria* species which have been planted on more than 80% of improved farming pastures with *Brachiaria decumbens* (*signal grass*) as the most favored species in Malaysia (Chung *et al.*, 2018). *B. decumbens* is considered an extremely productive tropical grass, which is common through Malaysia, Vanuatu, Australia, Indonesia, and South America, because of its adaptation to a wider range of soil varieties and environments as well as due to its high nutritive value (Chung *et al.*, 2018). Owing to the aggressive growth habit and high nutritive values, it has been commonly implemented as a pasture for grazing ruminants in the tropics. However, there have been many reports stating that *B. decumbens* can lead to sporadic outbreaks of photosensitivity, general ill-thrift, and deaths in ruminants due to the naturally occurring compound identified as steroidal saponins isolated from leaf and stem fractions of *B. decumbens* (Muniandy *et al.*, 2020).

On the other hand, saponins can be found in at least 400 plant types and forage like legumes, lupins, red clover, soybean, fodder plants, lucerne, and ladino clover (Wina *et al.*, 2005). The commercial saponins that are often used are commonly derived from *Quillaja saponaria* and *Yucca schidigera* (Hassan *et al.*, 2010). Saponins have anti-carcinogenic, anti-microbial, anti-inflammatory, anti-oxidant, hypocholesterolemic, and immunomodulatory effects on poultry, among other biological and pharmacological properties. In addition, saponins have anti-parasitic and anti-fungal effects (Chaudhary *et al.*, 2018). As a result, when compared to synthetic antibiotics, this compound has proven to be natural, residue-free, less toxic, and is thought to be an appropriate growth promoter in animal diets (Hashemi and Davoodi, 2011). In the poultry industry, saponins have been used as feed additives to increase the body weight gain of commercial broiler chicks during all stages of growth with better feed intake and improved carcass quality (Miah *et al.*, 2004).

1.2 Problem statement

The world poultry industry is growing exponentially in line with the increase in the human population. Thus, any disruptions such as poor growth rate and disease outbreaks could potentially harm the supply and demand which will eventually affect the food security of the world. In the poultry industry, using antibiotics as growth promoters has significantly increased feed conversion efficiency and growth performance. Nevertheless, using antibiotics as a growth promoter in the poultry production cycle may also lead to antimicrobial resistance in both poultry and humans. Concerning food safety reasons, developed countries have started to ban the practice of antibiotics in all animal feed. Consequently, it may be necessary to explore other preventive alternatives for disease prevention and to stimulate a fast growth rate in poultry. Therefore, phytobiotics such as commercial saponins have been used as a growth promoter instead of using antibiotics in poultry feed. However, there is a gap of knowledge regarding the possible utilization and the effect of different saponins on the growth performance, digestibility, gut histomorphology, carcass characteristic, meat quality, blood biomarkers, serum lipid profile, and liver function of broilers reared under tropical conditions. Based on the limitation and significance of using saponins in broiler diets, it is necessary to determine the best concentration of saponins especially from local grass such as *B. decumbens* saponins, since no study was conducted before using *B. decumbens* or its saponins extraction as feed additives in broilers despite its high nutritive value and high concentration of saponins, that may improve the feed nutritive value.

1.3 Hypotheses

This study will give a significant improvement in getting a better understanding and much knowledge of the effects of different phytobiotics supplementation as well as their different concentrations on broiler growth performance, digestibility, gut morphology, carcass, meat quality, blood biomarkers, as well as serum biochemistry. As a result, plant extracts (saponins) could be utilized instead of antibiotics as a natural and safe growth promoter.

Ho: There is no significant difference between different saponins extraction, and also no significant difference between low and high saponins levels.

Ha: There is a significant difference between different saponins extraction, and also a significant difference between low and high saponins levels.

1.4 Objectives

1. To measure the nutritive values and digestibility of the starter and finisher diets supplemented with different types and different levels of phytobiotics.

2. To evaluate the impacts of different types and levels of phytobiotics on the growth performance, gut histomorphology, and gut microbial population of commercial broilers.

3. To evaluate and compare the impacts of different types and levels of phytobiotics on the carcass characteristic and meat quality of commercial broilers.

4. To determine the influences of different types and levels of phytobiotics on the acute phase proteins, corticosterone, and heat shock proteins responses, as well as serum lipid profile and liver function of commercial broilers reared under tropical conditions.

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