LOCAL GRASSES AND THEIR PHYTOCOMPOUNDS AS NATURAL ALTERNATIVES TO ANTIBIOTICS FOR IMPROVING GROWTH PERFORMANCE AND HEALTH IN COLOURED BROILERS

By

ONG YEE LYN

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

This thesis is dedicated to my mother and my family, whose unwavering support and encouragement have been my greatest source of strength.

To my supervisor, Assoc. Prof. Dr. Eric Lim Teik Chung, who have guided me with wisdom and patience. To my friends who have always cheered me on and lifted me up during challenging times.

And finally, to my late father, Ong Sing Jew.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Eric Lim Teik Chung, PhD
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Antibiotics are widely used in the broiler industry to promote growth and prevent diseases, potentially leading to antimicrobial resistance in both livestock and consumers. Phytobiotics, including phytocompounds from grasses, may serve as natural alternatives to antibiotics. However, their effects in poultry production remain unexplored in Malaysia. This study assesses the impact of grass meals derived from *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* on the production performance of coloured broilers. Grass samples were collected from Universiti Putra Malaysia's Farm 15, fertilized, and harvested at four weeks old. They were analysed for dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash content. A completely randomized feeding trial was conducted using Sasso broilers fed commercial feed supplemented with different grass meals. Six treatment groups were tested for each grass: (1) negative control, (2) positive control with oxytetracycline (100mg/kg feed), and (3–6) increasing levels of grass meal (1.25g, 2.5g, 3.75g, and 5.0g/kg feed). Growth parameters, including body weight

(BW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR), were recorded weekly.On day 56, twelve broilers per treatment were slaughtered to analyse nutrient digestibility, gut histomorphology, cecal microbiota, carcass characteristics, and meat quality. Blood samples were collected to assess serum lipid profile, acute phase proteins (APP), corticosterone, and heat shock protein HSP70.

Statistical analysis revealed significant differences ($p<0.05$) across various parameters in all experiments. The highest grass meal inclusion (5.0g/kg feed) resulted in improved growth performance, nutrient digestibility, gut morphology, and cecal microbiota. Birds in this group exhibited reduced feed intake but higher BWG, leading to better FCR. Nutrient digestibility showed the highest apparent ileal digestibility for CF, CP, and EE, while DM and ash were the lowest. Gut analysis indicated longer villi height and shorter crypt depth, alongside distinct cecal microbial populations. In carcass characteristics, no significant changes were observed in Experiment A (*P. purpureum*), though numerical improvements were noted. However, Experiments B and C (*B. decumbens* and *M. maximus*) displayed notable changes. All three experiments demonstrated improved meat quality, lower APP, corticosterone, HSP70, and serum lipid levels in the highest grass meal inclusion group.

In conclusion, under tropical conditions, coloured broilers supplemented with 5.0g/kg feed of *P. purpureum*, *B. decumbens*, and *M. maximus* showed enhanced growth performance, nutrient digestibility, gut health, meat quality, and physiological markers. Among the three, *P. purpureum* exhibited the most consistent improvements. These findings suggest that locally available grasses and their phytocompounds have the potential to replace antibiotics as feed additives in poultry production.

Keywords: Pennisetum purpureum, Brachiaria decumbens, Megathyrsus maximus, phytobiotics, coloured-broilers.

SDG: GOAL 2: Zero Hunger, GOAL 3: Good Health and Well-Being, GOAL 12: Responsible Consumption and Production

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

RUMPUT TEMPATAN DAN FITOKOMPAUNNYA SEBAGAI ALTERNATIF SEMULAJADI KEPADA ANTIBIOTIK UNTUK MENINGKATKAN PRESTASI PERTUMBUHAN DAN KESIHATAN DALAM AYAM BERWARNA

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Antibiotik sering digunakan dalam penternakan ayam pedaging untuk pertumbuhan dan pencegahan penyakit, tetapi boleh menyebabkan perintang antibiotik dalam haiwan dan pengguna. Fitobiotik, termasuk fitokomponen daripada rumput, berpotensi sebagai alternatif semula jadi kepada antibiotik. Namun, kesannya dalam industri ayam di Malaysia masih belum dikaji. Kajian ini menilai kesan rumput *Pennisetum purpureum, Brachiaria decumbens,* dan *Megathyrsus maximus* terhadap prestasi ayam kampung kacuk. Rumput yang ditanam di Ladang 15, Universiti Putra Malaysia, dituai pada usia 4 minggu dan dianalisis bagi bahan kering (DM), serat mentah (CF), protein mentah (CP), ekstrak eter (EE), dan abu. Ujian pemakanan secara rawak dilakukan ke atas ayam Sasso yang diberi makanan komersial dengan tambahan rumput *P. purpureum* (Eksperimen A), *B. decumbens* (Eksperimen B), dan *M. maximus* (Eksperimen C). Enam kumpulan rawatan diuji bagi setiap rumput: (1) kawalan negatif,(2) kawalan positif dengan oksitetrasiklin (100mg/kg makanan), dan (3–6) peningkatan tahap aditif rumput (1.25g, 2.5g, 3.75g, dan 5.0g/kg makanan). Prestasi pertumbuhan dinilai berdasarkan berat badan (BW), pengambilan makanan

(FI), pertambahan berat badan (BWG), dan nisbah penukaran makanan (FCR). Pada hari ke-56, dua belas ayam setiap kumpulan disembelih untuk analisis pencernaan nutrien, histomorfologi usus, mikroflora cecal, ciri karkas, dan kualiti daging. Sampel darah dikumpul untuk menilai profil lipid serum, protein fasa akut (APP), kortikosteron, dan HSP70.

Keputusan statistik menunjukkan perbezaan signifikan ($p<0.05$) dalam prestasi pertumbuhan, pencernaan nutrien, histomorfologi usus, flora mikro cecal, karkas (kecuali Eksperimen A), kualiti daging, biomarker darah, dan profil lipid dalam semua eksperimen. Penambahan 5.0g/kg makanan menghasilkan prestasi produksi terbaik, dengan pertumbuhan setara atau lebih baik daripada kawalan positif. Ini disebabkan oleh pengurangan pengambilan makanan dan/atau peningkatan berat badan, meningkatkan FCR. Kecekapan pencernaan ileum tertinggi dicatat bagi CF, CP, dan EE, manakala DM dan abu paling rendah. Ketinggian villi lebih panjang dan kedalaman kripta lebih pendek turut diperhatikan bersama perbezaan mikroflora cecal. Dalam ciri karkas, tiada perubahan signifikan dalam Eksperimen A, tetapi terdapat kesan numerikal positif. Eksperimen B dan C menunjukkan perubahan ketara. Semua eksperimen melaporkan kualiti daging lebih baik dalam kumpulan 5.0g/kg makanan, serta pengurangan APP, kortikosteron, HSP70, dan profil lipid serum berbanding rawatan lain.

Kesimpulannya, di bawah keadaan tropika, ayam kacukan kampung yang diberi 5.0g *P. purpureum*, *B. decumbens*, dan *M. maximus* setiap kg makanan menunjukkan peningkatan prestasi pertumbuhan, pencernaan nutrien, kesihatan usus, mikroflora cecal, karkas, kualiti daging, dan parameter darah. *P. purpureum* memberikan hasil

paling konsisten, menjadikannya pilihan terbaik. Kajian ini mencadangkan bahawa rumput tempatan dan fitokomponennya berpotensi sebagai alternatif kepada antibiotik dalam industri ayam.

Kata Kunci: Pennisetum purpureum, Brachiaria decumbens, Megathyrsus maximus, phytobiotics, coloured-broilers.

SDG: MATLAMAT 2: Kelaparan Sifar, MATLAMAT 3: Kesihatan Baik dan Kesejahteraan, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawap

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%	percentage
µg	micro gram
µm	*micrometer*
a*	redness
A. anguistissima	*Acacia anguistissima*
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 gain
C. jejuni	*Campylobacter jejuni*
Ca	calcium
CAL	calibrator
CD	crypt depth
CF	crude fibre
CFU	colony-forming units
ChT	chestnut tannins
cm	centimeter
CP	ceruloplasmin

CP	crude protein
CRD	completely randomized design
d	day
DM	dry matter
DVS	Department of Veterinary Services
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
FAO	Food and Agriculture Organisation
FCR	feed conversion ratio
Fe	ferrous
FI	feed intake
FOA	Federation of Agriculture
g	gram
GGT	gamma-glutamyl transferase
GHz	gigahertz
h	hour
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
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
IU	international unit
kcal	kilo calories
Kg	kilogram
Kg/ha	kilogramme per hectare
L	litre
L*	lightness
L. javanica	*lippia javanica*
LD	drip loss
LDL	low-density lipoprotein cholesterol
M	Molar
M. maximus	*Megathyrsus maximus*
M. oleifera	*Moringa oleifera*
MDA	serum malondialdehyde
ME	metabolisable energy
MeOH	methanol
Mg	magnesium
mg	milligram
mg/L	milligrams per liter
min	minutes
mL	milliliter
mm	millimeter

mmol/L	millimoles per liter
MOLM	*Moringa oleifera* leaf meal
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. betle	*Piper betle*
P. guajava	*Psidium guajava*
P. multocida	*pasteurella multocida*
P. odorata	*Persicaria odorata*
P. purpureum	*Pennisetum purpureum*
POLM	*Persicaria odorata* leaf meal
ppm	parts per million
PSE	pale, soft, and exudative
Q. saponaria	*quillaja saponaria*
S. aureus	*staphylococcus aureus*
S. enteritidis	*salmonella enteritidis*
S. typhimurium	*salmonella typhimurium*
SAA	serum amyloid A
SEM	standard error of mean
SSP	soapnut shell powder
T. catappa	*Terminalia catappa*
TAC	total antioxidant capacity

TG	triacylglycerol and triglycerides
TiO_2	titanium dioxide
USDA	United States Department of Agriculture
VH	villi height
VLDL	very low-density lipoprotein cholesterol
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

The Malaysian livestock industry is an ever-growing industry that is important as it contributes to the economy of the country and is a source of protein for the nation. The expanding population growth of Malaysia and urbanisation are some of the factors that would further influence the growth of the livestock sector in order to provide for market demand. In the year 2020, the total population of livestock in Malaysia was 312,850,896 with chickens representing the largest livestock population in Malaysia comprising of 95.95%, followed by ducks (3.08%), swine (0.6%), cattle (0.21%), goats (0.10%), sheep (0.04%), and buffaloes (0.03%) (Zayadi, 2021). Despite pork being the most highly consumed meat globally (FAO, 2018), due to religious and cultural restrictions in Malaysia, poultry meat reigns as the top source of protein consumed in our country. Poultry meat ranks in as the highest per capita consumption of 47.4 kilograms per year in comparison to pork, beef and mutton at 17.5 kg, 5.6 kg and 1.3 kg respectively (Department of Statistics Malaysia, 2021). This is largely attributed to poultry meat being the cheapest form of meat available as poultry production in Malaysia continues to progress into an integrated and commercialised system that is cost-effective.

There are two types of chicken breeds that are supplied into the Malaysian market demand for chicken meat which are high performance breeds and the low performance breeds (Lokman et al., 2011). Examples of high-performance breeds available in the Malaysian market are Cobb and Ross, which are products of selective breeding.

Intensive selective breeding over the decades has resulted in modern strains of broiler chickens that are highly efficient with better genetic potential (Zuidhof et al., 2014). On the other hand, coloured-broilers or village chickens (also known as Ayam Kampung) fall under the category of low performance breeds as they are a slow growing type of chickens. While commercial broilers' meat has been concluded as being much more tender than the slow growing chickens, there is a niche group of consumers who continue to favour the chicken meat of these slow growing chickens as they believe that it is more nutritious and tastes better (Aini, 1990; Lokman et al., 2015). The Department of Veterinary Services Malaysia (DVS) reported that the retail price of a live commercial broiler in September 2021 was RM7.80 per kilogram whereas a village chicken was sold at RM15.10 per kilogram (Department of Veterinary Services Malaysia, 2021). Therefore, this market demand allows for the continuous production of these indigenous breeds as they fetch a premium price for broiler meat.

Like most South East Asian countries, poultry keeping in Malaysia started as a backyard operation among rural families whereby village chicken would be raised as a free-range chicken where the chickens are allowed to scavenge for food mainly comprising of fallen grains, insects, worms, kitchen scraps, and local grasses or weeds. The practise of keeping these chickens under the backyard farming system is still prevalent in today's culture especially in the rural areas. As the market demand for these indigenous breeds continue rise, there is a move towards a semi-intensive farming system whereby these chickens have an enclosed area for shelter whilst being provided with feed and a scratch area. Due to the nature of these chickens, one would rarely find an operation raising them in an intensive system such as a deep-litter or

caged system. These village chickens (*Gallus domesticus)* are said to be a descendent of the red jungle fowl (*Gallus gallus*) after evolving from random and indiscriminate crossbreeding between the original Malay fowl, the jungle fowl, and exotic commercial breeds (Nematbaksh et al., 2021). In Malaysia, these chickens are reared for dual-purposes which are for both meat and eggs. They are usually marketed at 15-20 weeks with a live weight of about 1.0-1.5kg (Tan et al., 2021). The great variability in production could be attributed to the different varieties of village chicken and the level of nutrition received (Ramlah, 1999). Furthermore, those chickens raised semi-intensively produced consistently lower results as compared to those raised in intensive systems (Ramlah, 1996). In contrast, coloured broilers like the Sasso breed are marketed at approximately 8-10 weeks at approximately 1.5kg for good fleshing and processing yield (Hendrix Genetics, 2022). DVS claims that there are 1015 farms in the whole of Malaysia providing village chickens; however, this number is mixed with coloured broilers. Consequently, some of these farms may be supplying coloured broilers instead of village chickens as there are no standard protocols to differentiate between the two (Nematbaksh et al., 2021). The variation of performances and indiscrimination between village chickens and coloured broilers shows that there is much room for improvement in the classification, nutrition, and health of these slow growing chickens that should be studied as rural farmers and the industry will benefit from it due to the increasing demand and consumer awareness towards organically grown chicken.

While the self-sufficiency ratio of broiler meat in Malaysia is 98.2% (Department of Statistics Malaysia, 2021), there still are prevalent issues surrounding the broiler industry such as production sustainability, accessibility and utilisation as well as food

safety (Shamsudin, 2013). The margin between the cost of production and sales is quite small which can be largely attributed to the high cost of feed (Rahman, 2003; Elsedig et al., 2015). This is because feeding plays an important role in broiler production as it covers 70% of the overall production cost. According to the Malaysian Feed Millers Association, Malaysia imports approximately 85% of feed ingredients which comprises of cereal grains, protein meals, fats and oils, and so on, while the other 15% is locally sourced (rice bran, crude palm oil, and palm kernel cake). 100% of the corn used in poultry feed are imported primarily from Argentina and Brazil and these prices are based on the Chicago Board of Trade (CBOT) Corn Future Prices (Malaysian Feed Millers Association, 2017). The heavy reliance on the imported feed materials creates a threat especially to small scale farmers having a smaller capital. Thus, multiple researchers (Elsedig et al., 2015; Abdurofi et al., 2017) have cited that in order for the local broiler industry to remain competitive internationally, the industry cannot rely on the importation of feed ingredients and must move towards a formulation that utilises local ingredients.

Globally, there has been efforts by researchers and industrial partners to find cheaper alternatives with the aim of supplementing or replacing feed materials in the poultry diet as conventional feed continues to increase in price (Paul et al., 2011). Several local alternatives have been proposed to be used in poultry feed ranging from plant-based materials such as broken rice, palm kernel cake, and sorghum to insects like the black soldier fly larvae and so on (Zahari & Wong, 2009; Rahmat, 2022). Yet, an issue with these alternative ingredients are the limitations of use due to inadequate supply for commercial feed production, deficiencies in nutrients or the presence of anti-nutritional factors (Koeleman, 2013). One particular alternative that may be a

promising alternative to be used in Malaysia is forage or plant derivatives as several advantages of forage consumption by poultry has been mentioned by Spencer and Tufarelli et al. in 2013 and 2018 respectively. Forages which are commonly utilised in the ruminant sector, such as Napier grass, Signal grass, and Guinea grass, could also play a pivotal role in poultry feed production. These grasses, widely cultivated in Malaysia, not only provide a cost-effective source of nutrients but also contribute to significant monetary savings for farmers. Secondary compounds found in forages could be a source of anti-nutrients or bring about nutritional benefits upon feeding value (Martens et al., 2012). Moreover, these secondary plant metabolites can be considered phytobiotics that may positively impact poultry performance. Thus, if the poultry sector could begin to utilise forages in feed production, this could open up another source of revenue for pasture farmers in Malaysia.

Generally, the use of antibiotics in livestock production includes treatment when ill (therapeutic), treatment of a group of animals when at least one is diagnosed with clinical infection (metaphylaxis) and preventative treatment (prophylaxis) (Bousquet-Melou et al., 2010). In the poultry sector, antibiotics are also used as a growth promoter whereby a small amount is administered in comparison to therapeutic use. However, this may cause bacteria to develop resistance to antibiotics (Selaledi et al., 2020). In light of calls by the World Health Organisation (WHO) to reduce the usage of antibiotics in food animal production, Malaysia has joined several other countries that bans the use of colistin in animal feed (Mcdougal, 2018). On top of that, in 2020, the Department of Veterinary Services Malaysia has also banned an additional six antibiotics (erythromycin, enrofloxacin, tetracycline, ceftiofur, tylosin, and fosfomycin) from being used on livestock in efforts to ensure animal health and

safeguard the consumer's well-being (TheSun, 2020). All these efforts to reduce the usage of antibiotics are essential to mitigate the increasing rates of antibiotic resistance that poses a global health threat (Frieri et al., 2017). Antibiotic bans in the livestock industry in Malaysia pushes researchers and industrial partners alike to move towards alternatives such as phytobiotics. Despite multiple researches on the possibility of phytobiotics as in alternative to replace antibiotics in feed additives, the information on the usage of phytocompounds in forages found in Malaysia for poultry use is still scarce (Grashorn, 2010; Gheisar and Kim, 2018; Alghirani et al., 2021). Hence, there is a need to investigate the local alternatives that can be incorporated into poultry diets to meet the growing demands for antibiotic-free meat consumptionwhile effectively preventing antibiotic resistance.

In Malaysia, it was found that 82.1% of farmers used free range system for the rearing of village chickens, followed by the semi-intensive system (15.4%) and the intensive system (1%). In a free range or semi-intensive farming system, broiler chickens have access to the outdoors where they can scratch and scavenge for most of their diet from the farm's pasture, backyard or field. As such, a majority of these chickens' diet would comprise of insects, seeds, leaves, and forages found with the occasional supplementation of grains or scraps from the table and kitchen (Ramlah, 1996). Over the years as the poultry industry continues to expand and with the rising cost of commercial feed, farmers and researchers continue to look for the best alternative feed that could be incorporated into the poultry's diet. In the tropics, locally grown forages and legumes may offer a potential solution or benefit to the poultry industry especially smallholders due to their availability and generally low production cost. While forages are more commonly utilised in the ruminant industry, forages such as *Pennisetum*

purpureum, *Brachiaria decumbens*, and *Megathyrsus maximus* that are locally available in Malaysia could be incorporated in the poultry diet as they contain a variety of secondary compounds. Secondary compounds or phytocompounds commonly found in plants can be grouped into polyphenols, alkaloids, cyanogenic glycosides, steroids, saponins, toxic proteins and amino acids, non-protein amino acids, triterpenes, phytohemagglutinins, and oxalic acid (Waghorn and McNabb, 2003). These secondary compounds could either exert both adverse or beneficial effects upon the forage feeding value (Martens et al., 2012). As such, the major phytocompound found in *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* are flavonoids, saponins, and alkaloids respectively (Okaraonye and Ikewuchi, 2009; Low, 2015; Jack et al., 2020; Abu Hafsa and Hassan, 2021)

In Asia, grasses are well-utilised in traditional medicine as they exhibit pharmocological and phytochemical properties (Gebashe et al., 2019). Several studies have shown that plant phytocompounds have antimicrobial effects when supplemented into poultry feed (Alghirani et al., 2021). The most important useful phytochemicals with antimicrobial activities can be divided into several categories, such as, terpenoids/essential oils, phenolics/polyphenols, alkaloids, and lectins/polypeptides (Lillehoj et al., 2018). Yang et al. (2015) suggested that these phytogenic feed additives vary in antimicrobial action based on the location of the functional hydroxyl or akyl groups. Saponins that are plant glycosides have been claimed to suppress growth, feed consumption, and egg production if used in poultry diets as saponin-protein complexes formed would cause reduction in protein digestibility (Attia-Ismail, 2015). On the other hand, it has the potential in favouring higher growth rate, better feed efficiency, lower serum cholesterol level and reducing

ammonia emission from animals' excreta when supplied at the optimum level in the diet. This is because saponins have shown the assistance in absorption of nutrients by increasing the permeability of the small intestinal mucosa (Miah et al., 2004). In addition to that, as saponins play a key role in the plant's defense system, saponin-containing plants and their extracts have also indicated antimicrobial activity (Wallace, 2004; Jin et al., 2017; Kołodziej et al., 2019; Zaynab et al., 2021). On the other hand, flavonoids are polyphenolic compounds that exhibit antimicrobial, anti-inflammatory, and antioxidant activities (Gebashe et al., 2019). They have been known to enhance the gut morphology and functionality, mucosal and cellular immunity, immune organs size and amelioration of heat stress (Kamboh et al., 2015). Thus, flavonoids as a purified molecule or those obtained from plant extracts could be potential options to improve the production of farm animals. In a review by Parthasarathy et al. (2021), it is claimed that plant defense molecules are one of the most promising drug candidates to combat current and emerging infections and diseases. Furthermore, alkaloids have been reported to bring about dynamic biological activity in both humans and animal body and possess antimicrobial activity (Debnath et al., 2018; Acet et al., 2021). A study by Quinteros et al. (2021) demonstrated that isoquinoline alkaloids granted some protection of layer hens from *Campylobacter hepaticus* (spotty liver disease). The same study also demonstrated an increase in feed consumption and body weight of the treated hens. In addition to that, alkaloids supplemented in poultry and swine diets increased their feed intake and weight gain, improve growth performance, and produce changes in their innate immune response (Ni et al., 2016; Kikusato et al., 2021). Henceforth, plants and their phytocompounds could be of great use to the animal industry as a feed additive.

1.2 Problem Statement

1. The rising cost of commercial feed and consumer awareness on antibiotic resistance has driven the movement in the search of organic alternative feed ingredients such as phytobiotics, but there are mixed reviews and outcomes on the usage of plant phytocompunds.

2. *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* are common local grasses used in the ruminant industry but is underutilised in the poultry sector.

3. Information on the usage of local grasses as a feed additive and its effects on the poultry productivity has yet to be explored, therefore poultry farmers are unable to utilise these grasses effectively in replacement of antibiotics.

4. In addition, information on the gut histomorphology, microbial population and blood profiles are not available, which could be used as an indicator for the benefits of utilising *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* as a feed additive.

1.3 Hypotheses

It is hypothesised that there will be different responses in coloured-broilers supplemented with various levels of forages compared to the control group.

1. Coloured-broilers supplemented with different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals will show different patterns of production performances.

2. Coloured-broilers supplemented with different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals will show differences in the blood profiles and health performance indicators.

1.4 Objectives

The general aim of this study was to investigate the potential of phytocompounds found in local grasses on the production and health performances of coloured-broilers. The specific objectives were:

1. To determine the nutritional content of starter and finisher diets supplemented with different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals, and their digestibility in Sasso broilers.

2. To study and compare the growth performance, gut histomorphology, and gut microbial population of Sasso broilers supplemented with different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals.

3. To evaluate the carcass traits, meat quality, and serum lipid profile of Sasso broilers supplemented with different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals.

4. To measure and compare the different levels of *Pennisetum purpureum*, *Brachiaria decumbens*, and *Megathyrsus maximus* leaf meals supplementation on the acute phase proteins, corticosterone as well as heat shock proteins responses of Sasso broilers.

CHAPTER 2

LITERATURE REVIEW

2.1 Malaysian Poultry Industry

On a global scale, the poultry industry is one of the fastest growing agricultural sectors and has gained significance in order to fulfil the increasing demands of poultry meat and eggs (Wasti et al., 2020; Kamaruzaman et al., 2022). In 2020, according to the Food and Agriculture Organization (FAO), the world poultry meat production recorded was 133 million tonnes and poultry meat represented 40% of global meat production. Poultry meat is rich in protein, vitamins, minerals and contains a low amount of saturated fatty acids (Wasti et al., 2020). Despite red meat being the best source of bioavailable iron with a high content of macro-nutrients, theq preference to poultry meat could be related to nutritional properties, competitive price of chicken meat compared to red meat and increased prevalence of diseases associated with the consumption of red meat (Akramzadeh et al., 2020). Consequently, the consumption of poultry meat has doubled in the past decade and is expected to continue to double by 2050 (Wasti et al., 2020). To meet the increasing demand for poultry meat, significant advancements have been made in chicken genetics over the decades. Through years of commercial intensive selective breeding, broiler chickens which used to weigh around 905g at 56 days old in 1957 achieved a weight of approximately 4202g in 2005 at the same age; showing an increase of 400% in growth rates with simultaneous 50% reduction in feed conversion ratio (FCR). Modern strains of broiler chickens are highly efficient as studies show genetic selection accounted for 85 to 90% of the body weight increase from 1957 to 1991 (Zuidhof et al., 2014). Besides genetics, application of novel and innovative technologies at the farm from rearing up to the

processing stage has played a key role for the vast growth of the poultry industry in order to keep up with the growing market demand of poultry meat (Chowdhury and Morey, 2019).

In Malaysia, the livestock industry plays an important and fundamental role that contributes to the socioeconomic sector of the country as it provides lucrative employment in addition to supplying the country with domestic demands of meat, eggs, and dairy products to the population. The livestock industry plays a vital role in the development of the agriculture sector as it continues to contribute to the gross domestic product (GDP) in a rising trend; increasing from 9.1% (2006), 12.4% (2013) to 14.9% (2019) (Benalywa et al., 2019; Amna et al., 2020; Ferlito, 2020). In the year 2020, the total population of livestock in Malaysia was 312,850,896 with chicken representing the largest livestock population in Malaysia comprising of 95.95%, followed by duck (3.08%), swine (0.6%), cattle (0.21%), goat (0.10%), sheep (0.04%), and buffalo (0.03%) (Zayadi, 2021). Correspondingly, the poultry sub-sector is a major contributor to the livestock GDP at a rate of 62.9% in comparison to the ruminant sub-sector at 12.1% (Ibrahim et al., 2021). In addition, Malaysia is one of the highest poultry consumers in the world with 47.4 kilograms per year in comparison to pork, beef and mutton at 17.5 kg, 5.6 kg and 1.3 kg respectively (Department of Statistics Malaysia, 2022; Selamat et al., 2022). This may be attributed to both urban and rural citizens majorly consume poultry meat as it is the cheapest form of meat protein in contrast to other sources such as beef and pork, coupled with the lack of religious and cultural restrictions surrounding chicken meat (Benalywa et al., 2019). Based on data obtained from the Department of Statistics Malaysia, chicken meat was able to have a SSR of more than 100% from the years 2017 (101.3%) till 2020

(101.7%). However, the SSR in 2021 for chicken meat dropped to 99.9%. A lower SSR score indicates that imports of animal products are required to meet the country's need. This is apparent from the fact that the Import Dependency Ratio (IDR) of chicken meat climbed to 6.1% in 2021, from 4.3% in 2020 due to the increased demand of chicken cuts.

Within a relatively short period, the broiler industry in Malaysia has evolved from a backyard subsistence level to a highly commercialised and efficient production system with 'integrators' that controls all activities. These activities such as feed manufacturing, breeding, hatchery, broiler farming and meat processing are all integrated into a system known as the vertical integration system (Ferlito, 2020). This development has led to a steady increase in production output and rapid business growth over the years, outpacing other livestock sectors. It is crucial for the nation as it ensures food security, promotes self-sufficiency, and reduces dependency on imports. Generally, there are two types of chicken breeds that are supplied into the Malaysian poultry market to meet the market demands of chicken meat. These can be categorised into the high-performance breeds and low performance breeds (Lokman et al., 2011). Low performance breeds such as the slow-growing coloured broilers that were developed from indigenous breeds have a higher demand among consumers that are more health conscious; as they believe that it is of better meat quality and tastes better (Aini, 1990; Lokman et al., 2015; Patbandha et al., 2017). The coloured broilers' meat has less fat, tastes well and performs better in tropical climates than the fast-growing broilers (Patbandha et al., 2017). Hence, a niche group of consumers that continue to favour the slow growing chickens allow the continuous production as they fetch a premium price in comparison to broiler meat. Whilst there is a continuing need

to supply the growing demand for chicken meat, sustainable meat production is required to offer safe and high-quality meat for human consumption. However, poultry producers and consumers are challenged with the threat of antibiotic resistance as the animal industry relies on antibiotics to treat diseases.

2.1.1 Antibiotic Usage in Malaysia

In livestock production, antibiotics are used for treatment when ill (therapeutic), treatment of a group of animals when at least one is diagnosed with clinical infection (metaphylaxis) and preventative treatment (prophylaxis) (Bousquet-Melou et al., 2010). In the poultry sector, in addition to vaccines, antimicrobials are used as growth promoters and for the treatment and prevention of infectious diseases in livestock (Ibrahim et al., 2021). Antibiotic growth promoter (AGP) is described as any medicine that is administered at a low, sub-therapeutic dose with the function of destroying or inhibiting bacteria (Hughes and Heritage, 2004). While there were early indications of beneficial effects of AGPs on the production efficiency in poultry and swine, the first reports of resistance in food animals were made in 1951 by Starr and Reynolds. Sub-therapeutic use in animal feeds have been associated with the increased rates of antimicrobial resistance (AMR) among pathogens isolated from animals as evidence showed that antibiotic resistance genes can be and are transmitted from animal to human microbiota (Dibner and Richards, 2005). AMR is a global health security concern as the persistence and emergence of AMR bacterial communities threaten the treatment options of microbial infections to both humans and animal health. On top of that, antimicrobial compounds used in poultry operations accumulate and are biomagnified through the food chain (Elmi et al., 2021). As a result, human health can be affected directly through antibiotic residues in meat, which may cause side effects,

or indirectly, through the selection of antibiotic resistance determinants that may spread to a human pathogen or zoonotic bacteria.

The consequences of selecting for resistance can range from prolonged illness and side effects from alternative treatments to death following complete treatment failure (Hughes and Heritage, 2004). Modern medicine has equipped humans with a wide array of antibiotics. Then again, as Elmi et al. (2021) has observed, *Eischerichia coli* isolates from poultry and environmental swabs across participating states along the east coast of Peninsular Malaysia have shown resistance against 12 panel antimicrobials with tetracycline having the highest prevalence (91.2%). Four types of bacteria most commonly associated with resistance due to antibiotic use are *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococci*, as these bacteria are most likely to be transmitted frequently from animals to humans (Hughes and Heritage, 2004). Another study by Ibrahim et al. (2021) on the prevalence of antimicrobial resistance of *Salmonella* spp. and *E. coli* isolated from broiler chickens on the East Coast of Peninsular Malaysia further confirmed the high prevalence of AMR in both pathogens. The excessive use of antimicrobials by poultry farmers could have attributed to these findings and suggest a concern over the misuse of veterinary antimicrobials that may further contribute to the threat of emergence of multi-drug-resistant pathogen isolates. For that reason, enhanced control measures, public health interventions and a monitoring system should be implemented to limit the emergence of antimicrobial resistance in local poultry farms.

To date, the Department of Veterinary Services (DVS) in Malaysia has prohibited the use of seven antibiotics in livestock due to high resistance rates in order to guarantee

animal health and consumers' safety (DVS, 2020). These banned antibiotics include colistin, erythromycin, enrofloxacin, tetracycline, ceftiofur, tylosin, and fosfomycin. Thus, this further accelerates the need to identify substitutes and alternatives for antimicrobials to prevent AMR and antibiotic residue in the poultry industry without compromising the performance of broiler chickens. Feed additives are non-nutritive compounds that are added to the baseline feed as an additional component in order to alter the feed presentation, digestibility, as well as better the animal's general health and production (Paraskeuas et al., 2017; Mnisi et al., 2023). With increasing pressure on the livestock sector to find alternatives, research and studies on natural feed additives have intensified in recent years (Abang et al., 2023). Common natural feed additives that could be used as an antibiotic alternative are phytogenic groups which include essential oils (Reis et al., 2018; Coles et al., 2023), enzymes (Rizwanuddin et al., 2023), prebiotics and probiotics (Fathima et al., 2023), herbal extract (Galli et al., 2020) and others (Reis et al, 2019; Reda et al., 2020; Bajagai et al., 2022). These phytogenics possess a plethora of bioactive chemicals or phytocompounds that account for their antioxidant and antimicrobial properties. Therefore, they could potentially be utilised to enhance the diet of broilers in order to establish a viable and sustainable production cycle.

2.1.2 Phytocompounds

The usage of plants and their extracts have long been used in traditional and alternative medicine to improve livestock production due to their phytogenic and phytobiotic properties (Kuralkar and Kuralkar, 2021). Phytochemicals or phytocompounds are bioactive plant chemicals that are abundant in fruits, vegetables, grains, and other plant foods that may provide protective properties to protect the plant. Additionally,

phytochemicals may also provide health benefits to the consumer beyond basic nutrition such as reducing the risk of major chronic diseases in humans (Ojo et al., 2022; Jimenez-Garcia, et al., 2018). Phytochemicals are of great interest as they have a wide range of beneficial pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic (Thakur et al., 2020). Compounds such as flavonoids, tannins, and alkaloids have been discovered to contribute to antimicrobial and physiological activities (Ojo et al., 2022). However, phytocompounds present in plants have been proven to be affected by the cutting intervals, fertiliser application rates, and seasonal variation (Onyeonagu et al., 2013; Onyeonagu and Ukwueze, 2012). Though it should be noted that varying results may also be due to different drying methods as drying conditions have been proven to significantly affect the retention phytocompounds such as the total flavonoid content, total phenolic content, tannin, and other antioxidant properties (Amba et al., 2013). This statement is further supported by Vargas et al. (2022) which studied the influence of different drying methods on vegetables. Both Saifullah et al. (2019) and Vargas et al. (2022) stated that the best drying method should be identified as it is product specific whilst taking quality retention and energy consumption into consideration. Additionally, Ojo et al. (2022) also described that exposure to high temperatures may initially cause a decline in antioxidant activity, but will later show a reversal, with an increase in flavonoid content.

Unlike temperate countries such as New Zealand, where high quality native forages are abundant, Malaysia is home to poor nutritive species such as *Impereta cylindrica*, also known as “lalang” by the locals. Despite this, a few grasses imported and naturalised in the nation have proven to be the current option of local farmers and

cattle producers as improved fodder or pasture. In 2004, Kong identified the top five grass genera that are extensively used in the ruminant sector due to their nutritional value and adaptability under Malaysian circumstances, which are Napier (*Pennisetum purpureum*), Guinea (*Megathyrsus maximus*), Setaria (*Setaria icazungula*), Signal (*Brachiaria decumbens*), and MARDI Digit (*Digtaria setivalva*). While Guinea grass is commonly used in the ruminant sector, Napier grass has been cited to be the most widely used fodder due to its nutritive qualities and promising yield (Halim et al., 2014). Besides, *Brachiaria* varieties have been cultivated on more than 80% of total improved pasture area in Malaysia, with *B. decumbens* being the most popular (Muniandy et al., 2020). Therefore, the focus of this systematic review will observe these three grasses as they are the most abundant and widely used grass in the ruminant industry.

2.2 Napier Grass (*Pennisetum purpureum*)

2.2.1 General Description

Native to the tropical regions of Africa, *Pennisetum purpureum* or more generally referred to as the Elephant grass or Napier grass is perceived as a productive tropical forage due to its high yield in terms of mass per unit area and good nutritional value (Farrell et al., 2002; Monção et al., 2020). Napier grass is a tall, stout, deep-rooted C4 perennial bunch grass that forms dense clumps with leaves that are flat, linear, and hairy at the base, 100cm to 120cm long (Karlen, 2014; Haryani et al., 2018). Napier has been introduced to all sub-tropical and tropical countries in the world and has naturalised in areas of Central and South America, tropical parts of Asia, Australia, the Middle East and the Pacific islands due to its wide agro-ecological adaptation

(Tessema et al., 2010; Negawo et al., 2017). It was first introduced to Malaysia in the 1920's from East Africa and in the period of two decades, various variety of cultivars have been established such as the Common Napier, Red Napier, Taiwan Napier, Dwarf Napier, Dwarf "Mott", Australian Dwarf, Indian Napier, Uganda Napier, Zanzibar Napier, Kobe Napier, and King grass (Halim et al., 2014; Zailan et al., 2018). Cultivars have been developed to suit local conditions and have a wide range of yield potential and nutritive value (Haryani et al., 2018). Napier grass that has been coded as IKN091, otherwise known as Indian Napier, is the most popular in West Malaysia possibly due to its high dry matter yield (Haryani et al., 2018; Bakar et al., 2019). The Napier grass is noted as a multipurpose grass. It is primarily fed to livestock such as cattle, sheep, and goats in the cut and carry system. Besides, several other uses for this plant have been explored such as using the young leaves and shoots for human food and traditional medication. Plus, there are indications that Napier grass has the potential to be used as biomass energy as well as its fibres studied to be used in composite materials such as automotive, construction, and aerospace industries (Burkill, 1985; Rengsirikul, et al., 2013; Bakar et al., 2019). Despite its popularity as a forage grass and wide range of other usage, Napier grass has never been applied in the poultry industry.

2.2.2 Chemical Composition and Nutritive Value

Napier grass cultivars yields have been reported to range from 20-60 tonnes of dry matter/ha/year, with some studies indicating significantly higher yields under high fertiliser input. Nevertheless, the yield potential, nutritional composition, and digestibility mainly depends on the type of cultivar which consecutively is highly influenced by the harvesting age, environmental conditions and management practices

implemented (Halim et al., 2014; Negawo et al., 2017; Haryani et al., 2018). Henceforth, a study performed in Malaysia that evaluated the nutritive composition of the Napier grass varieties confirmed that there were significant differences between the tall or medium height varieties (>130cm), and short or dwarf varieties (<95cm). In summary, a correlation analysis showed that the taller varieties had a higher dry matter yield but had less tillering and leaves than the shorter varieties. The tall varieties out yielded the shorter varieties but produced lower nutritive quality (Halim et al., 2014; Negawo et al., 2017). Napier has also been stated to be the superior grass in terms of a higher dry matter yield in comparison to other tropical grasses, such as the Guinea grass (*Megathyrsus maximus)* and Rhodes grass (*Chloris gayana)* with higher nutritive value compared to *Brachiaria* sp. and *Panicum* sp. (Negawo et al., 2017; Haryani et al., 2018). On average, Napier grass from 10-15 weeks old plant samples contain 9% CP, 20% DM, 70% NDF, 50% ADF, 9% ash and 6% lignin (Negawo et al., 2017). However, it should be noted that CP and ME shows an inversely proportional relationship with the cutting age. On the same note, DM and CF increases as the cutting age increases (Haryani et al., 2018). Furthermore, there are reports on the protein content of Napier grass ranging from 4.4% to 20.4% due to the age of harvest. Therefore, it has been recommended in multiple studies that the recommended age to harvest Napier is at 6-8 weeks of growth to optimise the DM yield and nutritive value as harvesting at an earlier age may harm the long-term sustainable regrowth of the grass (Rusdy, 2016; Haryani et al., 2018). On the other hand, in another study by Zedwu et al. (2002), they suggested cutting Napier grass at a height of 0.5m to obtain the highest results in which has the lowest DM yield with the highest CP yield. They also concluded that cutting the Napier grass at a young stage with fertilisation results in the highest nutritive value. In another experiment conducted by Zailan et al. (2016),

Napier cultivars harvested at different ages showed that the cultivars harvested at 4 weeks' old had the highest nutritive value despite its limited DM yield.

2.2.3 Associated Phytocompounds

Multiple studies have shown that the young leaves and tender shoots of Napier grass are potential sources of dietary protein, minerals, vitamins, and antioxidants (Ng et al., 2021). Phytochemical screening of *P. purpureum* indicated the presence of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, and tannins (Akuru et al., 2015; Sangeetha and Rajendran, 2019; Jack et al., 2020; Ojo et al., 2022). *P. purpureum's* extracts have proven to be effective against *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Apergillus niger*, and *Trichophyton mentagrophyte*, among others. The extracts of *P. purpureum* have demonstrated antifungal activities against isolates of tested fungi, and this activity is attributed to the phenolic compounds present (Ojo et al., 2022). In a study by Jack et al. (2020), a quantitative estimate of the phytochemicals presents in sundried, fresh young shoots of n-hexane, ethylacetate and methanolic *P. purpureum* extracts showed that flavonoids compounds were the highest (0.021%), followed by glycosides (0.008%), and saponins (0.002%) as the lowest. On the other hand, in another study by Akuru et al. (2015), phytochemical analysis of aqueous stem extract of *P. purpureum* showed that there were very high concentrations of tannins (67.89%), alkaloids (lunamarine 26.21%), followed by flavonoids (4.47%) comprising of catechin (2.49%), rutin (2.10%), kampferol (0.09%) and anthocyanin (0.06%) and phenols (1.16%). While tannins are known to be an anti-nutritional factor when present in high amounts, this compound have been shown to increase immunological competence, intestinal microbial ecology, and gut heath in broiler chicks when present at a modest dose

(Huang et al., 2018). Previous research has explored the use of plant-derived flavonoids in treating various physiological problem. For instance, when administered to animals, certain flavonoids demonstrate atypical hormonal activity, such as oestrogens. Besides, findings from animal studies indicated that flavonoids could have beneficial effects related to lipid metabolism due to their antioxidant properties (Cao et al., 2012). Similarly, supplementation of flavonoids-rich diets may contribute to the overall improvement of broiler health on top of increasing immune organ indices and humoral immunity against Newcastle disease and Avian influenza virus (Sugiharto et al., 2019). In this regard, the proper quantities of Napier leaf meals in broiler feed should be investigated in order to determine the optimal quantity to be provided in order to balance the good benefits without harming the chickens' productivity.

2.3 Signal Grass (*Brachiaria decumbens*)

2.3.1 General Description

Over the last 50 years, in regards to its taxonomic information, there has been uncertainties surrounding the *Brachiaria* species names being substitutable, with *B. eminii, B. decumbens* and *B. ruziziensis* all being used for the same plant (Low, 2015). On top of that, several *Brachiaria* species, including *B. decumbens*, have been placed in the *Urochloa* genus by several writers, hence the taxon *Urochloa decumbens* is widely accepted. However, these alterations are still being debated, and many current articles still refer to the Signal grass as *B. decumbens* (Torres Gonzalez and Morton, 2005). While *B. decumbens* and *B. brizantha* have been mistaken for one another due to their similar morphology, *B. decumbens* differs in that it is decumbent, less tufted, and creates a looser cover (Heuze et al., 2021). As a result of its high nutritional

content and aggressive growth capable of suppressing weeds, Signal grass (*Brachiaria decumbens*) is widely utilised in the ruminant sector across Latin America, Australia and Southeast Asia (Low, 2015; Pedreira et al., 2017). In comparison to other *Brachiaria* species, *B. decumbens* is more commonly accessible as it is planted in more than 80% of improved farmed pastures in tropical climates. *B. decumbens*, which originated in Africa, has become an important tropical C4 fodder because of its productivity, adaptability to varied settings, and excellent tolerance to acidic, infertile soils. (Pedreira et al., 2017; Chung et al., 2018). Owing to its high nutritional content and tolerance to pests and drought, Signal grass, is a major source of fodder for cattle in Malaysia. *B. decumbens* of the *Brachiaria* grass family is also the most favoured species in Malaysia because of its high yields despite subpar management standards (Hasiah et al., 2000; Assumaidaee and Mustapha, 2012; Suhaimi et al., 2017; Wassie et al., 2018). Signal grass has several uses in the agricultural industry, including grazing pasture, cutting for fresh feeding, and making hay or silage (Shamim et al., 2021). Signal grass is a robust, stoloniferous, perennial grass species with a dense root-system that possesses aggressive growth behaviour which can cause pastures to become monocultures (Low, 2015; Heuze et al., 2021). Due to this trait, it may be used as a cover crop to reduce erosion and to control weeds (Mollot et al., 2012; Procópio et al., 2015). In addition, because of its capacity to endure shade, *B. decumbens* has become an essential companion plant species to *Leucaena*, and it has been successfully planted as a pasture in coconut farms (Low, 2015).

2.3.2 Chemical Composition and Nutritive Value

A study by Guenni et al. (2002) revealed that short-term dry periods have little influence on plant biomass output because *B. decumbens* redistributes biomass,

adjusts growth rates, and compensates leaf expansion or growth after re-watering. Furthermore, *B. decumbens* was shown to generate double as much digestible DM as other species of plants during the dry period in East Indonesia; concluding that out of all the grass species studied, *B. decumbens* has the most potential for seasonal challenges such as a drought (Bulo et al., 1994). According to Low (2015), *B. decumbens* has the potential to produce 15-27 metric tonnes of dry matter (DM)/hectare/year depending on the environment. This statement is similar to FAO (2017) which reported that the annual yield of *B. decumbens* in Honduras was 23 tonnes DM/hectare; however, in Fiji's warm tropical climate, 34 tonnes DM/hectare were yielded over an 11-month period. On the same note, Signal grass in Malaysia has been found to generate an average dry matter yield of 25.5 tonnes DM/hectare yearly and when pasture is fertilised and adequately managed, it offers excellent quality, palatable forage that promotes animal performance (Assumaidaee and Mustapha, 2012). A study of data gathered over a 5-year period on the proximate analysis findings of local *B. decumbens* in Malaysia discovered that the average percentage values of crude protein were 12.26 percent with a crude fibre content of 33.8 percent (Suhaimi et al., 2017). The chemical composition of *B. decumbens*, like that of many other tropical grasses, varies greatly depending on the stage of maturity (Wassie et al., 2018). It is also important to note that seasonal variations or management would alter the nutritive value and digestibility of the grass. Although harvesting intervals of less than three weeks should be avoided to ensure plant persistence and adequate ground cover (Vendramini, et al., 2013; Hughes et al., 2022), it is important to note that younger leaves generally contain a higher concentration of phytocompounds compared to older leaves (Low, 2015).

2.3.3 Associated Phytocompounds

Signal grass is one of the plants often utilised in traditional medicine in South Africa due to its pharmacological and phytochemical characteristics (Gebanshe et al., 2019). The phytochemicals found in this grass, such as total soluble phenolics, flavonoids, iridoids, condensed tannins, and antioxidant activity (DPPH radical-scavenging activity and ferric-reducing power), are ascribed to its pharmacological and medicinal qualities (Gebanshe et al., 2020). On top of that, several investigations on secondary hepatogenous photosensitisation from plants, including Signal grass, identified the substance as steroidal saponins (Patamalai et al., 1990; Miles et al., 1992; Munday et al., 1993; Meagher et al., 1996; Cruz et al., 2000; Low, 2000; Pires et al., 2002; Brum et al., 2009, Lee et al., 2009; Barbosa-Ferreiria et al., 2011; Moore, 2014; Muniandy et al., 2020). Steroidal saponins, a hazardous chemical linked with secondary hepatogenous photosensitisation in ruminants, can be isolated from *B. decumbens* leaf and stem fractions (Low, 2015). While there are several types of steroidal saponins found in *Brachiaria* spp., the primary saponins are dichotomin, protodioscin, and dioscin. Due to the prevalence of these saponins, they have been correlated to intoxication predominantly in grazing ruminants, namely goat and sheep, particularly during the wet seasons when the grass develops new growth fast (Chung et al., 2018). This may be because saponins levels in *B. decumbens* have been shown to have the highest average concentrations when the plant is young and immature; 3.15% at 60 days and 1.11% at 360 days. Furthermore, saponins levels in *Brachiaria* spp. have been observed to be higher during periods of heavier rainfall (Lima et al., 2012). This is supported by De Oliveira et al. (2013) as they discovered that saponins levels in *B. decumbens* pasture reached 3.54% during rainy seasons and 0.8% during dry seasons. This is corroborated by other research that discovered increased protodioscin

concentrations during seed set and seed fall, as well as other studies that reported the greatest quantities in sprouting leaf shoots in comparison to mature ones (Brum et al., 2007; Brum et al., 2009; Furlan, et al., 2012). Despite the negative effects of saponins when ingested by ruminants, optimum inclusions of saponins in poultry diet have been reported to demonstrate beneficial effects such as an increase in growth rate, better feed efficiency, reduced odour in excreta, and overall improvement in health of the bird (Chaudhary et al., 2018). Alghirani et al. (2021) found that broiler chickens treated with *Yucca shidigera*, which contains steroidal saponins, outperformed non-supplemented chickens in terms of growth performance, ileal nutrient digestibility, gut health, carcass features, and meat quality. In addition to that, saponins extracts from lemongrass have been shown to exhibit phytotoxic and antibacterial effects that is particularly efficient against Gram-negative bacteria equivalent to ampicillin, a common antibiotic (Kolawole et al., 2007). The addition of steroidal saponins to feed has also been demonstrated to benefit growth production and improve characteristics including cholesterol reduction in monogastrics (Makkar et al., 2007). Although saponins have been noted to affect palatability due to the bitter taste, several studies have shown interesting results that inclusion of dietary saponins in poultry diets at an appropriate level may prove to be a useful ingredient as a feed additive (Smithard, 2002).

2.4 Guinea Grass (*Megathyrsus maximus*)

2.4.1 General Description

Megathyrsus maximus, or known as Guinea grass and green panic grass is a forage grass that is native to tropical and subtropical parts of Africa that was introduced

across Asia, Europe, North America and South America (Soti and Thomas, 2022). As a major pantropical grass, it is widely used throughout the tropics for pasture, cut-and-carry, silage and hay due to its fast growing and leafy grass, which is palatable and nutritious to livestock (Aswanimiyuni et al., 2018). The Guinea grass has a lengthy history of taxonomic changes at both generic and species levels; originally classified to genera: *Panicum*, *Urochloa*, and *Megathyrsus*. Previously known as *Panicum maximum*, the plant was renamed *M. maximus* in 2003 after the sub-generic name *Megathyrsus* was elevated to a generic rank. (Benabderrahim and Elfalleh, 2021). However, some databases have not been updated thus alternate generic placements like *P. maximum* is still retained and found in literature (Heuze and Tran, 2020; Rhodes et al., 2022). Guinea grass is a large, tufted, perrenial C4 bunchgrass that has a wide variety of cultivars with broad morphological traits that have been developed for various agronomic purposes (Benabderrahim and Elfalleh, 2021; Rhodes, et al., 2021). Forage quality, excellent palatability and digestibility, highly viable seeds, insect resistance, adaptability to low fertility savannah soils, drought tolerance, quick seedling growth, and creeping features with stoloniferous rooting are commonly selected traits that have been developed through hybridization and selection (Rhodes et al., 2021). Guinea grass has been listed as an invasive grass due to its robust nature and its ability to quickly spread (Benabderrahim and Elfalleh, 2021). Nonetheless in Malaysia, Guinea grass is among the most common species of grass that is used as a source of animal fodder particularly in the ruminant livestock industry (Jusoh et al., 2014; Benabderrahim and Elfalleh, 2021). It has been hailed as a good fodder plant since it thrives in a wide range of soils, including mild shade from trees and bushes, and can withstand lengthy dry spells and fast-moving fires without harming the underground roots (Jusoh et al., 2014; Aswanimiyuni et al., 2018). Guinea grass has

an excellent reputation in the ruminant livestock industry due to its nutritional content and ease of cultivation, yet its potential in the poultry industry has yet to be explored.

2.4.2 Chemical Composition and Nutritive Value

The yield and nutritive value highly depend on the type of cultivar, stage of harvesting and fertilization rate of the plant (Aswanimiyuni et al., 2018). As the cutting interval increases, the dry matter output increases, but the forage quality, in terms of DM digestibility and CP content, decreases significantly (Ngo and Wiktorsson, 2003). The maximum yield of Guinea grasses has been estimated to be about 18 to 21 tonnes per hectare per year if treated with 150 to 200 kg/ha of nitrogen; with total digestible nutrients (TDNs) ranging from 40 to 60% of DM (Benabderrahim and Elfalleh, 2021). According to the findings of a study by Jusoh et al. (2014), results revealed that a shorter cutting interval generated better quality of grass in terms of nutritive value. However, a longer cutting interval produce a greater number of tillers per plant and a higher DM output. This statement is supported by Hare et al. (2013) whereby two different cultivars of Guinea grass showed the same outcome in addition to showing an increase in ADF and NDF concentrations with longer harvesting frequency. The same study also suggested cutting Guinea grass at 30-day intervals for the best quality forage with the highest CP content and despite a reduced DM production; which is consistent with Aswanimiyuni et al. (2018)'s recommendation for long-term management. On the other hand, Jusoh et al. (2014) found that with a longer cutting interval, weed infestation was reduced attributed to the shaded condition created by the grass canopy, but fodder quality would be compromised. As the yield of forage highly depends on the forage management and variety, there would be several reports of varying result. In a review paper, CP of Guinea grass has been reported to range

from 2.8% to 16.8% with NDF and ADF values ranging from 33% to 75.5% and 29.1% to 60.9% respectively. However, only five out of fifteen papers reported on ADL value, whereby the data ranged from 9.21% to 13.89% (Benabderrahim and Elfalleh, 2021). To summarise, farmers should set to harvest the grass at appropriate intervals to obtain the best mix of quality and quantity for their animal production demands.

2.4.3 Associated Phytocompounds

A review by Rhodes et al. (2021) addressed the duality of *M. maximus* as a significant contributor to the livestock industry and as an extremely destructive invasive species. Nonetheless, they concluded that an effective cultivation method is required to achieve the most appealing results while minimising harm to surrounding agricultural land and/or the existing native ecosystem. Henceforth, besides being a key fodder species for ruminants, phytocompounds present in the plant may be potential alternative to antibiotics in the broiler industry (Grashorn, 2010). A study by Abu Hafsa and Hassan (2021) presented that alkaloids are present in the Guinea plant at 29.6 mg/g, followed by tannins (17.2 mg/g), saponins (11.4 mg/g), phytates (4.8 mg/g), and hydrogen cyanide (3.1 mg/g). Another quantitative analysis performed on Guinea plant leaves revealed that phytocompounds present are alkaloids (2.83%), closely followed by saponins (2.60%), tannins (0.85%) and flavonoids (0.54%) (Kanife and Doherty, 2012). The varying results could be attributed to the different management practices such as the cutting age or fertilization application (Onyeonagu and Ukwueze, 2012; Onyeonagu et al., 2013) as neither study reported on this matter in their paper. According to the study by Kanife and Doherty (2012), alkaloids, tannins, saponins, and flavonoids which are the principal physiologically active constituent prevalent in

Guinea grass may have antifungal effects and alkaloids present may contribute to antimicrobial actions against pathogens such as *Klebsiella pnueumoniae* and *Candida albican*. Beneficial effects to the growth of broilers have also been observed when broiler chickens are fed with alkaloid-rich leaf meals such as *Sauropus androgynus* and *Polyalthia longifolia* as they may be employed as antibiotic replacements (Sugiharto et al., 2019). It was also reported that phyocompounds such as alkaloids and saponins in the aqueous leaves extract of *Azadirachta indica* may have contributed to the reduction of *Eimeria* oocyte counts as they bind to critical macromolecules on the membrane of the parasite, causing disruption and subsequently death (Onyiche et al., 2021). Thus, the implications of introducing Guinea grass to broiler rations should be explored further since the plant contains beneficial phytocompounds and may be a practical strategy as an alternative feed additive for the poultry industry.

2.5 Functional Properties of Leaf or Grass Meal

This systematic research was finalised according to the guidelines of "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement" (PRISMA). A systematic literature search was conducted via the electronic database of Scopus where articles were identified using those key words related to production. Articles were then refined by restricting the publication year to 2013 to 2023, a range of 10 years.

2.5.1 Growth Performance

During the last few decades, phytobiotics which are plant-derived bioactive compounds have arisen and gained traction as an antibiotic replacement in animal feed. This is largely due to its ability to provide considerable benefits in poultry

production, including improved growth performance as well as meat and egg quality (Kikusato, 2021). While the exact mechanism of how these phytobiotics influence growth in poultry have yet to be completely understood, Valenzuela-Grijalva et al. (2017) has outlined four key methods by which the addition of phytobiotics may promote growth, which are: 1) increased feed status and feed intake (FI) due to the improved flavour and palatability; 2) antimicrobial effects modulating gut fermentation; 3) enhanced nutrient digestion and absorption via modification of gut functions; and 4) anabolic action on target tissues, both direct and indirect, through activation of endocrine and antioxidative defence systems. Supporting that, previous studies by Jang et al. (2004) and Czech et al. (2009) proposed that these enhancements in poultry development might be linked to the production of digestive enzymes and an increase in antimicrobial activity, resulting in improved gut function. According to Kuralkar and Kuralkar (2021), the beneficial effect of these phytogenics is due to some essential bioactive chemical components such as alkaloids, tannins, flavonoids, saponins, and phenolic compounds that result in defined physiological actions in animal bodies. These secondary metabolites are important because they function as nutrition additives, growth stimulants, immune boosters, increase animal fertility, and aid in the reduction of methane and ammonia emissions. In addition to that, an increase in FI and improvements in FCR could be linked to the active compounds in herb or plant extracts as the benefits include stimulation of appetite and enhanced secretion of digestive enzymes, which in turn facilitates the digestion of nutrients and eases absorption (Rahimi et al., 2011). Furthermore, several studies have linked an increase in FI to the addition of phytogenics, which may be due to improved flavour and palatability of the feed (Jamroz et al., 2002; Windisch et al., 2008). Numerous studies

that utilised leaf or grass meals found that they had a good influence on chicken growth performance.

Recently, Alghirani et al. (2022) conducted a study on the effects of *B. decumbens* as a novel supplementation on the production performance of broiler chickens. The study provided commercial Ross308 broilers with *B. decumbens* ground leaf powder at rates of 0, 25, 50, 75, and 100 mg/kg of basal diet without antibiotics. It was discovered that supplementing *B. decumbens* at a rate of 25mg/kg generated broilers with the best growth performance, as they had improved FCR, body weight gain (BWG), and final body weight (BW) throughout both the starter and finisher phases when compared to the other treatment groups. FI (1.200-1.237kg) was not substantially different ($p>0.05$) across treatment groups during the starter phase, but BWG was significantly greater ($p<0.05$) in the group with 25mg/kg of *B. decumbens* (0.901kg) compared to the other treatment groups (0.853-0.889kg), resulting in a lower FCR. These positive effects were credited to steroidal saponins, which are found in high concentrations in *B. decumbens* and have beneficial effects on the digestive tract such as increasing nutrient absorption by increasing villi height, thus elevating nutrient uptake that is not normally absorbed. Furthermore, other phytocompounds present, such as tannins, flavonoids, and alkaloids, possess traits that lead to the improvement of growth performance by enhancing immunity, which maintains microflora balance, and antimicrobial activity, which reduces pathogenic load and stabilises intestinal health (Chaudhary et al., 2018a; Tonda et al., 2018; Redondo et al., 2022). However, supplementation of *B. decumbens* at higher levels showed lower feed intake which could be caused by the bitter taste of steroidal saponins and anti-nutritional effect of both saponins and tannins (Hassan et al, 2013). Besides, Abang et al. (2023) conducted a study to determine the

effect of graded levels of dried guava (*Psidium guajava*) leaf meal on productive performances of broilers. During the experiment trial of a four-week finisher period, 240 unsexed 5-weeks-old Cobb broiler chickens were randomly grouped and supplemented with 0, 1.5, 3.0 and 4.5g of guava leaf meal per kg of basal diet. It was concluded that the final BW and average daily BWG of broiler chickens increased correspondingly with those fed with higher levels of guava leaf meal($p<0.05$). Chickens fed with 4.5g of guava leaf meal recorded a difference of +200g of the final BW and +4.67g average daily BWG in comparison to the control group. On top of that, FCR and protein efficiency ratio (PER) followed the trend of response whereby performances improved significantly ($p<0.05$) as the inclusion level increased. Average daily FI amongst treatment groups did not vary ($p>0.05$), averaging between 97.42-97.47g per bird indicating that the inclusion of guava leaf mealwas palatable to the chickens. Similarly, with the basal diets reported that broiler chickens fed with the experimental diets showed significantly ($p<0.05$) higher FCR and growth performance parameters as compared to the control group. In another prior research by Daing et al. (2021), supplementing 10g and 20g of guava leaf meal per kg of basal diet to broiler chicks significantly ($p<0.05$) improved BWG and FCR. While the latter study did not discuss on physiological changes in broilers with guava leaf meal supplementation, Abang et al. (2023) credited these beneficial effects in broiler gut health to PGLM's anti-inflammatory and antimicrobial properties. This is as there is an abundance of flavonoids present in guava leaf meal GUAVA LEAF MEAL which possess antimicrobial and anti-oxidative properties. Similarly, as demonstrated by Xue et al. (2021), flavonoid-rich supplement, in the form of Kudzu-leaf (KLF) treatment was found to demonstrated significant decrease ($p<0.05$) in FCR when compared to control group. Xue et al. (2021) attributed this to the improved proliferation of bacteria and hindered

pathogens as gut flavonoids content increased; which in return promote the substrates available for absorption by gut microbiota, thus improving the feed efficiency. The findings this study is in line with previous studies which demonstrated that phytocompounds, namely flavonoids, phenolic groups and alkaloids contribute to the antimicrobial effect which would improve the animals' performance (Taraz et al., 2015).

On top of that, studies on *Moringa oleifera* leaf meal,which has a wide spectrum of medicinal importance and phytochemical value, have demonstrated impacts on production performances (Modisaojang-Mojanaga et al., 2019; Meel et al., 2021). Its leaves are believed to be an excellent source of natural antioxidants and anti-inflammatory compounds such as flavonoids, phenolics, and carotenoids, as well as a good supply of carotene, protein, vitamin C, calcium, and potassium (Hussein and Jassim, 2019). However, the presence of phytate and other anti-nutrients in the leaves may limit nutrient bioavailability and absorption in animal guts. Tannins, which are also found in Moringa leaves, has also been shown to disrupt the biological utilisation of protein and, to a lesser extent, accessible glucose and lipids (Esonu et al., 2001). Therefore, in a research conducted by Meel et al. (2021) to assess the efficacy of supplementing MOLM with multienzyme on broiler chick development performance, concluded that including 15g of MOLM with 0.5g of multienzyme per kg of feed improved the broiler chicks' growth performance and net profit, causing a 13.3% of overall reduction in feed cost/kg gain. Results from the study showed that the body weight gain of broilers supplemented with 15g of MOLM per kg of feed and multienzyme (0.5g/kg of feed) were higher ($p<0.01$) at the starter (726.05g) and finisher (2267.02g) phase. On top of that, chickens fed with MOLM and multienzymes

had better FCR (1.60-1.65; 1.70-1.74) ($p<0.01$) in comparison to the control group (1.81 and 2.03) at both the starter and finisher phase respectively. The decrease in FI was linked by Kakengi et al. (2003) to the lower palatability of the diets supplemented with MOLM. Despite a lowered feed intake, Sharifi et al. (2013) noted that the enzyme supplementation may have helped to overcome the negative effects of anti-nutritional factors and promote digestion of fibre and non-starch polysaccharides. These findings are further backed up by Mikhail et al. (2020) which found that with the addition of MOLM without the supplementation of enzymes had a significantly lower ($p<0.05$) broiler performance compared to those supplemented with the enzymes. Despite that, MOLM-fed chickens without enzyme still managed to acquire considerably more weight and had a better FCR ($p<0.05$) than control-fed chickens, suggesting that the improvement might be related to MOLM's nutritional content and Moringa's antibacterial characteristics (Fahey et al., 2001).

Another experiment comparing the effects of MOLM, a probiotic, and an organic acid as an AGP alternative on the growth performance suggested that the supplementation of MOLM affected the BW similarly to an AGP and organic acid ($p>0.05$). It was only during the first seven days, broilers fed with MOLM had a lower FI (20.13g/day) ($p<0.05$) in comparison to those chickens fed with organic acid (22.01g/day), but did not have any significant differences ($p>0.05$) in comparison to the other groups (Nduku et al.,2020). Similar results were found in the experiment conducted by Alshukri et al. in 2018, whereby the inclusion of MOLM at ranges of 2.5g-10g per kg of feed significantly ($p<0.05$) improved the final body weight (2641g-2750g) and total body weight gain (2492g-2599g) of broiler chicks in comparison to the control chickens (2497g and 2349g respectively). FI was comparable ($p>0.05$) amongst all

groups, resulting in a lower FCR (1.59-1.69) ($p<0.05$) for treated groups that received MOLM compared to the control group (1.82). The increased average daily gain of MOLM-fed chickens may be due to phenolics found in the leaves, which have a moderating effect on the chickens' average daily gain. Given that the major site of activity in plant extracts such as MOLM is in the gastro-intestinal tract through alterations in gut micro-flora, phenolics found in MOLM may promote enhanced glycolysis and utilisation of glucose for energy generation (Mbikay, 2012; Nkukwana, 2012). On top of that, Moringa leaves are high in protein, amino acids, vitamins, and minerals, with antibacterial and therapeutic qualities which may have act as a growth promoter, contributing to the final body weight and weight gain of the chicks (Alshukri et al.,2018). Nevertheless, Alidou et al. (2016) attributed the lower FI of MOLM-fed chickens to anti-nutritional components that limit nutrient absorption, hence altering production metrics, which Alshukri et al. (2018) stated may be related to phytate, which functions as an anti-nutritional element.

Although high levels of tannin-rich leaf meals in broiler diets may have an adverse effect on animal productivity and FI (McSweeny et al., 2008; Ncube et al., 2012; Martens et al., 2013; Ncube et al., 2017), lower and appropriate levels of tanniniferous leaf meals in broiler diets might benefit development metrics and gut morphology (Huang et al., 2018; Miya et al., 2019). Tanniniferous plants, such as *Acacia*, have been shown to improve animal performance due to their high quantities of natural antioxidants and possibly be employed as a phytobiotic (Jiang et al., 2016; Huang et al., 2018; Atiba et al., 2021). In the experiment conducted by Kolobe et al. (2022), it was discovered that the inclusion of 0, 0.5, 1.0, 1.5g *Acacia karroo* leaf meal per kg DM of feed did not affect ($p>0.05$) the FI, FCR, growth rate, and live weight of both

sexes across all treatments for the first 2 weeks and the last 3 weeks of the feeding trial. Likewise, in another research by Madzimure et al. (2018) reported that *Acacia anguistissima* at 0, 50, and 100g/kg of feed as a protein source had no effect ($p>0.05$) on FI in broiler chicks across all treatment diets but treatment groups with higher inclusion levels recorded greater total FI value. Conversely, Gudiso et al. (2019) found that adding 0, 30, 60, 90, 120, and 150 g/kg DM of *Acacia anguistissima* to the broiler diets showed a linear decrease in the average daily FI and average daily gain. Contradicting results from these studies shows that the variations in FI and other productivity indices are influenced by a variety of factors, including feed texture, palatability, retention time in the GIT and bioavailability of nutrients (Madzimure et al., 2018; Gudiso et al., 2019; Kolobe et al., 2022). Elevated FI rates suggests that the animal consumes more feed in order to fill its empty gut as feed moves quickly through the GIT to balance body nutrients (Madzimure et al., 2018). Despite the disparities in findings, all three studies on the addition of *Acacia* in broiler diets emphasised the effects of tannins, which are abundant in the plant's leaves and are thought to be the most important component influencing chicken performance. This is due to the fact that tannins, which are natural antioxidants, have a high affinity for proteins, with which they connect via hydrogen bonding, hydrophobic association, or covalent bonding, limiting the utilisation of proteins that are essential for chicken development (Frutos et al. 2004; Garcia et al. 2004; Kolobe et al., 2022). A previous study by Smith et al. (2003) in rats corroborates this statement by reporting that there are two mechanisms that can lead to decreased feed intake: decreased palatability due to astringency (tannin and salivary glycoprotein complexes) and aversive post-ingestive feedback due to nutrient deficiency (tannin-protein complexes), leading to decreased protein availability. Therefore, more studies should be conducted to determine the

appropriate levels of *Acacia* in broiler diets to avoid the disruption of protein digestion and absorption as tannins present may cause a retardation in growth despite a higher feed intake.

Studies on copra meal (CM) and cassava leaf meal (CLM) supplementation at 100g/kg and 200g/kg to broiler bird in the starter and finisher diet found that there were no effects ($p>0.05$) on the FI and BWG (Diarra and Anand, 2020; Diarra et al., 2023). In addition, the experiment conducted in 2023 found significantly poorer ($p<0.05$) FCR (1.81-2.13) in the treatment groups during the finisher phase whereas in the previous study, the FCR (1.46-1.40) significantly increased ($p<0.05$) during the starter phase. Nonetheless, both experiments carried out the trial comparing the supplementation of CM and CLM alone or with enzymes, found that the addition of enzymes improved or restored ($p<0.05$) the FCR and BWG in comparison to the control group. Diarra et al. (2023) credited the supplementation of enzymes to the improvements in broiler performance due to increased fibre hydrolysis in the digestive tract (Diarra, et al., 2015; Devi and Diarra, 2017). Bakare et al. (2020) which included CLM in broiler diets at 0, 100, 200 and 300 g/kg found similar results whereby FI was not affected ($p>0.05$) by the inclusion of CLM. But, the average daily gain (0.97g/kg) and FCR of chickens fed with 300g/kg CLM decreased ($p<0.05$) in comparison to the control group (1.23g/kg average daily gain). While the previous two studies explored the use of enzymes as a technique of boosting high fibrous elements like CM and CLM in broiler diets, Bakare et al. (2020) discussed how the phytocompounds found in CLM influenced broiler productivity. This is because CLM is known to contain significant amounts of antinutritional substances such as hydrocyanic acid (HCN), tannin, and

phytin, which may have interfered with nutrient absorption (Ravindran et al. 1986; Wobeto et al. 2007; Oresegun et al. 2016).

Lipiński et al. (2017) have also hypothesised that polyphenols have been shown to suppress digestive enzyme production, increase protein secretion, and decrease protein and amino acid digestibility, resulting in opposite metabolic consequences such as lower body weight and feeding efficiency As demonstrated by Amer et al. (2022) in which the study looked at the effects of utilising anthocyanin-rich roselle extract (ARRE) as an additive on broiler chicken development found that there were no significant differences ($p>0.05$) on the growth performance between the groups treated with AARE and those that were not, despite improving the intestinal morphometric.

Table 2.1 summarises the favourable and harmful impacts of including leaf meals into chicken diets on growth parameters. The differing findings might be attributed to the diverse sorts of sources employed, which could have influenced the chickens' acceptance of the meal. Furthermore, variable quantities of phytocompounds and their combination with nutrients in diet may impact broiler bird production indices. Although plant-based phytobiotics may be a viable alternative to antibiotics and growth promoters, further research is needed to discover the optimal dosage for each type of phytobiotic. Furthermore, antinutritional factors, harvesting age, continuous supply, storage period, stability, and microbial contamination must be addressed before it can be used commercially in the chicken business. The scarcity of studies on the use of locally available grasses needs to be looked at as well since this might be a viable option owing to its availability. As a result, further studies and research are needed to shed light on the use of grass or leaf meal as an effective poultry feed

additive. A better and deeper knowledge of this novel feed additive will eventually increase broilers production performance.

Table 2.1 : The Different Effects of Various Leaf Meal Supplementation on Poultry Production Indices

Source	Inclusion levels	Effect	References
B. decumbens ground leaf powder	25mg/kg of basal diet without antibiotics	Improved FCR, body weight gain (BWG), and final body weight (BW) throughout both the starter and finisher phases	Alghirani et al. (2022)
Guava (*Psidium guajava*) leaf meal	1.5, 3.0 and 4.5g of dried guava leaf meal per kg of basal diet	Improved final BW, ADG, FCR and PER in all treatment groups No improvements in average daily FI	Abang et al. (2023)
	10g and 20g of guava leaf meal per kg of basal diet	Improved BWG and FCR	Daing et al. (2021)
Kudzu-leaf	0.06% of the total diets	Lower FCR	Xue et al. (2021)
Moringa oleifera leaf meal	15g of *Moringa oleifera* leaf meal with multienzyme (0.5g) per kg of feed	Improved BWG, net pro.fit, and FCR	Meel et al. (2021)
	2.5, 5, and 7.5% with enzymes	Higher live BW, BWG, FI, and FCR.	Mikhail et al. (2020)
	1000 g of leaf meal per ton of feed	Lower FI during first 7 days.	Nduku et al. (2020)
	0.25, 0.50, 0.75 and 1.0%	Improved final and total BWG, comparable FI and lower FCR.	Alshukri et al. (2018)
Acacia	0.5, 1.0, 1.5g *Acacia karroo* leaf meal	No effect on FI, FCR, growth rate, and live weight of male and female broiler chickens.	Kolobe et al. (2022)
	50, and 100g of *Acacia anguistissima* kg of feed	No effect on FI.	Madzimure et al. (2018)
	30, 60, 90, 120, and 150 g/kg DM of *Acacia anguistissima*	Decrease in ADG and average daily FI.	Gudiso et al. (2019)
Copra meal	100 and 200g of copra meal per kg of feed	No effect on FI and BWG. Decreased FCR during starter phase.	Diarra et al., 2023; Diarra and Anand, 2020
Cassava leaf meal	100 and 200g of copra meal per kg of feed	No effect on FI and BWG.	Diarra et al., 2023; Diarra and Anand, 2020
	100, 200 and 300 g of cassava leaf meal per kg of feed	No effect on FI. Decreased ADG and FCR of chickens fed with 300g/kg	Bakare et al. (2020)

2.5.2 Nutrient Digestibility

Animal digestive systems are made up of hollow organs connected by a long passage that starts at the mouth and ends at the anus. Along the process, organs and enzymes play key roles in mechanical and chemical digestion and nutritional absorption. Digestibility of nutrients with the addition of leaf meals in poultry are still highly debated due to the fibre content and secondary metabolites present which may act as anti-nutritive compounds. The amount of fibre in the diet is an important component that influences the rate at which the feed travels through the gastrointestinal system, which in turn influences the digestion parameters (Mateo et al., 2002). There has been some contradictory data and claims when it comes to the digestibility of leaf meals. On one hand, plant phytocompounds with antioxidant and immunomodulatory qualities are claimed to aid the breakdown and absorption in the gastrointestinal system (Suresh et al., 2017). As a result, the addition of phytobiotic-rich leaf meals is believed to improve feed intake and digestibility. This is due to the fact that they boost nutritional digestion and absorption in the colon by increasing the release of digestive enzymes (Khan et al., 2012). Furthermore, the favourable effects of phytobiotics on the structure of the intestine, as well as the decrease of harmful bacteria and inflammation in the gut, help to increase nutrient uptake and absorption, as detailed further in the subsequent sections. A handful of previous research has claimed that the inclusion of bioactive substances like as tannins, flavonoids, and polyphenols improve digestibility through gut regulation, hence improving growth performance (Basit et al. 2020b). Other studies have claimed that apparent digestibility is improved with the inclusion of dietary fibre but there have been inconsistencies with the data as phytocompounds such as phytate, tannins and polyphenols have also been said to reduce digestibility (Nkukwana et al., 2014). Phytate and tannins may bind to proteins

in the gastrointestinal tract, limiting protein digestibility and hence nutritional intake (Selle et al., 2010; Moyo et al., 2011). Furthermore, polyphenols were discovered to form complexes or denature enzymes when taken in excess, limiting digestibility (He et al., 2006). Having said that, phytocompound-rich herbs have been shown to improve digestion by stimulating saliva secretion, increasing bile acid synthesis in the liver and extraction in the bile, stimulating pancreatic enzyme function, increasing the activity of digestive enzymes in the gastric mucosa, and accelerating digestion (Frankic et al., 2009).

Alghirani et al. (2022), showed that during both starter and finisher phases, there were significant variations ($p<0.05$) found in the AID. CP (59.81%; 62.36%), CF (35.24%; 32.29%), and EE (76.06%; 76.99%) were significantly greater ($p<0.05$) in the group supplemented with 25mg of *B. decumbens* per kg of basal diet, whereas DM (63.45%; 62.36%) and ash (41.11%); 31.90%) were lower in comparison to the other treatment groups. Grasses and forage plants have been shown to increase feed intake and digestibility by enhancing endogenous secretion production in the small intestine, liver, and pancreas (Cross et al., 2007; Martens et al., 2012; Akande et al. 2010). However, Alghirani et al. (2022) observed that higher levels of *B. decumbens* supplementation reduced digestibility, which they attributed to anti-nutritional factors such as steroidal saponins and tannins, which could potentially hinder broilers' growth potential by reducing nutrient digestibility (Dei et al., 2007; Hidayat et al., 2021). Furthermore, high fibre dietary content increases feed volume and, as a result, nutritional density, as well as increases digesta transit rate and lowers feed retention 92 time in chickens, which may reduce intestinal size while lowering nutrient availability for development (Rahman et al., 2020). Manyelo et al. (2022a) studied the

effects of incorporating 0, 50, 100, 150, and 200g of Amanranth leaf meal per kg diet into the meals of Ross308 broiler chicks. All inclusion amounts had no influence on DM and GE digestibility ($p>0.05$). Although there was no linear or quadratic effect observed in the digestibility of increasing levels of Amanranth leaf meal, those fed 50g had higher ($p<0.05$) CF, CP, and ash digestibility values, while those fed 100g had higher ($p<0.05$) EE digestibility. With the above information, it should be emphasised that the inclusion levels had no effect on the broilers' performance. In another experiment by Manyelo et al. (2022b) which included the same levels of Amanranth leaf meal fed to indigenous Boschveld chickens, it was found that the digestibility of DM and GE were not affected ($p>0.05$) but CP, CF, EE and ash were different ($p<0.05$) in the group of chickens fed with 50g of Amanranth leaf meal. Because there are no other studies that are comparable to these, the authors can only speculate that the inclusion level of 50g Amanranth leaf meal in the meals is related to the chickens' ability to tolerate the phytocompounds found in the leaves. They also determined that Amanranth leaf meals may be included in the diets of indigenous Boschveld hens at doses of 0, 50, 100, and 150g per kg of basal food since the chickens' nutrient utilisation and performance indices were good. On top of that, the supplementation of 50g and 100g of Azolla leaf meals per kg of diet were also found to produce a linear increase in DM, OM, CP and EE digestibility with the increase of *Azolla* leaf meals (Abdelatty et al., 2020). The authors attributed these findings of improved nutrient digestibility to the interaction between the supplementation of *Azolla* leaf meal and the intestinal morphology. Similarly, the increasing addition of *Ginkgo biloba* leaves (0 to 60 g/kg of diet) that contain bioactive compounds such as flavonoids, bilobalides, and polyphenols increased the apparent digestibility and true digestibility of EE, threonine, valine, leucine, histidine, and methionine linearly

($p<0.05$) and tended ($0.05<p<0.1$) to increase the true metabolisable energy (TME), nitrogen corrected TME (TMEn) and arginine (Ren et al., 2018).

In another experiment, Sebola et al. (2018) studied the apparent digestibility of *Moringa oleifera* mature and tender leaf meals supplemented at 25, 50, and 100g to three different strains of chicken (Potchefstroom koekoek (PK), Ovambo (OV) and Black Australop (BA) chickens). The results revealed that adding MOLM at all levels had no negative impact on the nutritional digestibility of PK and OV chickens, and for these two strains, higher inclusion levels of MOLM had better ($p<0.05$) CP digestibility. It did, however, reduce CP digestibility in BA chickens. This might be related to the genetic differences in each strain's ability to absorb and digest nutrients in high fibre diet. On the contrary, Gakuya et al. (2014) found lower ($p<0.05$) feed intake and DM digestibility in those supplemented with MOLM above 750g MOLM per kg of diet. While Gukaya et al. (2014) did not disclose the strain of broiler chickens used in their experiment, Nkukwana et al. (2014) recommended that MOLM to be supplemented up to 25g per kg of feed as there were no apparent ($p>0.05$) differences in digestibility but it improved growth performance parameters of Cobb500 broiler chickens. Furthermore, supplementing broiler chicks with 4g/kg *Piper betle* and 8g/kg *Persicaria odorata* enhanced ($p<0.05$) digestibility of EE and DM compared to the control group (Basit et al., 2020a). The digestibility of CP was found to be higher ($p<0.05$) in the group fed 8g/kg *Persicaria odorata*, which is said to be high in flavonoids and polyphenols that improve cellular and mucosal immunity by regulating circulatory and endocrine markers, thus improving broiler health and digestibility (Kamboh et al., 2018). In contrast, the group fed with 8g/kg *Piper betle* had the

significantly ($p<0.05$) least CP digestibility. High concentration of tannins could have caused this reduction in digestion of CP (Sav´on et al., 2006).

Table 2.2 summarises the various outcomes of incorporating several leaf or grass meals. Because various sources include distinct phytocompounds and nutritional values, they will alter the digestion of each broiler strain differently. Not to mention that genetic variations in chicken breeds may have an impact on tolerance and intestinal digestibility. As previously stated, indigenous poultry are thought to be more tolerant of high CF diets. As a result, additional studies should be undertaken on the benefits of leaf or grass meals on broiler bird strains, particularly local species, since farmers who rear these strains may profit from the inclusion of phytobiotics in the diet.

Table 2.2 : The Different Effects of Various Leaf or Grass Meal Supplementation on Digestibility of Poultry

Source	Inclusion levels	Effect	References
B. decumbens ground leaf powder	25mg/kg of basal diet without antibiotics	Improved digestibility of CP, EE, CF but lowered digestibility of DM and ash	Alghirani et al. (2022)
Amanranth leaf meal	0, 50, 100, 150, and 200g of Amanranth leaf meal per kg diet	All levels had no effect on DM and GE Ross308 broilers fed with 50g had higher CF, CP, and ash digestibility Ross308 broilers fed with 100g had higher EE digestibility	Manyelo et al. (2022a)
		All levels had no effect on DM and GE in indigenous Boschveld chickens Improved digestibility in CP, CF, EE and ash in indigenous Boschveld chickens	Manyelo et al. (2022b)
Azolla leaf meals	50g and 100g of *Azolla* leaf meals per kg of diet	Linear increase of digestibility in DM, OM, CP and EE	Abdelatty et al. (2020)
Ginkgo biloba leaf meal	0, 20, 40, 60g of *Ginkgo biloba* leaves per kg diet	Increasing supplementation of *Ginkgo biloba* leaves increased apparent digestibility and true digestibility of EE, threonine, valine, leucine, histidine, and methionine linearly	Ren et al. (2018)
Moringa oleifera mature and tender leaf meal	25, 50, and 100g of *Moringa oleifera* leaves per kg diet	Higher inclusion levels had better CP digestibility in Potchefstroom koekoek and Ovambo strain chicken Reduced CP digestibility in Black Australop chicken	Sebola et al. (2018)
Moringa oleifera leaf meal	0, 75, 150, 300g per MOLM per kg diet	Lower DM digestibility in those supplemented with more than 750g of MOLM	Gakuya et al. (2014)
Moringa oleifera leaf meal	Low (1, 3, 5g), Medium (3, 9, 15g), High (5, 15, 25g) of MOLM per kg diet	No differences in digestibility	Nkukwana et al. (2014)
Piper betle and *Persicaria odorata* leaf meal	4g *Piper betle*, 4g *Persicaria odorata*, 8g *Piper betle*, 8g *Persicaria odorata* per kg of basal diet	4g of *Piper betle* and 8g of *Persicaria odorata* enhanced digestibility of EE and DM 8g of *Persicaria odorata* leaf meal improved CP digestibility 8g *Piper betle* decreased CP digestibility	Basit et al. (2020a)

2.5.3 Gut Histomorphology

As previously stated, one of the hypothesised ways phytobiotics may stimulate development is through modifications in the gastrointestinal system (Valenzuela-Grijalva et al., 2017). Though the method by which phytocompounds alter gut structure is not fully known, there have been multiple theories on how changes in the intestinal morphology affects chicken production performance. A greater villi height: crypt depth ratio (VH:CD) may indicate that the broiler's small intestine has superior digesting and absorption capabilities due to a larger absorptive surface area, allowing for increased nutrient uptake. Besides enhancing VH:CD, changes in gut pH, epithelial cell proliferation, acid secretion, gastrin synthesis, and dietary modifications may alter gut histomorphology and health by decreasing microbial pressure, stabilising bowel health, and preventing intestinal illnesses (Chrubasik et al., 2005; Pohl et al., 2012). The increase in villus height and VH:CD was also found to have a direct correlation with the increase in epithelial cell turnover and thus activated cell mitosis (Khan et al, 2017). Alghirani et al. (2021) further explained that the improved growth performance due to decreased turnover rate in intestinal mucosal cells, results in a shorter crypt depth and lower maintenance needs, thus, allowing more energy to be dedicated to development. In addition to changes in the gut morphology, a rise in production of enzymes such as proteases and amylases improves growth performance in the gastrointestinal tract by increasing absorptive cell development (Jamroz et al., 2006). As a result, including phytobiotics into chicken feed has been proven to increase VH:CD ratio and promote digestive enzyme secretions. Several studies have found that incorporating phytobiotics in poultry feed can improve the morphological features of a chicken's intestine, hence enhancing its production ability and promoting food safety (Jamroz et al., 2006; Ferdous et al. 2021; Gilani et al., 2021).

Supplementation of 25mg/kg of *B. decumbens* powdered meal were observed to show an increase ($p<0.05$) in villus height while simultaneously decreasing ($p<0.05$) crypt depth in the duodenum, jejunum, and ileum of Ross308 broilers on at both starter and finisher phase (Alghirani et al., 2022). This might be explained by saponins' antioxidant properties, which may have changed intestinal shape, enhancing lumen conditions (Tavangar et al., 2021). Antioxidant qualities may also promote digestive tract growth and development by increasing villus height, which enhances absorptive surface area, enzyme secretions, and nutrition transfer (Olukosi and Dono, 2014). In the same line, the administration of 15 or 30 mL/L nanoencapsulated *Terminalia catappa* leaf extract which are rich in various phytocompounds into broilers' drinking water did not affect the crypt depth but improved the villus height ($p<0.01$) and thus, a higher VH:CD ratio ($p<0.05$). Results showed that the extract capsulated or not, produced higher ($p<0.01$) villus height (1521.01-1723.00µm) in comparison to the usage of tetracycline (1407.15µm) as antibiotics. There were no apparent differences ($p>0.05$) between the villus width and crypt depth throughout all treatments, therefore, VH:CD ratio of nanocapsulated or non-nanocapsulated treatments (7.48-10.61) produced better results than the control (5.68) and antibiotic (6.15) group (Hadayati et al., 2022). Similar results were discovered by Saharan et al. (2022) which studied the influence of supplementing 15, 25 and 35g of PLGM per kg of feed found that VH:CD ratio were significantly improved ($p<0.05$). On top of that, when *Azolla* leaf meal (ALM) was added to broiler diets at 50g and 100g per kg of baseline food, villi length rose in the duodenum ($p=0.09$), while in the jejunum, treatment groups given 50g of ALM per kg of basal diet increased villi length by 29.8% compared to the control group. However, compared to the control group, villi length fell by 8% in the ileum ($p=0.03$). Because the jejunum is the primary location of nutrient absorption in

chicken, increase of the villi length of the jejunum with the addition of 50g ALM improved the intestine's absorptive capacity, which would be beneficial to the poultry (Abdelatty et al., 2021). These positive results could be explained by the addition of dietary phytobiotic with antibacterial action that would diminish pathogenic microbe growth, which produces toxic chemicals that inhibit intestinal mucous production by Goblet cells, minimising intestinal damage and lumen repair (Hadayati et al., 2022). This theory is further supported by Ogwuegbu et al. (2021) which studied the effects of sodium butyrate and rosemary leaf meal on the gut histology and microflora. They discovered that supplementing chickens with 5.0g of rosemary leaf meal per kg of basal diet improved ($p<0.05$) gut histological traits which include villus length, crypt depth, epithelial thickness, muscularis thickness in the duodenum, jejunum, and ileum. Rosemary leaf meal is said to have antioxidant, antimicrobial, and antifungal properties derived from bioactive compounds such as carvacrol, thymol, capsaicin, cineole, rosmanol, carnosol, and their acid forms, or flavonoids. Basit et al. (2020b) also reported that with the supplementation of phytobiotics (4g/kg of *Piper betle* and 8g/kg *Persicaria odorata* leaf meal) improved ($p<0.0001$) the villus height in the jejunum and duodenum up to 8.6% similarly to the addition of tetracycline as antibiotics and 0.03g of halquinol/kg of basal diet in comparison to the negative control. For the CD, no change ($p>0.05$) was seen of jejunum but improvements were significantly ($p<0.0.5$) in the duodenum and ileum of groups supplemented with *Piper betle*, *Pesicaria odorata*, halquinol and antibiotics. Therefore, significantly ($p<0.05$) positive results were seen in the VH:CD ratio of duodenum, jejunum and ileum of broilers with treatment diets in comparison to the negative control (basal diet only).

On the other hand, Sugiharto et al. (2020) compared the effects of four diet groups: corn-soybean-based feed with no additive (CONT), corn-soybean-based diet supplemented with 0.1% zinc bacitracin (BACI), diet containing 20% FCPMO (FERM), and diet containing 20% FCPMO and added with 0.1% *Bacillus subtilis* (FERB). Although FERM enhanced other criteria such as immunological responses, antioxidative state, and physiological circumstances, the findings for intestinal morphology were less than favourable. While the villi height was not significant across treatment groups ($p>0.05$), the CD in FERM was larger ($p<0.05$). Thus, the VH:CD ratio in FERM (4.54) was lower ($p<0.05$) than in CONT (6.60) and FERB (6.09). The precise explanation of the lower VH:CD ratio is obscure; however, it is thought to be owing to the high fibre content in FERM, since Saadatmand et al. (2019) showed that dietary fibre (rice hull) would result in a lower VH:CD. This negative effect would result in a decreased absorptive capacity, resulting in poor development performance in broiler chickens. On top of that, in another experiment by Yang et al. (2019), found that the supplementation of cinnamon essential oil or a combination with bamboo leaf flavonoid extracts has no significant differences ($p>0.05$) in the VH:CD ratio of duodenum, jejunum and ileum as there were no differences ($p>0.05$) in the villus height throughout the small intestine.

A summary of the effects of grass or leaf meals on the gut histomorphology of chickens can be found in Table 2.3 below. Based on previous experiments, there were more positive or non-significant results produced with the addition of plant-based phytobiotics in the diets of broilers. Negative findings could be explained by the high fibre content which is why it is crucial for thorough studies to be conducted on grass and leaf meals. This is necessary in order to determine the optimum inclusion level of

feed additive that will produce beneficial results in the gut morphology without compromising the growth performance of the bird. Future research might benefit from a deeper examination of the relationship between fibre content and villus height, as well as the significance of intestinal mucus and the microbial community to gain the chicken industry's trust and acceptance of phytobiotics as an antibiotic substitute.

Table 2.3 : The Different Effects of Various Leaf Meal Supplementation on Poultry's' Gut Histomorphology

Source	Inclusion levels	Effect	References
B. decumbens ground leaf powder	25mg/kg of basal diet without antibiotics	Increased villus height and decrease in crypt depth.	Alghirani et al. (2022)
Terminalia catappa leaf extract	15 or 30 mL of *Terminalia catappa* leaf extract (nanoencapsulated or not) per L of drinking water	Improved villus height. No effect in crypt depth. Higher VH:CD ratio.	Hadayati et al. (2022)
Guava (*Psidium guajava*) leaf meal	15, 25 and 35g of PLGM	Improved VH:CD.	Saharan et al. (2022)
Azzola leaf meal	50g and 100g of *Azzola* leaf meal per kg of baseline food	Improved villi length in duodenum and jejunum. Depressed villi length in ileum.	Abdelatty et al. (2021)
Rosemary leaf meal	5.0g of rosemary leaf meal per kg of basal diet	Enhanced villus length, crypt depth, epithelial thickness, muscularis thickness in the duodenum, jejunum, and ileum	Ogwuegbu et al. (2021)
Piper betle and *Persicaria odorata* leaf meal	4g/kg of *Piper betle* and 8g/kg *Persicaria odorata* leaf meal	Higher villus height in the jejunum and duodenum. Improved crypt depth of jejunum and ileum. No change in crypt depth of jejunum. Better VH:CD ratio of duodenum, jejunum and ileum.	Basit et al. (2020b)
Cassava pulp and *Moringa oleifera.*	20% fermented mixture of cassava pulp and *Moringa oleifera*	No significant differences in crypt depth. Lower VH:CD ratio	Sugiharto et al. (2020)
Cinnamon essential oil (CEO) or a combination with bamboo leaf flavonoid (BLF) extracts	50, 100, 200, 400, or 800 mg of CEO/kg of basal diet 100 mg of CEO and 16.7 mg of BLF/kg of basal diet 200 mg of CEO and 33.3 mg of BLF/kg of basal diet	All treatment groups did not show any significant differences in VH:CD ratio.	Yang et al. (2019)

2.5.4 Gut Microbial Population

The chicken gastrointestinal system is home to a diverse variety of microorganisms that live in symbiotic partnerships which ultimately impacts the host's nutrition, metabolism, and immunity. The balance of pathogenic and non-pathogenic bacteria in the gut could disrupt the microflora equilibrium and compromise the intestinal barrier, allowing toxic compounds or pathogenic bacteria to enter the intestinal lumen. (Gilani et al., 2021). As such, another way to enhance the general health and performance of broiler chickens is to improve gut health conditions, particularly by lowering the potentially harmful microbe population in the gut. Previous research seems to indicate that plant-based feed additives have some good impacts on the gut bacteria community when incorporated into the chicken diet due to their active components. The potential of bioactive substances to break down the cellular membrane and interfere with the cytoplasm ecology of infections is connected to the promise of phytobiotic as a natural antibiotic (Gheisar and Kim, 2018). This is because when phytobiotics with antimicrobial action come into contact with the microbial cell membrane, they modify permeability for H+ and K+ cations, resulting in a reduction in the population of enteropathogens (Gilani et al, 2021). For example, tannins' antibacterial mode of action is thought to restrict metal ions and interact with the cell membrane of microbes through complexes. Whereas saponins form interactions with membrane sterols, causing membrane damage, whereas alkaloids block topoisomerase and disrupt DNA synthesis (Suresh et al., 2017). Basit et al. (2020b) further explains that as the permeability of cell membranes and morphology of the cell walls are modified, tannins disrupt the permeability and because these microbes require iron for development. The tannin process of iron chelation results in decreased metal ion availability, therefore restricting microbial growth other than *Lactobacillus*, which does not require iron for

development (Yilmaz et al., 2018). In addition, according to Jamroz et al. (2006) with the incorporation of plant-origin active substances, increased mucus production on the jejunum wall supports villi-related protective characteristics of phytocompounds in broiler diet, which might explain the lower quantity of detrimental microbiomes to the epithelial wall. These aforementioned phytobiotic qualities will result in bacteriostatic and bacteriocidal actions. Therefore, the decrease in pathogen population should enhance the development and number of helpful microorganisms in the broiler's gut.

Alghirani et al. (2022) discovered that supplementing Ross308 broiler chicks with 25, 50, 75, and 100mg/kg of *B. decumbens* ground leaf meal suppressed the development of *E. faecalis* during the starter phase, similar to the antibiotic-fed group. The presence of saponins, tannins, flavonoids, and *alkaloids*, all of which regulate the cecal microbiota and have antibacterial properties, implies that *B. decumbens* has antimicrobial properties that protect against harmful bacteria such as *E. faecalis* (Viveros et al., 2011; Zdunczyk et al., 2010; Low, 2015; Lee et al., 2021; Hidayat et al., 2021). Furthermore, when compared to the other treatments, the treatment group that received 25mg/kg of *B. decumbens* had the highest standard plate count (3.1×10^6; 1.9×10^6) and coliform count (4.9×10^5; 2.7×10^4) during both starter and finisher phases. These findings could be attributed to the decrease in faecal coliforms and/or a decrease in *Lactobacilli*, anaerobes, aerobes, and enterococci groups could have contributed to increased feed utilisation. In another experiment, Hidayati et al. (2022) studied the effect of nanoencapsulation of *Terminalia catappa* leaf extract (NETLE) as an antibacterial agent on the intestinal health of broilers. Its extracts are known to suppress the growth of *Staphylococcus aureus, Escherichia coli, Salmonella thypii, Bacillus cereus, Zymomonas mobilis,* and *Serratia marcescens* as they contain

phytochemicals such as flavonoids, tannins, saponins, phytosterols, β-carotene, glycosides, cyanogenic glycosides, alkaloids, phenols, and steroids (Rajesh et al., 2015; Offor et al., 2015). The addition of 15-30 mL *T. catappa* leaf extract per L of broilers' drinking water, whether nano-encapsulated or not, effectively halted the development of *Salmonella* sp. population ($p<0.01$) and encouraged the growth of lactic acid bacteria (LAB) (4.23-5.19 log cfu/g) ($p<0.01$), comparable to the use of 50 ppm tetracycline (3.49 log cfu/g LAB). Mandal et al. (2014) discovered that adding 5g, 10g, 15g and 20g of *Moringa oleifera* leaf meal, which also contains a wide variety of phytocompounds to day-old broiler chicks reduced microbial load in comparison to the other groups, including the control and antibiotic supplemented groups. The incorporation of *Azolla* leaf meal (ALM) into broiler diets was also demonstrated to linearly enhance *Lactobaccili* species and reduce *Bacilli* as ALM inclusion levels rose, but decrease *Enterobacteriaceae* independent of ALM inclusion level. Abdelatty et al. (2021) were unable to determine if the alteration in mucus was driven by the change in microbiota with the addition of ALM or vice versa; feeding ALM impacted the mucus composition and, as a result, influenced the microbiota, since ALM contains a large amount of silica, which might have affected the mucous contents. Gut microflora composition was also affected ($p<0.05$) in chickens fed with rosemary leaf meal. During the starter and finisher phase, *Lactobacillus* count in the ileum and caecum was higher in chickens treated with rosemary leaf meal regardless of inclusion level in comparison to those fed with the negative control diet. Conversely, those fed with the negative control diet had the highest ($p<0.05$) count of *E. coli* and *Salmonella*. Beneficial bacteria in the gut, such as *Lactobacillus*, contribute to the production of antibiotic agents, bile salt hydrolase chemicals, and the preservation of gut integrity via their probiotic properties (Ogwuegbu et al., 2021). According to Saeed et al.

(2019), increased *Lactobacillus* and decreased *E. coli* and *Salmonella* aid to manage the intestinal microflora balance by reducing the numbers of hazardous microbes. This promotes the development of intestinal absorptive cells, which in turn promotes the growth of chickens. Furthermore, in another experiment, it was reported that the supplementation of phytobiotics (*Piper betle* and *Pesicaria odorata*) significantly increased ($p<0.001$) the *Lactobacillus* population count compared to other groups treated with halquinol, antibiotics and negative control group. Phytobiotics treated group also significantly decreased ($p<0.05$) population count of *E. coli*, *Staphylococcus aureus, Clostridium,* and *Salmonella* (Basit et al., 2020b). The inclusion of phytobiotics might result in a healthy gut microbiota population since they include beneficial secondary metabolites such as flavonoids and phenolic compounds that favourably influence the microbiota population (Mustafa, 2019; Rahman et al., 2018; Liaqat et al., 2016).

Broiler chickens fed with diets containing fermented cassava pulp and *Moringa oleifera* leaf meal and 0.1% of *Bacillus subtilis* were found to have lower ($p<0.05$) coliform, lactose-negative-enterobacteria and enterobacteria levels than the other treatment groups (Sugiharto et al., 2020). However, Manafi et al. (2017) suggested that the lowered levels of potentially harmful bacteria are related to the *B. subtilis'* probiotic activity rather than the fermented leaf meal in this present study. Another study found that adding copra meal or cassava leaf meal at 100g and 200g/kg of feed to broiler diets had no effect ($p>0.05$) on *E. coli* counts, despite the fact that the fermentation of dietary fibre in the ceca is thought to supress pathogenic bacteria by lowering gut pH through the production of short-chain fatty acids, mainly acetic, propionic, and butyric acid. (Khan and Iqbal, 2016; Jha et al., 2019; Diarra and Anand,

2020). A recent experiment by Diarra et al. (2023) found similar results with the supplementation of copra meal, palm kernel meal and cassava leaf meal with or without enzymes has no effect ($p>0.05$) on the *E. coli* count. Diarra et al. (2023) deduced that the supplementation doses were insufficient to provide substantial fibre content for relevant bacterial fermentation to occur in the ceca. This theory is supported by Saharan et al. (2022), who found that guava leaf meal supplementation significantly reduced ($p<0.001$) *E. coli*, *Clostridia* and increased ($p<0.001$) *Bifidobacteria* and *Lactobacilli* counts in the ceca as the intake of crude fibre was significantly ($p<0.05$) higher with the increase of guava leaf meal AS supplementation rate. Despite some insignificant results with the addition of these leaf meals on the gut microbial population, it can also be said that the inclusion of plant-based products has no deleterious effects on the gut microbial population in broiler chickens.

Generally, the addition of leaf meals and/or their extracts have proven to show beneficial effects in the gut microbial population while some studies show insignificant results. However, appropriate levels for the inclusion of leaf meals should be carefully studied as poultry are known to have lower tolerance towards high fibre diets. This goes hand in hand with finding supplementation levels that are sufficient to stimulate bacterial fermentation in the gut. On top of that, secondary metabolites like tannin which have antibacterial properties may also work against the targeted performance level as they are also classified as antinutrients. Thus, future studies should identify phytocompounds and nutrient profiles of plants that could potentially be used as effective feed additives in broiler diets. Table 2.4 summarises the different effects of various leaf meal supplementation on poultry's gut microbial population.

Table 2.4 : The Different Effects of Various Leaf Meal Supplementation on Poultry's' Gut Microbial Population

Source	Inclusion levels	Effect	References
B. decumbens ground leaf powder	25mg/kg of basal diet without antibiotics	Inhibited the development of *E. faecalis* during the starter phase.	Alghirani et al. (2022)
Terminalia catappa leaf extract (NETLE)	15 or 30 mL of *Terminalia catappa* leaf extract (nanoencapsulated or not) per L of drinking water	Stopped the growth of *Salmonella* sp. Encouraged the growth of lactic acid bacteria.	Hidayati et al (2022)
Moringa oleifera leaf meal	5g, 10g, 15g and 20g of *Moringa oleifera* leaf meal	Reduced microbial load and coliform count in all treatment levels	Mandal et al (2014)
Azolla leaf meal	50g and 100g of *Azzola* leaf meal per kg of baseline food	Increased *Lactobacilli* sp. Reduced *Bacilli* and *Enterobacteriaceae.*	Abdelatty et al. (2021)
Piper betle and *Persicaria odorata* leaf meal	4g/kg of *Piper betle* and 8g/kg *Persicaria odorata* leaf meal	Increased *Lactobacillus* population. Decreased *E. coli*, *Staphylococcus aureus*, *Clostridium*, and *Salmonella*	Basti et al. (2021)
Guava (*Psidium guajava*) leaf meal	15, 25 and 35g of PLGM	Increased ($p<0.001$) *Bifidobacteria* and *Lactobacilli* Reduced *E. coli* and Clostridia	Saharan et al. (2022)
Cassava pulp and *Moringa oleifera* leaf meal	20% fermented cassava pulp and *Moringa oleifera* leaf meal and 0.1% of *Bacillus subtilis*	Lowered ($p<0.05$) coliform, lactose-negative-enterobacteria and enterobacteria levels	Sugiharto et al. (2020)
Copra meal or cassava leaf meal	100g and 200g/kg of feed	No effect on *E. coli* population count	Diarra and Anand (2020)
Copra meal, palm kernel meal and cassava leaf meal	100g and 200g/kg of feed	No effect on *E. coli* population count	Diarra et al. (2023)

2.5.5 Carcass Traits

Carcass weight is strongly associated to poultry's' body weight as well as other carcass characteristics such as dressing percentage and cut portions weight (Chung et al., 2020). Because of their antibacterial and antioxidant capabilities, the inclusion of phytobiotics in broiler diets may be beneficial in improving carcass quality while increasing value to the products (Adriani et al., 2019). Additionally, adding plant extracts to chicken feed has been proven to enhance the eviscerated carcass breast muscle percentage by 1.2% (Puvača et al., 2011). Phytocompounds have been shown to exhibit anabolic effect via two possible mode of actions, by altering animal metabolism by acting similarly to β-adrenergic agonist chemicals and/or boosting plasma norepinephrine levels by inhibiting catechol-o-methyltransferase. In short, these two mechanisms alter the animals' metabolism by boosting protein synthesis and lipolysis while lowering lipogenesis, both of which favour muscle tissue growth (Valenzuela-Grijalva et al., 2017; Oloruntola et al., 2022).

On day 21, Alghirani et al. (2022) observed significant differences ($p<0.05$) on the carcass characteristics of Ross308 broilers supplemented with *B. decumbens* in comparison to the control groups in final live weight, kill-out weight, de-feathered weight, carcass weight, dressing percentage, breast, drumstick, wing, head, neck, shank, GIT, heart, and liver weight, whereas on day 42, all parameters showed significant differences ($p<0.05$). In comparison to the other treatment groups, broilers fed with 25mg/kg of *B. decumbens* had the highest values for both the starter and finisher phases, suggesting the best carcass features across treatment groups. These findings were attributed to the favourable effects of saponins and other phytochemicals found in grass, since saponins have been associated to improved

nutrient absorption along the gastrointestinal system, leading in superior body weight increase and carcass features. Diarra et al. (2022) studied the effect of including copra meal, palm kernel meal and cassava leaf meal with or without enzyme supplementation at 100g/kg (starter) and 200g/kg (finisher) on Cobb500 broiler chickens. They discovered that chickens given cassava leaf meal had a lower ($p<0.05$) abdominal fat recording (0.9%) than the other groups (1.1-1.5%), likely owing to variations in the sources, namely micronutrients. This is in line with Diarra and Anand's (2020) previous experiment with copra meal and cassava leaf meal with or without enzyme supplementation at similar supplementation levels which reported that the lightest ($p<0.05$) abdominal fat was recorded in the group fed with cassava leaf meal with enzymes. On top of that, Faria et al. (2011) found that supplementing Pescoço Pelado broilers with 100g/kg of cassava leaves resulted in higher ($p<0.05$) carcass yield, lower back and abdominal fat, as well as better economic efficiency in comparison to other treatments which are rice bran and ground lead tree hay. However, there were no significant ($p>0.05$) differences for the neck, wings, drumstick, thighs and feet of the broiler chickens across all treatments. Similarly, *Lippia javanica* leaf meal inclusion at 5 and 12g/kg of feed had no effect ($p>0.05$) on broiler breast weight, thigh weight, carcass weight or dressing percentage, but significantly increased abdominal fat weight when compared to those whose diets did not contain *L. javanica* leaf meal. Nonetheless, broilers fed 12g/kg *L. javanica* leaf meal exhibited the greatest ($p<0.05$) proventriculus and gizzard weights as well as the longest small intestines (Mpofu et al., 2016).

Oloruntola et al. (2022) supplemented Arbor Acres broiler chicks with 5, 10, and 15g/kg of Mucuna leaf meal which are rich in alkaloids, flavonoids, saponins, tannins,

terpenoids, and other phytocompounds. They reported a higher dressing percentage with increasing levels of Mucana leaf meal supplementations (72.30-76.43%) in comparison to the groups supplemented with oxytetracycline (71.08%) and control diet (70.35%) despite not showing significant differences ($p=0.45$). Additionally, there were no significant ($p>0.05$) differences in the relative organ weights which indicates that the supplementation did not interfere with the healthy development of the broilers. However, the supplementation of neem leaf meal in broilers at 25g/kg of feed resulted in higher average giblet percentage and is cited to be attributed to the bioactive compounds present in the leaf such as isoprenoids and polyphenolics despite not fully understanding the mode of action (Biswas et al., 2002; Kumari et al., 2014).

On the other hand, the addition of kudzu leaf meal and alfalfa leaf meal at 60 and 73g per kg of starter feed over 21 days resulted in lower ($p<0.05$) carcass weights (822.96-850.90g) in comparison to the control group (894.10g). Correspondingly, the whole breast weight and breast percentage yield were also lower ($p<0.05$) in the treatment groups compared to the control group. Generally, the organ weights of chickens on the control diet had a higher liver weight percentage than the chickens fed with kudzu and alfalfa leaf meals possibly in response to increased fibre content that reduces liver lipid accumulation and plasma lipid content (Zaefarian et al., 2019). Inclusion of *Gliricidia sepium* leaf meal into broiler diets increased the weight of gizzard and liver significantly ($p<0.05$) likely due to the presences of tannins, alkaloids and nitrates in the leaves that act as anti-nutritional factors. Thus, the increase in liver weight could serve the purpose of aiding absorption of nutrients from the gut. Besides, abdominal fat weights of the control chickens were also significantly ($p<0.05$) higher than those supplemented with 100g and 150g/kg of *Gliricidia sepium* leaf meal but broilers fed

with 150g/kg of *Gliricidia sepium* leaf meal had lower ($p<0.05$) carcass dressing percentage (76.5%). The dressing percentage of those fed with 50 and 100g/kg were comparable (79.3 and 77.6%) with the control (82.6%) (Kagya-Agyemang et al., 2007). The addition of *Chromolaena odorata* leaf meal at 0, 25, 50, and 75g per kg diet of broiler chickens also showed detrimental effects ($p<0.05$) on the carcass yield which reflected the poor production performances. The tannins present, according to the investigators, bind to enzymes and restrict the availability of dietary proteins. Organ weights such as the spleen, liver, heart, gizzard and intestine however, were not affected and were similar ($p>0.05$) to those fed on the control diet (Donkoh et al., 2002).

Table 2.5 below summarises the different effects of various leaf or grass meal supplementation on carcass traits of poultry. Carcass quality is a very important aspect of broiler production as it determines the economical profit of the industry. As seen in the past studies, there have been conflicting results on the use of phytogenics in broiler feed and its effects on the carcass qualities and organs. More studies need to be undertaken in order to fully understand how phytobiotics and their biological compounds affect the carcass traits and organs in order to fully reap its benefits for the broilers.

Table 2.5 : The Different Effects of Various Leaf or Grass Meal Supplementation on Carcass Traits of Poultry

Source	Inclusion levels	Effect	References
B. decumbens ground leaf powder	25mg/kg of basal diet without antibiotics	Improved carcass traits across all indicators during finisher phase.	Alghirani et al. (2022)
Cassava leaf meal	100g/kg (starter) and 200g/kg (finisher)	Lower abdominal fat percentage	Diarra et al. (2022)
	100g/kg (starter) and 200g/kg (finisher)	Lower abdominal fat percentage	Diarra and Anand (2020)
	100g/kg diet	Higher carcass yield, lower back and abdominal fat No effect on neck, wings, drumstick, thighs and feet	Faria et al. (2011)
Lippia javanica leaf meal	5 and 12g/kg of feed	Significantly increased abdominal fat weight, proventriculus, and gizzard weight. No effect on breast weight, thigh weight, carcass weight or dressing percentage.	Mpofu et al. (2016)
Mucuna leaf meal	5, 10, and 15g/kg of feed	Higher dressing percentage. No differences on relative organ weight.	Oloruntola et al. (2022)
Neem leaf meal	25g/kg of feed	Higher average giblet percentage	Kumari et al. (2014)
Kudzu leaf meal	60g/kg of feed	Lower carcass weight, whole breast weight, breast percentage yield. Higher liver weight.	Zaefarian et al. (2019)
Alfafa leaf meal	73g/kg of feed	Lower carcass weight, whole breast weight, breast percentage yield. Higher liver weight.	Zaefarian et al. (2019)
Gliricidia sepium leaf meal	50,100 and 150g/kg feed	Higher abdominal fat, liver, and gizzard weight. Lower carcass dressing percentage.	Kagya-Agyemang et al. (2007)
Chromolaena odorata leaf meal	0, 25, 50, and 75g per kg diet	Lower carcass yield. No effect on organs.	Donkoh et al. (2002)

2.5.6 Meat Quality

Consumer purchasing decisions are frequently impacted by the visual appeal of the product, particularly the colour of meat (Mancini and Hunt, 2005). pH-value, colour, and cumulative drip loss are all critical factors in evaluating meat quality (Janisch et al., 2012). According to Fletcher (2002), threshold pH levels below 5.7 represent pale, soft, and exudative (PSE) meat, values 5.7 to 6.1 represent ordinary quality, and values over 6.1 represent dark, firm, and dry (DFD) meat. However, according to Abdulla et al. (2017), they observed that the pH of fresh broiler meat ranged from 6.34 to 6.47. Aside from microbial-related quality loss in meat, oxidation is a primary cause of meat quality decline. Under stressful conditions, excess formation of free radicals beyond what endogenous antioxidant enzymes can control results in brownish coloration of meat from its natural red hue (Falowo et al. 2014). The inclusion of antioxidant-rich feed additives has been proven to promote the preservation of normal meat colour (red) in chickens as a well as increase meat tenderness (Contini et al., 2014). These secondary metabolites are thought to prevent or postpone oxidation via two key pathways: 1) donating electrons to break or terminate the oxidation cycle at the propagation step, preventing the formation of additional lipid and protein radicals; 2) removing free radical (ROS) initiators in order to quench chain-initiating catalysts (radicals); or limiting the radicals initiators by binding metals such as iron and copper as metal chelators to stabilise them in an inactive or insoluble form (Falawo et al, 2014). Furthermore, it was discovered that by directly applying natural antioxidants to meat products, the phytocompounds of these plant-based products could reduce meat spoilage bacteria and decontaminate beef, pork, and poultry against *Salmonella* (Mani-López et al., 2012; Sant'Ana et al., 2014).

The inclusion of MOLM at 1g/kg of feed were found to improve meat quality of chicken meat as its effects were comparable to probiotics and organic acid. Despite not showing any significant differences ($p>0.05$) in pH values among treatments, meat from chickens fed with MOLM had a higher ($p<0.05$) redness (a*) value (1.43) in comparison to the other treatments (0.73-1.19) which could be attributed to the higher levels of dietary iron in *Moringa oleifera* (Nduku et al., 2020). This may be attributed to *Moringa oleifera* leaves that are high in antioxidant activity due to phytocompounds present such as saponins, glucosinolates, flavonoids and phenolic acids (Modisaojang-Mojanaga et al., 2019). In another experiment using fermented copra meal and *Moringa oleifera*, results showed that there were no effects ($p>0.05$) on the pH and WHC of breast meat (Sugiharto et al., 2020). Additionally, the authors could not explain the differences ($p<0.05$) in cooking loss and moisture as meat of broiler chickens fed with fermented copra meal and *Moringa oleifera* added with *B. subtilis* had a higher cooking loss and lower moisture compared to the control and zinc bacitracin groups, yet the group with only fermented copra meal and *Moringa oleifera* showed no significant differences ($p>0.05$) when compared to the control and zinc bacitracin groups. Similarly, Cobb500 chickens that were supplemented with low (1-5g/kg of feed, depending on growth stage), medium (3-15g/kg feed), and high (5-25g/kg feed) had no significant differences ($p>0.05$) in the pH measured at post-mortem. Cumulative drip loss was also significantly decreased ($p<0.05$) in chicken meat fed with high MOLM, and lightness was significantly reduced ($p<0.05$) while increasing redness, yellowness, chroma, and the hue angle during storage over time with the supplementation of MOLM (Nkukwana et al., 2015). Sebola et al. (2018) studied the effects of MOLM (0, 25, 50, 100g/kg feed) on the meat quality of three different chicken strains (Potchefstroom Koekoek, Ovambo and Black Australorp)

and found that there were no 3-way interactions (diet x strain x gender) that affected ($p>0.05$) redness (a*), yellowness (b*), pH, and cooking loss but affected ($p<0.05$) lightness (L*) and shear force. Variations in chicken strains within diets were detected, as pH was unaffected ($p>0.05$), while colour, shear force, and cooking loss were substantially altered ($p<0.05$). Because of the high carotene content of MOLM, higher inclusion levels of MOLM resulted in increased b* of breast meat in the Black Australorp strain. Male and female Ovambo strains generated meat with lower shear force (41.29-61.03) than the other strains (51.06-86.82), which might be attributed to differences in muscle fibre size and genetic diversity across the strains.

Similarly, Ross308 broilers fed feeds containing 0 and 100g Amaranth leaf meals (ALM) exhibited greater ($p>0.05$) cooking loss than those fed diets containing 50, 150, and 200g ALM per kg feed. All inclusion levels but had no effects ($p>0.05$) on the pH value (5.57-6.75) and breast meat a* (-0.99-1.05) or b* (7.33-10.45). But, L* of breast meat from chickens fed with 50 (51.11) and 100g (50.16) of ALM recorded lighter ($p<0.05$) breast meat colour than the other treatments (44.56-49.14) which did not differ from each other ($p>0.05$). In addition, there were no significant differences ($p>0.05$) on the meat tenderness, juiciness, flavour and overall acceptability of the meat across all ALM inclusion levels. Broilers that were fed with 50,100 ad 200g of ALM/kg feed had lower ($p<0.05$) cooking loss in comparison to the other group. On top of that, meat from chickens supplemented with 200g of ALM had higher shear force values than the other chickens. ALM inclusion to the diet might be responsible for the darker breast meat colour due to higher chlorophyll and carotene levels present in the leaves (Manyelo et al., 2022). Another study by Ncube et al. (2017) discovered that when *Acacia angustissima* leaf meal levels increased, L* values declined from

53.66 to 49.23, b* values increased from 12.93 to 19.97, shear force increased from 14.14 N to 14.54 N, and cooking loss increased from 5.95% to 7.64%. Yavaş and Malayoğlu (2018) fed 0, 12.5, 25 and 50g of olive leaf (oleuropein) supplementation to Ross308 broiler chicks found that the addition of oleuopein at different levels had no effect ($p>0.05$) on breast meat brightness (L*), yellowness (b*), and pH after 24 hours, while redness (a*) increased significantly ($p<0.05$) compared to the control group. Another experiment demonstrated that the addition of broccoli stem and leaf meal at 40, 80, and 120g per kg diet enhanced b*, concentrations of xanthophyll in abdominal fat and breast skin, total antioxidant capacity, while decreasing malondialdehyde concentration, and decreased drip loss percentage of breast muscle compared to the control (Hu et al., 2012).

Generally, the addition of different sources of leaf meals produced varying results as the inclusion level and composition of each source fluctuates. Despite the mixed outcomes, it could be said that there were pros and cons or neutral outcomes of supplementing leaf meals to the diet of broilers. These effects must be studied further in order to determine if any individual or mixed phytobiotic source could be effectively utilised as a feed additive. A summary on the effects of leaf meals supplementation on meat quality of poultry can be found in Table 2.6.

Table 2.6 : The Different Effects of Various Leaf Meal Supplementation on Meat Quality of Poultry

Source	Inclusion levels	Effect	References
Moringa oleifera leaf meal	1g of MOLM per kg of feed	No effect on pH Higher a* value	Nduku et al. (2020)
	low (1-5g/kg of feed, depending on growth stage) medium (3-15g/kg feed), and high (5-25g/kg feed)	No effect on pH Decreased cumulative drip loss in high MOLM Reduced L*, a*, b*, chroma and hue	Nkukwana et al. (2015)
	0, 25, 50, 100g/kg feed	No effects in a*, b*, pH and cooking loss Affected L* and shear force	Sebola et al. (2018)
Fermented copra meal and *Moringa oleifera*	20% fermented mixture of cassava pulp and *Moringa oleifera*	No effects on pH and WHC	Sugiharto et al. (2020)
Amaranth leaf meals	50, 100, 150, and 200g per kg of feed	Had no effects on pH, breast a* and b*, meat tenderness, juiciness, flavour and overall acceptability Recorded differences in cooking loss and L*.	Manyelo et al. (2022)
Acacia angustissima leaf meal	0, 50 and 100g/kg feed	L* declined as supplementation increased b*, shear force, and cooking loss increased linearly along with supplementation	Ncube et al. (2017)
Olive leaf	0, 12.5, 25 and 50g of olive leaf (oleuropein)	No effect on L*, b*, and pH_{24} Increased a*	Yavaş and Malayoğlu (2018)
Broccoli stem and leaf meal	40, 80, and 120g per kg diet	Improved b*, xanthophyll concentrations, and total antioxidant capacity. Decreased malondialdehyde concentration and drip loss percentage	Hu et al. (2012)

2.5.7 Serum Lipid Profile, Blood Biomarkers and Biochemistry

Heat stress has a substantial influence on animal well-being and impacts animal output globally because it encourages animals to restrict heat generation by lowering feed intake. This leads to a detrimental impact on growth performance (Hansen, 2009). Other forms of stress like environmental, pathogenic, and nutritional issues, for example, can induce a state of stress, prompting a series of behavioural and physiological reactions that would reduce poultry performance (Tamzil et al., 2013). Because of their antioxidant activity, phytobiotics could be utilised during heat stress and other stressor conditions to influence poultry serum biochemistry by lowering serum concentrations of cholesterol, triglycerides, and LDL while increasing HDL levels in broilers (Gilani et al., 2018). This may be due to the distinct features of phytobiotics, which have been linked to hypocholesterolaemic effects as volatile oils found in plants can impede the function of 3-hydroxy-3-methylglutaryl-coenzyme, a reductase (HMGCoA reductase) liver enzyme that controls cholesterol production, thus lowering blood cholesterol levels (Fujioka et al., 2003). On top of that, most therapeutic plants have been proven to have medicinal effects, either owing to phytochemical ingredients or dose (Addy et al., 2013) and blood parameters provide valuable insight for the health status of broiler chickens (Rehman et al., 2017). Plant secondary metabolites have also been examined for their immunomodulatory effects, which are assumed to be linked to the stimulation of heat shock proteins, which improve protein translation efficiency (Suresh et al., 2017). Furthermore, the study on hepatic function indicators such as AST, ALT, and ALP levels would offer information on liver function and the occurrence of liver damage caused by toxins (Muazu and Aliyu-Paiko, 2020).

Serum cholesterol was discovered reduced ($p<0.05$) in chickens supplemented with 10 and 15g of mucuna leaf meal compared to chickens in other treatments. Additionally, the supplementation of mucuna leaf meal had no effects ($p>0.05$) on the AST levels of broilers across all treatments, signifying that there were no negative impacts on the liver's normal physiological and anatomical function (Oloruntola et al. 2022). Widiastuti et al. (2021) found that the addition of acidified papaya leaf and seed meal (APLS) at 10, 25, and 50g per kg of broiler diet linearly increased ($p<0.05$) HDL to LDL ratio and linearly decreased ($p=0.06$) cholesterol to HDL ratio with higher inclusion levels of APLS. These favourable results would prevent unwanted physiological conditions in broilers such as cardiovascular problems (Bueno et al., 2017). Similarly, broilers supplemented with 10, 20 and 30g of *Vernonia amygdalina* leaf meal per kg diet had lower total cholesterol (107.3-118.7 mg/dl) and LDL values (93.9-113.7 mg/dl) than those in the control group (139.3 mg/dl and 122.3 mg/dl respectively) suggesting the reduction in triglyceride synthesis and improved redistribution of cholesterol between lipoprotein molecules (Tokofai et al., 2020).

Another experiment that included *Moringa oleifera* leaf meal at 0, 25, 50, and 100g/kg DM of diet of broiler chickens discovered that the diet had significant effect ($p<0.05$) effect on AST, ALT ad ALP. Higher levels of MOLM resulted in lower AST and ALP levels which indicated that there were no toxic effects in the liver (Sebola and Mokoboki., 2019). In the same way, dietary supplementation of *Persicaria odorata* leaf meal at 2, 4, and 8g/kg diet showed a linear decrease ($p<0.05$) of AST, ALT, and ALP on day 21 and linear decrease of AST, ALT, cholesterol, and triglycerides ($p<0.05$) on day 42 with higher inclusion levels of *Persicaria odorata* leaf meal. Reduced ALT and AST levels suggest hepatoprotective action, which is thought to be

associated with flavonoids and secondary metabolites in the leaf meal. Likewise, the improved serum levels of triglycerides and cholesterol are thought to be from the phytobiotic effect of *Persicaria odorata* leaf meal (Basit et al., 2020c).

When compared to the negative control group, broiler chicks given 1.25, 2.5, and 5.0g of *Azadirachta indica* leaf meal per kg of feed and antibiotics exhibited reduced ($p<0.05$) ALP values. Furthermore, when leaf meal levels increased, blood and tissue cholesterol levels reduced dramatically ($p<0.05$) suggesting a decrease in lipid mobilisation (Ansari et al., 2012). Dietary inclusion of 0.1 and 0.2g/kg of *Morinda lucida* leaf meal were found to have lower ($p<0.05$) levels of AST and ALT, with or without routine medication included. However, serum cholesterol and triglyceride results were not affected ($p>0.05$) across all treatments. The authors ascribed the reduced AST levels to the presence of tannins and saponins, which are considered to have hepatoprotective properties (Lala et al., 2018). On the other hand, the addition of *Accacia angustissima* leaf meal at 50 and 100g/kg diet had no effect ($p>0.05$) on the serum AST, ALP, TC, HDL, and LDL of broiler chickens (Ncube et al., 2018). But at 4 and 6 weeks, ALT levels were elevated with increased levels of *A. angustissima* leaf meal after the starter phase. As ALT is the most sensitive hepatocyte damage marker, this may indicate liver damage when the liver attempts to metabolise the antinutritional substances (Giannini et al. 2005; Ojo et al. 2013; Hassan et al. 2016).

An experiment by Song et al. (2017) which studied the effects of enzymatically treated *Artemisia annua* on the intestinal inflammatory response of heat-stressed Arbor Acres broilers aged 21 days' old found that supplementing *Artemisia annua* at 1g/kg of feed

significantly decreased ($p<0.05$) the mRNA expression of HSP70. *Artemisia annua* is an annual weedy herb that has been demonstrated to boost antioxidant capacity and immunological function due to its high antioxidant content, which includes flavonoids and phenolics (Brisibe et al., 2009; Cherian et al. 2013; Gholamrezaie Sani et al., 2013). The presence of higher levels of HSP70 mRNA in the heat stress model group in the current study shows that heat stress was induced, and the results indicate that supplementation of enzymatically treated *Artemisia annua* could be linked to the modification of pro-inflammatory cytokine production. The research also examined plasma diamine oxidase (DAO), which is prominent in the top section of the intestinal mucosa and revealed a significant increase ($p<0.05$) due to heat stress (Li et al., 2015). DAO levels that are elevated suggest necrosis of the intestinal mucosal cells, which ultimately impairs intestinal function and permeability (Li et al., 2002). More importantly, enzymatically treated *Artemisia annua* supplementation was observed to significantly ($p<0.05$) lower plasma DAO levels, indicating that enzymatically treated *Artemisia annua* may enhance intestinal barrier function.

Another study that looked at the effects of pure rosemary extracts on heat stress in broilers discovered that rosemary created a higher antioxidative condition (Tang et al., 2018). Antioxidant activity of rosemary are due to the leaves containing antioxidant compounds such as carnosol, carnosic acid, rosmaridiphenol, rosmanol, isorosmanol, epirosmanol, rosmariquinone, and rosmarinic acid which could be comparable to butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are synthetic antioxidants (Zhang et al., 2010). The results showed that HSP70 levels in broiler chickens' hearts were raised ($p<0.0001$) before and during heat stress by introducing purified rosemary extracts at a rate of 3%, providing a protective effect to

the chicken heart during heat stress. According to the authors, the addition of rosemary stimulated the development of HSP70, which adheres to and inhibits apoptotic proteins while also repairing unfolded or misfolded proteins in response to diverse environmental stresses.

Furthermore, Alghirani et al. (2023) found that supplementing powdered *Y. schidigera* saponins to broiler chickens at 25, 50, 75, and 100mg per kg of basal feed resulted in significant differences ($p<0.05$) in serum lipid profile, liver function, acute phase protein, hormone, and heat shock protein analyses. When compared to the other treatment groups, broilers given 100mg/kg of *Y. schidigera* saponins had the lowest levels of cholesterol, TG, and LDL, as well as the greatest concentration of HDL. On top of that, 100 mg/kg *Y. schidiger*a saponins supplemented broilers revealed the lowest levels of SAA, AGP, and corticosterone which are stress biomarkers, indicating a reduced inflammatory reaction and stress response, possibly leading to enhanced growth performance. Conversely, no significant variations in CP concentrations were seen between treatments ($p>0.05$). Saponins' cholesterol-lowering impact, which inhibits pancreatic cholesterol esterase, bile acid binding, and decreases cholesterol solubility in micelles, potentially delaying cholesterol absorption in the gut, was linked to the positive effects of those lipid profiles in this experiment (Ngamukote et al., 2011). SAA concentration, which is the most sensitive protein of APP was found to be lower in the group supplemented with 100mg/kg of *Y. schidigera*. Similarly, AGP which is a major and more sensitive APP in poultry (Nazifi et al., 2010) also showed a decrease in concentration on day 42. The reduced APP levels could be explained by saponins' anti-inflammatory and anti-microbial effects. Additionally, corticosterone levels were lower at 100mg/kg of *Y. schidigera* powder because saponins have a

hypocholesterolemic effect, and since serum cholesterol is a precursor to serum corticosterone, lower serum corticosterone levels could be linked to lower serum cholesterol levels. Since this experiment was conducted in an open sided house in a tropical environment, serum HSP70 was measured and found to be lower in the highest concentration of *Y. schidigera* supplementation (100mg/kg) as saponins present could scavenge radicals, metal chelate, and synergize with other antioxidants, thus lowering HSP70 levels.

Table 2.7 summarises the effect of leaf meals on serum lipid profile, blood biomarkers and biochemistry of broilers. While a majority of the previous experiments indicated positive or neutral effects with the inclusion of leaf meals, further studies should be carried out in order to determine the phytocompound or nutritional compositions responsible for these effects as well as the dosage required for that could potentially allow the replacement of antibiotics in feed. Moreover, there were no previous researches that studied the effects of leaf or grass meals on blood biomarkers such as APP, corticosterone and HSP in broiler chickens. Therefore, with the plethora of plants available, more viable options should be discovered and studied in order to ensure a continuous supply should the usage of phytobiotics be adopted in the commercial poultry industry.

Table 2.7 : The Different Effects of Various Leaf or Grass Meal Supplementation on Serum Lipid Profile, Blood Biomarkers and Biochemistry of Poultry

Source	Inclusion levels	Effect	References
Mucuna leaf meal	10 and 15g per kg diet	Reduced serum cholesterol. No effect on AST.	Oloruntola et al. (2022)
Acidified papaya leaf and seed meal	10, 25, and 50g per kg diet	Increased HDL: LDL with higher inclusion levels. Linear decrease of cholesterol: HDL with higher inclusion levels.	Widiastuti et al. (2021)
Vernonia amygdalina leaf meal	10, 20 and 30g per kg diet	Lower total cholesterol and LDL	Tokofai et al. (2020)
Moringa oleifera leaf meal	25,50, and 100g/kg DM of diet	Higher inclusion levels resulted in lower AST and ALP levels.	Sebola and Mokoboki. (2019)
Persicaria odorata leaf meal	2,4, and 8g/kg diet	Decrease of AST, ALT, and ALP with higher inclusion levels.	Basit etl al. (2020c)
Azadirachta indica leaf meal	1.25, 2.5, and 5.0g/kg diet	Reduced ALP, blood and tissue cholesterol.	Ansari et al. (2012)
Morinda lucida leaf meal	0.1 and 0.2g/kg diet	Lower AST and ALT. No effect on serum cholesterol and triglyceride.	Lala et al. (2018)
Acacia angustissima leaf meal	50 and 100g/kg diet	No effect on serum AST, ALP, TC, HDL, and LDL.	Ncube et al. (2018)
Enzymatically treated *Artemisia annua*	1g/kg of feed	Lowered mRNA expression of HSP70.	Song et al. (2017)
Pure rosemary extracts	3% rosemary nanoemulsion liquid	Higher HSP70 levels in the heart.	Tang et al. (2018)
Powdered *Y. schidigera* saponins	100 mg/kg of feed	Lowered levels of cholesterol, TG, LDL, SAA, AGP, corticosterone and serum HSP70. Higher levels of HDL.	Alghirani et al. (2023)

2.6 Summary

As the Malaysian poultry industry continues to expand and grow, the agricultural sector is under greater stress to explore alternative feed additives that can replace antibiotics in chicken diets in order to prevent antibiotic resistance and the emergence of super bugs. With that, natural feed additives such as phytobiotics, which contain a plethora of bioactive compounds with pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic, might have potential to serve as an antibiotic alternative. Based on this review paper, compounds such as flavonoids, saponins, tannins, and alkaloids have been discovered to contribute to antibacterial and physiological activities that might boost growth performance, enhance the immune system, and product quality. Although phytobiotics are thought to be superior, several issues, including optimal dosages, complex composition, harvesting time, extraction method, storage conditions, antagonistic effects, antinutritional factors, and microbial contamination, warrant further investigation.

In order to avoid competition with humans for phytobiotic-rich sources, local forages and grasses that are already present in abundance in Malaysia could be adopted as an alternative to antibiotics. Grasses such as Napier, Signal, and Guinea are already widely employed as fodder or pasture in the ruminant industry due to their nutritional properties and prospective production. Based on literature findings, Napier and Signal grass have already been used as traditional medicine for human consumption and the phytocompound profiling of these three grasses have already been explored. All three grasses have proven to contain bioactive compounds that could have the potential to induce beneficial effects on poultry. However, there were no other prior studies on the

effects of these grasses in broiler chickens besides one experiment conducted by Alghirani et al. in 2022 which studied the effects of supplementing *B. decumbens* at a rate of 25, 50, 75, and 100 mg/kg of feed to Ross308 broiler chickens. On top of that, only one study was found to supplement indigenous broiler chickens with leaf meals even though it has been hypothesised that this strain of chickens can tolerate phytocompounds and fibre better in comparison to commercial chickens (Manyelo et al., 2022a; Manyelo et al., 2022b).

Furthermore, with the ban of AGPs, poultry producers are challenged with the growing demand for chicken meat whilst balancing a sustainable and safe meat production for human consumption. Thus, for economic considerations, the antibiotic alternative of choice should be one with the least possible cost and have equivalent effects to synthetic antibiotics at a lower cost. Feed cost reduction through the use of less expensive and unconventional sources is a key part of commercial poultry production. This technique might lower production costs and assure cheaper meat production, whilst avoiding competition between humans and animals. With the incorporation of Napier, Signal and Guinea grass as a feed additive in broiler feed, it is critical to evaluate the gross profit, net profit, economic efficiency, and total feed cost to determine the viability of using these grasses. These feed additives should give economic benefits while also improving growth performance and/or sensory attributes of the end-product, such as meat quality.

Since there is a clear lack of information on how various phytobiotic sources and their distinct phytocompounds profile impact different strains of broilers, more studies and researches should be undertaken to better understand the interaction and implication

of these grasses and their phytocompounds may have on the broiler industry, as well as enhance food safety. Because Malaysia has a market demand for slow-growing broilers which retail for a premium price, tapping into this niche area of the poultry business could benefit local farmers, consumers, as well as the Malaysian socioeconomic standing overall.

CHAPTER 3

MATERIALS AND METHOD

3.1 Ethical Approval

The experimental protocols and ethics were approved by the Institutional Animal Care and Use Committee, University of Putra Malaysia (Approval number: UPM/IACUC/AUP-R047/2022).

3.2 Plant Preparation

All three grasses (*P. purpureum, B.decumbens,* and *M. maximus*) were planted at Field 15, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. Plots were randomised to minimise bias, and soil preparation and planting spacing were standardised across all plots, each measuring at $18.2m^{2.}$ To ensure uniform nnutrient availability, all plots were fertilised with a balanced NPK fertiliser at a ratio of 50:30:30, applied at the beginning of the experiment across all treatments. At four weeks old, leaves were harvested manually and dried in a 60 °C oven until constant weight was achieved. The grasses's leaves were then processed and ground into powder (50-300 mesh) using an electric grinder (RRH-800A), which were then stored at room temperature for further use. The levels of dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash content of each grass were determined with reference to The Official Methods of Analysis of AOAC International (Horwistz, 2010). The nutritional composition of *P. purpureum, B.decumbens,* and *M. maximus* are presented in Table 3.1.

Table 3.1 : Nutrient Composition of *P. purpereum, B. decumbens* and *M. maximus* Leaf Meal

Parameters	*P. purpereum*	*B. decumbens*	*M. maximus*
Metabolisable energy (MJ/kg)	17.01±0.56	17.23±0.35	18.24±0.23
Dry matter (%)	28.00±0.26	28.57±0.67	26.10±0.15
Crude protein (%)	13.63±0.40	11.31±0.22	8.61±0.28
Crude fibre (%)	29.5±0.67	28.00±0.45	30.00±0.45
Ether extract (%)	2.02±0.05	1.28±0.07	1.75±0.15
Ash (%)	7.50±0.09	5.84±0.12	6.42±0.16

All values were expressed as mean ± standard error.

3.3 Phytochemical Qualitative Analysis

The qualitative analyses of *P. purpureum, B.decumbens,* and *M. maximus* for various phytochemical compounds was then conducted according to Osuntokun et al. (2016). For the qualitative method of analyses, the plant filtrate was first prepared by boiling 20g of fresh plant in distilled water. A vacuum pump was used to filter the solution. The filtrate was then tested for alkaloids, flavonoids, tannins, saponins, and anthocyanosides. Results of the presence and absence of tested phytochemical compounds in *P. purpureum, B.decumbens,* and *M. maximus* are shown in Table 3.2.

3.3.1 Test for Alkaloids

About 0.2gram were warmed with 2% H2SO4 for two minutes before filtering and adding a few drops of Dragendoff's reagent. Alkaloids is determined by the presence of orange red precipitate.

3.3.2 Test for Flavonoids

One millilitre of plant filtrate was combined with two millilitres of 10% lead acetate; a brownish precipitate showed that the phenolic flavonoids were present. For

flavonoids, 1 ml of the plant filtrate was combined with 2m1 of dilute NaOH; the presence of flavonoids was indicated by a golden yellow hue.

3.3.3 Test for Tannins

A dark green tint indicated a positive tannin test when one millilitre of the filtrate was combined with two millilitres of FeCl.

3.3.4 Test for Saponins

One millilitre of plant filtrate was diluted with two millilitres of distilled water; the combination was rapidly agitated and let to stand for ten minutes, during which time the formation of foam on the top of the mixture lasting more than ten millimetres shows the presence of saponins.

3.3.5 Test for Anthocyanosides

One millilitre of plant filtrate was combined with five millilitres of weak HCI; a faint pink tint indicates a positive test.

Table 3.2 : Qualitative Analysis of the Phytochemical Screening of *P. purpereum, B. decumbens,* and *M. maximus* Leaf Meal

Parameters	*P. purpereum*	*B. decumbens*	*M. maximus*
Saponins	+ve	+ve	+ve
Tannin	+ve	+ve	-ve
Alkaloid	-ve	+ve	+ve
Anthocyanoside	-ve	-ve	-ve
Phenolic flavonoid	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve

All values were express as + ve and – ve represent present and absent respectively.

3.4 Phytochemical Quantative Analysis

The quantitative studies of saponins, tannins, flavonoids, and alkaloids in *P. purpureum*, *B. decumbens*, and *M. maximus* were carried out using the approach described by Osuntokun et al. (2016) with some modifications. The procedures for producing each phytochemical compound are described in the following subsections. Table 3.3 summarises the quantitative data for *P. purpureum*, *B. decumbens*, and *M. maximus.*

3.4.1 Quantitative Test for Saponins

About 20 grams of dried plant materials were crushed and placed in a conical flask, followed by 100ml of 20% aqueous ethanol. A hot water bath was used to heat the mixture. At around 55°C, for 4 hours with constant stirring, the mixture was filtered and the residue was re-extracted with 200ml of 20% ethanol. The mixed extracts were reduced to 40ml over a 90°C water bath. The concentrate was transferred to a 250 ml separatory funnel, and 20 ml of diethyl ether was added and vigorously agitated. The aqueous layer was retrieved, while the ether layer was discarded. The purifying procedure was carried out three times. n-butanol (60 ml) was added. The mixed n-butanol extracts were washed twice with 5% aqueous sodium chloride solution (10 ml). The remainder of the solution was heated in a water bath. The samples were dried in the oven to a consistent weight after evaporation, and the saponin content was determined as a percentage of the starting material.

3.4.2 Quantitative Test for Flavonoids

At room temperature, 10 g of the plant material was extracted overnight with 100 ml of 80% aqueous methanol. The whole solution was filtered using No. 42 Whatman filter paper. The filtrate was then transferred to a crucible and dried over a water bath; the dry content was weighed to a consistent weight. Then the flavonoid content was calculated as a percentage of the starting material.

3.4.3 Quantitative Test for Tannins

A 50 ml plastic bottle was filled with 500 mg of the plant sample. A mechanical shaker was used to shake 50ml of distilled water for 1 hour. This was filtered into a 50 ml volumetric flask and filled to the indicated level. The filtrate was then combined with 2 ml of 0.1M FeCl in 0.1M Hcl and 0.008M potassium ferrocyanide in a test tube. Within 10 minutes, the absorbance was measured at 605nm (Baliyan et al., 2022). The tannin content was determined using an extract standard curve.

3.4.4 Quantitative Test for Alkaloids

A total of 5g plant material were weighed into a 250 ml beaker, and 200ml of 10% acetic acid in ethanol was added. The reaction mixture was then covered and left to stand for four hours. This was filtered, and the extract was concentrated to one-quarter of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until the precipitation was complete. Allowing the entire solution to settle, the precipitate was collected, washed with dilute ammonium hydroxide, and then filtered; the residue was the alkaloid, which was dried and weighed to a constant weight.

Table 3.3 : Quantitative Analysis of the Phytochemical Screening of *P. purpereum, B. decumbens,* and *M. maximus* Leaf Meal

Parameters	*P. purpureum*	*B. decumbens*	*M. maximus*	p-value
Saponins (%)	1.16±0.23[b]	2.40±0.34[a]	1.06±0.04[b]	0.0124
Tannin (%)	1.50±0.07[a]	1.09±0.04[b]	1.41±0.02[a]	<0.000
Alkaloid (%)	1.45±0.14[b]	1.81±0.13[b]	4.59±0.17[a]	<0.000
Flavonoid (%)	1.96±0.03[a]	0.95±0.03[b]	1.00±0.04[b]	<0.000

All values were expressed as mean ± standard error.

3.5 Determination of Antioxidant Activity by DPPH Scavenging Activity Test

Table 3.4 summarises the antioxidant activity of *P. purpureum*, *B. decumbens*, and *M. maximus* by measuring DPPH radical scavenging activity. DPPH radical scavenging activity of the *P. purpureum*, *B. decumbens* and *M. maximus* leaf meals were estimated in line with the method designated by Jack et al. (2020) with some minor changes. First, 1.5g of sample was weighed on an analytical balance and homogenised with 6mL of methanol: water (80:20, v/v) using a homogeniser (Wiggen Hauser® D-500, Germany) at 10,000 rpm for one minute. Then, the mixture was centrifuged at 9840 G force for ten minutes using a micro-refrigerated centrifuge, before filtering the supernatant through Whatman filter paper No 1. 200µL aliquot of the supernatant was then added to 800 µL of distilled water and 1 mL of 0.2 mM methanolic DPPH solution. Next, the solution was vortexed using a test tube shaker at high speed for two minutes before incubating in the dark for 20 minutes. The control was prepared with DPPH solution in methanol only without samples. The absorbance was then measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was then calculated using the equation below:

$$Radical\ scavenging\ activity = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100$$

Table 3.4 : Antioxidant Activity of *P. purpureum*, *B. decumbens,* and *M. maximus* Leaf Meals

Sample grass	DPPH radical scavenging activity (%)
P. purpureum	67.73±0.01
B. decumbens	38.24±0.00
M. maximus	12.78±0.00

All values were expressed as mean ± standard error.

3.6 Animals Housing, Experimental Design, and Diets

All procedures involving animal care, handling, and sampling were carried out in accordance with the guidelines and regulations, and were authorised by the Institutional Animal Care and Use Committee (IACUC) of University of Putra Malaysia prior to the start of the research. In a fully randomised design (CRD) experimental approach, a total of 648-day-old broiler chicks (Sasso) were acquired from a local hatchery to be weighed and randomly distributed into three treatment groups with six treatment levels each, with six replications for each level and six broilers in each replicate. The broilers were raised in an open-sided house for 56 days in stainless-steel tiered cages of 113 cm x 82 cm x 45 cm with a stocking density of six chickens per cage. The mean temperature and relative humidity were observed throughout the research period with an average of 30.9°C and 71.24% respectively. Lighting program was implemented based on guidance from the Sasso Management Guide Rural Poultry by Hendrix Genetics (2022) and were maintained with natural day light, 40-watt fluorescent lamps, and a 60-watt bulb placed centrally in each cage cell, above the heads of the chickens. After two weeks, the 60-watt bulb was turned off and lighting was sustained with natural day light and florescent lamps. Light hours were provided constantly for 24 hours from the day of chick arrival until the seventh day, then lowered to 20 hours each day from the eighth to the fourteenth, and then maintained at 12 hours of natural daylight until the day of slaughter. On day 7, all

chicks were immunised intraocularly against infectious bronchitis (IB) and Newcastle disease (ND), and on day 14, they were immunised against infectious bursal disease (IBD) also via eye drop. Feed and freshwater were freely accessible.

The broilers were fed commercial diets that included maize and soybean meal as the base diet in crumble form. All feed was purchased from a local vendor and the starter diet were utilised from day 0 to day 28, while the finisher diet was used from day 29 to day 56. Treatment 1 (negative control) broilers were fed commercial diets devoid of antibiotics. Treatment 2 (positive control) broilers were fed commercial feeds containing 100mg/kg oxytetracycline antibiotic. Treatments 3, 4, 5, and 6 were fed the same commercial diets supplemented with 1.25, 2.5, 3.75, and 5.0 g/kg of *P. purpereum* grass meal but without antibiotics (A). The grass meals prepared were thoroughly mixed into the commercial feed with a commercial mixer (Manesty300) for a standardised time of 10 minutes to ensure uniform distribution. The same experimental design was conducted for another two studies using *B. decumbens* (B) and *M. maximus* (C) grass meals.

3.7 Feed Proximate Analysis

The AOAC (2006) dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash content were determined using the AOAC (2006) technique. Table 3.5 shows the composition of the basal diets for starter and finisher phases. The nutritional composition of starter and finisher diets supplemented with *P. purpereum*, *B. decumbens* and *M. maximus* grass meals at various levels are shown in Tables 3.6, 3.7 and 3.8.

Table 3.5 : Composition of the Basal Diets for Starter and Finisher Phases from Day 1 to Day 28 and Day 29 to Day 56 Respectively

Ingredients (%)	Starter	Finisher
Corn	46.00	52.1
Wheat bran	4.50	6.0
Soybean meal	40.00	32.0
L-Lysine	1.32	1.05
DL-Methionine	0.55	0.45
Choline chloride	0.18	0.20
Calcium carbonate	0.80	0.80
Palm oil	3.35	5.10
Dicalcium phosphate	2.60	1.60
Salt	0.30	0.30
Mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.15	0.15
Toxin binder	0.10	0.10
Total	100	100

[1] Mineral mix (provided per kg of the product): Selenium 0.30 g; iron 80.0 g; manganese 100.0 mg; zinc 80.0 g; copper 16.0 g; potassium 6.0 g; sodium 1.80 g; iodine 1.25 g and cobalt 0.25 g; [2] Vitamin premix (provided per kg of the product): Vitamin D3 9.0 MIU; vitamin A 35.0 MIU; vitamin K3 6.0 g; vitamin E 90.0 g; vitamin b2 22.0 g; vitamin B1 7.0 g; vitamin B12 0.070 g; vitamin B6 12.0 g; nicotinic acid 120.0 g; pantothenic acid 35.0 g; folic acid 3.0 g; cobalamin 0.05 mg; biotin 300.000 mg; phytase 25,000.0 FTU; folic acid 0.56 mg; thiamine 1.43 mg; riboflavin 3.44 mg; pantothenic acid 6.46 mg; biotin 0.05 mg; niacin 40.17 mg, and pyridoxine 2.29 mg.

Table 3.6 : Nutrient Composition of Broiler Diets Added with Different Levels of *P. purpereum* Grass Meal

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Starter diet (1-28 days)						
Metabolisable energy (MJ/kg)	12.67±0.22	12.45±0.19	12.67±0.22	12.45±0.19	12.67±0.22	12.45±0.19
Dry matter (%)	90.97±0.33	91.33±0.71	91.33±0.33	90.83±0.70	91.17±0.31	91.17±0.48
Crude protein (%)	22.46±0.20	22.13±0.07	22.42±0.09	22.42±0.18	22.60±0.51	23.20±0.19
Crude fibre (%)	3.50±0.50	3.50±0.56	3.50±0.50	4.33±0.42	3.67±0.33	4.00±0.52
Ether extract (%)	2.10±0.12	1.70±0.27	2.10±0.12	1.72±0.22	2.10±0.12	1.72±0.22
Ash (%)	5.53±0.28	5.95±0.19	5.95±0.26	6.45±0.30	5.83±0.35	5.45±0.16
Finisher diet (29-56 days)						
Metabolisable energy (MJ/kg)	19.05±0.13	19.01±0.15	19.05±0.03	19.09±0.19	18.79±0.19	19.01±0.12
Dry matter (%)	89.18±0.21	88.38±0.29	88.10±0.19	89.10±0.26	89.38±0.34	88.46±0.09
Crude protein (%)	19.78±0.43	19.53±0.02	19.55±0.11	19.97±0.21	19.86±0.11	19.79±0.38
Crude fibre (%)	3.29±0.11	3.69±0.14	3.61±0.36	3.23±0.25	3.55±0.29	3.75±0.04
Ether extract (%)	5.24±0.17	5.58±0.42	5.39±0.41	5.09±0.26	5.01±0.48	5.09±0.82
Ash (%)	4.76±0.19	5.59±0.06	4.68±0.45	4.75±0.08	5.38±0.41	5.71±0.17

All values were expressed as mean ± SE. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetraxycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Table 3.7 : Nutrient Composition of Sasso Broilers Diets Added with Different Levels of *B. decumbens* Grass Meal

	Treatments					
Parameters	T1	T2	T3	T4	T5	T6
Starter diet (1-28 days)						
Metabolisable energy (MJ/kg)	12.88±0.15	12.55±0.14	12.88±0.28	12.50±0.27	12.66±0.14	12.57±0.24
Dry matter (%)	90.67±0.33	91.33±0.71	90.50±0.22	91.50±0.22	91.17±0.65	90.00±0.45
Crude protein (%)	23.92±0.44	23.13±0.16	23.85±0.34	23.99±0.38	24.20±0.13	24.34±0.16
Crude fibre (%)	3.23±0.08	3.27±0.10	3.37±0.09	3.28±0.10	3.20±0.10	3.17±0.06
Ether extract (%)	4.10±0.12	4.03±0.08	4.23±0.14	4.32±0.07	4.15±0.16	4.02±0.09
Ash (%)	5.70±0.34	5.94±0.24	5.52±0.01	5.67±0.21	6.31±0.23	5.96±0.08
Finisher (29-56 days)						
Metabolisable energy (MJ/kg)	13.04±0.09	13.00±0.11	13.39±0.04	13.28±0.05	13.40±0.07	13.17±0.04
Dry matter (%)	89.25±0.32	88.23±0.35	88.80±0.26	88.18±0.09	89.14±0.14	88.86±0.10
Crude protein (%)	19.78±0.23	19.86±0.20	19.98±0.31	19.97±0.42	19.94±0.34	19.94±0.14
Crude fibre (%)	3.29±0.11	3.42±0.08	3.46±0.09	3.33±0.09	3.06±0.22	3.38±0.23
Ether extract (%)	6.85±0.02	6.97±0.02	6.95±0.03	6.89±0.02	6.85±0.05	6.92±0.03
Ash (%)	5.17±0.07	5.59±0.06	4.92±0.21	5.42±0.09	5.00±0.11	5.01±0.29

All values were expressed as mean ± SE. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetraxycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Table 3.8 : Nutrient Composition of Sasso Broilers Diets Added with Different Levels of *M. maximus* Grass Meal

	Treatments					
Parameters	T1	T2	T3	T4	T5	T6
Starter diet (1-28 days)						
Metabolisable energy (MJ/kg)	13.00±0.84	12.45±0.51	12.15±0.50	12.17 ±0.79	12.13±0.66	18.02±0.69
Dry matter (%)	90.67±0.33	91.33±0.71	90.83±0.60	91.17±0.31	90.50±0.34	90.67±0.21
Crude protein (%)	21.96±1.70	22.13±0.08	22.56±0.09	22.65±0.40	22.93±1.42	21.02±0.95
Crude fibre (%)	3.50±0.50	3.40±0.68	2.80±0.20	2.60±0.40	2.25±0.63	2.83±0.17
Ether extract (%)	4.93±0.12	4.70±0.27	4.86±0.31	4.57±0.87	4.53±0.19	4.60±0.33
Ash (%)	5.70±0.34	5.94±0.24	6.43±0.35	7.13±0.46	6.99±0.36	6.07±0.25
Finisher (29-56 days)						
Metabolisable energy (MJ/kg)	13.04±0.13	12.00±0.15	12.71±0.36	12.00±0.11	12.76±0.25	12.09±0.25
Dry matter (%)	89.85±0.43	88.04±0.34	89.00±0.17	88.55±0.20	89.34±0.44	89.20±0.23
Crude protein (%)	19.78±0.43	19.36±0.17	19.92±0.25	20.16±0.33	17.63±1.34	18.63±0.82
Crude fibre (%)	3.29±0.11	3.69±0.14	4.03±0.36	1.76±0.32	2.68±0.46	3.46±0.21
Ether extract (%)	5.24±0.17	5.78±0.42	5.13±0.15	6.11±0.41	7.52±0.33	5.09±0.62
Ash (%)	13.00±0.84	12.45±0.51	12.15±0.50	12.17 ±0.79	12.13±0.66	18.02±0.69

All values were expressed as mean ± SE. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetraxycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

3.8 Growth Performance

Throughout the eight-week study period, body weight (BW) and feed intake (FI) were recorded weekly for each replicate using a digital weighing scale with a measurement accuracy of two decimal points (Mettler Toledo Industrial Scale, BBA211 series, Greifensee, Switzerland) to determine body weight gain (BWG) and feed intake. Feed conversion ratio (FCR) were then calculated and recorded according to the formula:

$$FCR = \frac{FI}{BWG}$$

3.9 Sample Collection and Analyses

On day 56, six chickens (three male and three female) were randomly selected and slaughtered from each treatment group level to determine nutritional digestibility, gut histomorphology, cecal microbiota population, carcass features, and meat quality. The broilers were slaughtered in accordance with Halal standards in the slaughterhouse of the Department of Animal Science, Faculty of Agriculture, UPM. Blood were also obtained to determine the concentration of acute phase proteins, corticosterone, and heat shock proteins, as well as other biochemical markers such as liver function and serum lipid profile.

3.9.1 Nutrient Digestibility

As an indigestible marker, 300 mg/kg titanium dioxide (TiO_2) was introduced to the starter and finisher diets three days before slaughter. Following slaughter, the ileal material were collected and kept at -20 °C for subsequent nutritional content analysis. 100 mg of each sample was placed in porcelain crucibles in a furnace heated at 580 °C for 13 hours, then 10 ml of 7.4 M sulphuric acid was added to each crucible after

cooling. The solution was put into a beaker containing 25 ml of distilled water after boiling for one hour or until dissolved. Whatman filter paper No. 541 was used to transfer the solution into a 100 ml volumetric flask. Each flask received 20 ml of 30% hydrogen peroxide (H_2O_2) and was topped up to 100 ml with distilled water.

Then, a 0.3 mg/ml working standard TiO_2 solution was created. The standard solution was made by boiling 150 mg of pure TiO_2 in 100 ml of concentrated H_2SO_4. After cleaning with 200 ml distilled water, the contents were carefully placed into a 500 ml volumetric flask. A further 100 ml of concentrated H_2SO_4 was added to the solution, followed by another 500 ml of distilled water. Pipette 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ml of 0.3 mg/ml TiO_2 into separate 100 ml volumetric flasks to create the standard curve. A combined volume of 10 ml was obtained by adding 10 ml of 7.4 M H_2SO_4. Each volumetric flask was filled with 20 ml (30%) H_2O_2 and was topped up to 100 ml with distilled water. The sample devoid of TiO_2 was used as a blank to calibrate the spectrophotometer. All samples and working standard solutions were measured using a spectrophotometer set to 410 nm, and the relationship between optical density and concentration was linear up to 0.03 mg/ml. (Alghirani et al., 2021).

Apparent ileal digestibility (AID) of dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash were calculated using titanium marker ratios in diet and ileal content using the given equations according to Hartinger, et al. (2022):

$$Apparent\ ileal\ digestibility\ (AID) = 100\% - [(\frac{\%\,\%\,TiO2\ in\ feed}{TiO2\ in\ ileal\ content}) \times (\frac{\%\ of\ nutrient\ in\ ileal\ content}{\%\ nutrient\ in\ feed}) \times 100]$$

Table 3.9 : Standard Solution of Titanium Dioxide

		Amount (ml)			
TiO_2 (0.3mg/ml)	7.4 M H_2SO_4	H_2O_2 (30%)	dH_2O	Total	TiO_2 concentration(mg/ml)
0	10	20	70	100	0
1	9	20	70	100	0.003
2	8	20	70	100	0.006
3	7	20	70	100	0.009
4	6	20	70	100	0.012
5	5	20	70	100	0.015
6	4	20	70	100	0.018
7	3	20	70	100	0.021
8	2	20	70	100	0.024
9	1	20	70	100	0.027
10	0	20	70	100	0.03

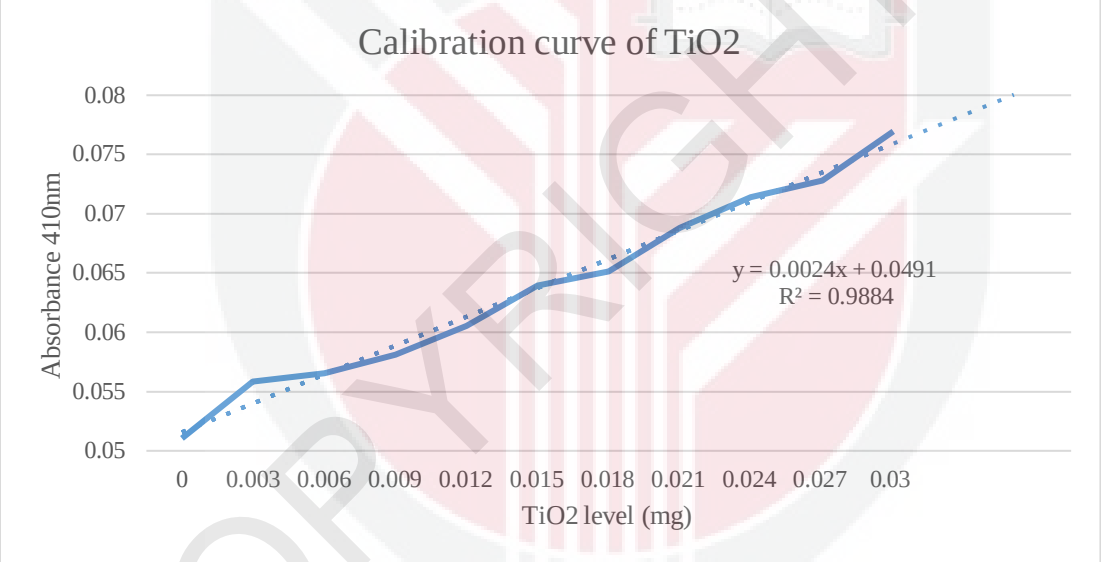

Figure 3.1 : Calibration Curve of TiO_2

3.9.2 Gut Histomorphology

For gut histomorphology, this procedure was carried out in accordance with a standard practice at the Histopathology Lab, Faculty of Veterinary Medicine, UPM (Alghirani et al., 2021). First, 5 cm of the duodenum, jejunum, and ileum were removed, flushed with neutral buffered saline, and stored in 10% formalin solution overnight. Before

excising, all samples were dried in a tissue processing machine and embedded in paraffin wax. Then, all intestinal samples were sliced at 4 µm on a glass slide, stained with hematoxylin and eosin on a hot plate at 60°C, mounted, to be observed under a Nikon DS-U2/L2 light microscope. NIS-Elements D software was used to investigate, capture, and determine the villi height and crypt depth. The height of the villi was measured from tip to crypt transition, while the depth of the crypt was calculated at the invagination between two villi.

3.9.3 Cecal Microflora Population

Upon slaughtering on day 56, cecal material were taken from each treatment group level and immediately transferred to the Microbiology Lab, Faculty of Veterinary Medicine, UPM, in an ice box with ice for microorganism isolation and identification, *Salmonella* identification, standard plate count, and coliform count (Park and Kim, 2020). The respective viable bacteria colonies were enumerated and expressed as the $\log_{10}$ of colony-forming units (cfu)g^{-1} of cecal content.

3.9.4 Carcass Characteristics

Following slaughter, all carcass parameters were manually dissected and the following were recorded: final live weight, kill-out weight, de-feathered weight, dressing percentage, the weight of the breast muscle (both left and right pectoralis major and minor), drumsticks, wings, head, shank, gastrointestinal tract, heart, liver, full and empty gizzard (Chung et al., 2020).

3.9.5 Meat Quality Analysis

Chung et al. (2019) protocols were followed to measure pH, colour, drip loss, cooking loss, and tenderness. After slaughtering, the right pectoralis major (breast muscle) and right soleus muscle (drumstick) were extracted from each replicate for all meat quality evaluations.

3.9.5.1 pH Measurement

The right pectoralis major (breast muscle) and right soleus muscle (drumstick) were removed for pH determination and snap-frozen in liquid nitrogen (-195 ℃) before being kept at -80 °C to retain the meat's pH characteristics before further examination. After 24 hours, the materials were mashed with a mortar and pestle and homogenised. A homogeniser (Wiggen Hauser® D-500, Germany) was used to homogenise about 0.5 g of each crushed sample for 20 seconds in 10 ml distilled water. The pH was then determined using a portable pH metre (Mettler Toledo, AG 8603, Greifensee, Switzerland). Prior to usage, the pH metre was calibrated with a pH 4.0 buffer and subsequently with a pH 7.0 buffer.

3.9.5.2 Colour Analysis

A colour flex spectrophotometer (Hunter Lab, Reston, VA, USA) was used to determine colour, using 30 g of breast and drumstick muscle from each replicate in each treatment group. After being transported from a -80 ℃ freezer, the meat samples were thawed overnight in a 4°C chiller and bloomed at ambient temperature 27 ℃ for 30 minutes before examination (AMSA, 2012). The device was calibrated at a wavelength of 400 to 700 nm, with three measurements obtained at three separate sites

for each breast and drumstick sample, and the data were averaged (Hunt, 1980). The colour flex spectrophotometer supplied L* (lightness), b* (yellowness), and a* (redness) based on the gross appearance of the colour of the meat samples.

3.9.5.3 Water Holding Capacity

Water holding capacity (WHC) was determined in terms of drip loss and cooking loss based on the method as described by Chung, et al. (2020).

3.9.5.4 Drip Loss Measurement

For drip loss, on day 0, approximately 40 g of breast and drumstick samples was weighed and recorded as the beginning weight from each replicate in each treatment group (W1). All samples were then placed in plastic bags that are vacuum-sealed and maintained in the cooler at 4 °C. The samples were removed from the bags after 24 and 48 hours, and the final weight was determined (W2). The percentage of drip loss was calculated using the following formula:

$$Drip\ loss\ percentage = \frac{(W1 - W2)}{W1} \times 100$$

3.9.5.5 Cooking Loss Measurement

The vacuum-packed frozen breast and drumstick were allowed to thaw for 24 hours in a chiller at 4 °C. For cooking loss, approximately 30 g of breast and drumstick muscle were extracted and weighed as the beginning weight from each replicate in each treatment group (W1). The samples were then placed in plastic vacuum-packed bags and soaked for 20 minutes in an 80 °C water bath. The samples were then removed from the water bath to enable the bags to cool before being weighed to

determine the final weight (W2). The following formula was used to calculate the cooking loss:

$$Cooking\ loss\ percentage = \frac{(W1 - W2)}{W1} \times 100$$

3.9.5.6 Meat Tenderness Measurement

After measuring the cooking loss, the cooked meat was tested for tenderness. The test was based on the mechanical force (kg) required to shear the muscle fibres of a cooked meat sample. The textural assessment of cooked poultry flesh softness was done using a Volodkovich blade set with the TA HD plus® texture analyser (Stable Micro Device, Surrey, UK), which was balanced by weight at 5 kg, have a return distance of 10 mm by height, and a blade speed of 10 mm/sec. Each sample was divided into three strips ($1 \times 1 \times 2$ cm^3) that are to be cut as parallel to the direction of the muscle fibres as feasible. The Volodkevitch jaw (a stainless steel probe fashioned like a jaw) was be used to shear each sample on a texture analyser (Stable Micro System, Surrey, UK).

3.9.6 Blood Samples Collection

On day 56, blood samples from the same six chickens from each treatment chosen at random and slaughtered was collected. Blood samples for plasma were collected into blood collection tubes containing the anticoagulant EDTA (BD vacutainer®, New Jersey, USA) and kept on ice. Blood samples were centrifuged at 3000 g for 10 minutes at 4 °C. For further analysis, the plasma was decanted into 1.5 ml microcentrifuge tubes and stored at -80 °C. Acute phase proteins, corticosterone, heat shock protein responses, and biochemical analyses were all measured from the blood samples.

3.9.6.1 Corticosterone Concentration

A double-antibody sandwich enzyme-linked immunoabsorbent one-step process assay (ELISA) kit was used to determine the level of chicken corticosterone (CS) in plasma samples. The ELISA kit for chicken CS was purchased from QAYEE-BIO for Life Science (China), and was used according to manufacturer's instructions. The standard diluents were first diluted into 2000, 1000, 500, 250, 125, and 0 ng/ml before being introduced to the standard wells, while the washing dilution was diluted by 1:20 distilled water. The calibrator was serially diluted to produce a six-point calibrator curve with concentrations ranging from 31.2 pg/ml-2000pg/ml. Next, the blank, standard, and test sample wells, were established in that order. In standard wells, 50 µl of standard was added, but in test sample wells, 40 µl of special diluent was added to 10 µl of sample. Then, 50 µl of horseradish peroxidise (HRP) was then added into all wells except blank wells before sealing, gentle shaking and incubating for 60 minutes at 37°C. Following that, surplus liquid was thrown, the plate was dried, filled with washing liquid, mixed and shaken for 30 seconds, then dumped the washing liquid once more and tapped the plate onto absorbent paper to dry. This technique was carried out five times. After drying, 50 µl of chromagen solution A and 50 µl of chromagen solution B were applied to each well. The plate was gently shaken and incubated at 37 °C away from light for 10 minutes. The reaction was then stopped by adding 50 µl of stop solution to each well. Finally, 15 minutes after applying the stop solution, the optical density (OD) values at 450 nm wavelength were assessed using a BioRad ELISA Reader. The standard curve linear regression equation was computed based on the concentration of standards and the corresponding OD values, and then the OD values of the sample were applied to the regression equation to calculate the concentration of the corresponding sample using MyAssays software.

3.9.6.2 Acute-Phase Proteins Concentration

3.9.6.2.1 Serum Amyloid A

A double-antibody sandwich enzyme-linked immunoabsorbent one-step process assay (ELISA) kit was used to determine the level of chicken serum amyloid A (SAA) in plasma samples. The ELISA kit for chicken SAA was purchased from QAYEE-BIO for Life Science (China), and was used according to manufacturer's instructions. The standard diluents were first diluted into 120, 60, 30, 15, 7.5, and 0 ng/ml before being introduced to the standard wells, while the washing dilution was diluted by 1:20 distilled water. The calibrator was serially diluted to produce a six-point calibrator curve with concentrations ranging from 0.312ng/ml-120ng/ml. After that, the blank, standard, and sample wells were assigned in that order. In standard wells, 50 µl of standard was added, but in test sample wells, 40 µl of special diluent was added to 10 µl of sample. Then, 50 µl of horseradish peroxidise (HRP) was then added into all wells except blank wells before sealing, gentle shaking and incubating for 60 minutes at 37℃. Following that, surplus liquid was thrown, the plate was dried, filled with washing liquid, mixed and shaken for 30 seconds, then dumped the washing liquid once more and tapped the plate onto absorbent paper to dry. This technique was carried out five times. After drying, 50 µl of chromagen solution A and 50 µl of chromagen solution B were applied to each well. The plate was gently shaken and incubated at 37 ℃ away from light for 10 minutes. The reaction was then stopped by adding 50 µl of stop solution to each well. Finally, 15 minutes after applying the stop solution, the optical density (OD) values at 450 nm wavelength were assessed using a BioRad ELISA Reader. The standard curve linear regression equation was computed based on the concentration of standards and the corresponding OD values, and then the OD

values of the sample were applied to the regression equation to calculate the concentration of the corresponding sample using MyAssays software.

3.9.6.2.2 Alpha-1-Acid Glycoprotein

A double-antibody sandwich enzyme-linked immunoabsorbent one-step process assay (ELISA) kit was used to determine the level of chicken alpha-1-acid glycoprotein (AGP) in plasma samples. The ELISA kit for chicken AGP was purchased from QAYEE-BIO for Life Science (China), and was used according to manufacturer's instructions. The standard diluents were first diluted into 400, 200, 100, 50, 25, and 0 ng/ml before being introduced to the standard wells, while the washing dilution was diluted by 1:20 distilled water. The calibrator was serially diluted to produce a six-point calibrator curve with concentrations ranging from 6.25 ng/ml-400ng/ml. Subsequently, the blank, standard, and test sample wells were determined, in that order. In standard wells, 50 µl of standard was added, but in test sample wells, 40 µl of special diluent was added to 10 µl of sample. Then, 50 µl of horseradish peroxidise (HRP) was then added into all wells except blank wells before sealing, gentle shaking and incubating for 60 minutes at 37°C. Following that, surplus liquid was thrown, the plate was dried, filled with washing liquid, mixed and shaken for 30 seconds, then dumped the washing liquid once more and tapped the plate onto absorbent paper to dry. This technique was carried out five times. After drying, 50 µl of chromagen solution A and 50 µl of chromagen solution B were applied to each well. The plate was gently shaken and incubated at 37 °C away from light for 10 minutes. The reaction was then stopped by adding 50 µl of stop solution to each well. Finally, 15 minutes after applying the stop solution, the optical density (OD) values at 450 nm wavelength were assessed using a BioRad ELISA Reader. The standard curve linear regression

equation was computed based on the concentration of standards and the corresponding OD values, and then the OD values of the sample were applied to the regression equation to calculate the concentration of the corresponding sample using MyAssays software.

3.9.6.2.3 Ceruloplasmin

A double-antibody sandwich enzyme-linked immunoabsorbent one-step process assay (ELISA) kit was used to determine the level of chicken ceruloplsamin (CP) in plasma samples. The ELISA kit for chicken CP was purchased from QAYEE-BIO for Life Science (China), and was used according to manufacturer's instructions. The standard diluents were first diluted into 100, 50, 25, 12.5, 6.5, and 0 µg/ml before being introduced to the standard wells, while the washing dilution was diluted by 1:20 distilled water. The calibrator was serially diluted to produce a six-point calibrator curve with concentrations ranging from 1.56 µ g/mL-400 µ g/mL. The blank, standard, and test sample wells was then established in that order. In standard wells, 50 µl of standard was added, but in test sample wells, 40 µl of special diluent was added to 10 µl of sample. Then, 50 µl of horseradish peroxidise (HRP) was then added into all wells except blank wells before sealing, gentle shaking and incubating for 60 minutes at 37°C. Following that, surplus liquid was thrown, the plate was dried, filled with washing liquid, mixed and shaken for 30 seconds, then dumped the washing liquid once more and tapped the plate onto absorbent paper to dry. This technique was carried out five times. After drying, 50 µl of chromagen solution A and 50 µl of chromagen solution B were applied to each well. The plate was gently shaken and incubated at 37 °C away from light for 10 minutes. The reaction was then stopped by adding 50 µl of stop solution to each well. Finally, 15 minutes after applying the stop solution, the

optical density (OD) values at 450 nm wavelength were assessed using a BioRad ELISA Reader. The standard curve linear regression equation was computed based on the concentration of standards and the corresponding OD values, and then the OD values of the sample were applied to the regression equation to calculate the concentration of the corresponding sample using MyAssays software.

3.9.6.3 Heat Shock Protein 70

A double-antibody sandwich enzyme-linked immunoabsorbent one-step process assay (ELISA) kit was used to determine the level of chicken heat shock protein (HSP70) high sensitivity in plasma samples. The ELISA kit for chicken HSP70 was purchased from QAYEE-BIO for Life Science (China), and was used according to manufacturer's instructions. Before adding the standard diluents to the standard wells, the standard diluents were diluted into 1600, 800, 400, 200, 100, and 0 pg/ml, while the washing dilution was diluted by 1:20 distilled water. The calibrator was serially diluted to produce a six-point calibrator curve with concentrations ranging from 15.6pg/ml-1600 pg/ml. The blank, standard, and sample wells were then arranged and established. In standard wells, 50 µl of standard was added, but in test sample wells, 40 L of special diluent was added to 10 µl of sample. Then, 50 µl of horseradish peroxidase (HRP) was added to all wells except the blank wells before sealing, gently shaking, and incubating at 37 °C for 60 minutes. Following that, surplus liquid was thrown, the plate was dried, filled with washing liquid, mixed and shaken for 30 seconds, then dumped the washing liquid once more and tapped the plate onto absorbent paper to dry. This technique was carried out five times. After drying, 50 µl of chromagen solution A and 50 µl of chromagen solution B were applied to each well. The plate was gently shaken and incubated at 37 °C away from light for 10 minutes.

The reaction was then stopped by adding 50 µl of stop solution to each well. Finally, 15 minutes after applying the stop solution, the optical density (OD) values at 450 nm wavelength were assessed using a BioRad ELISA Reader. The standard curve linear regression equation was computed based on the concentration of standards and the corresponding OD values, and then the OD values of the sample were applied to the regression equation to calculate the concentration of the corresponding sample using MyAssays software.

3.9.6.4 Blood Biochemical Analysis

3.9.6.4.1 Liver Function

Alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST) parameters were determined in the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Putra Malaysia using a BA400 biochemical and turbidimetry analyser from Spain (Manual code TEUS00048-07-EN).

3.9.6.4.2 Lipid Profile

The plasma total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured using an automatic analyser (Automatic analyser 902, Hitachi, Germany). The Friedewald Equations (Friedewald et al., 1972) were used to estimate LDL and VLDL:

$$LDL = Total\ cholesterol - HDL - VLDL$$

$$VLDL = \frac{Triglycerides}{5}$$

3.10 Statistical Analysis

All data collected was uploaded in RStudio version 4.1.3 where the data was analysed using one-way analysis (ANOVA) based on the completely randomised design model (RStudio Team, 2020). Tukey's HSD post-hoc test was used to determine the significant difference among treatment groups if any. Results would be considered significant at $p<0.05$. For mortality, Chi-square test was used as a non-parametric statistical test.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Growth Performance

4.1.1 Experiment A (*P. purpereum*)

Table 4.1 presents the effect of *P. purpereum* leaf meal supplementation on the growth performance of Sasso broilers on days 28 and 56. During the starter phase, only cumulative FCR had significant difference ($p<0.000$) whereas during the finisher phase feed intake ($p=0.037$) and cumulative FCR ($p<0.000$) had significant differences amongst treatment groups. Broilers in T6 supplemented with 5.00g/kg of *P. purpureum* had the lowest FCR during both phases. T6 chickens had the lowest feed intake (3.11kg) during the finisher phase but was not significantly different ($p>0.05$) compared to the other treatments except T3 (3.46kg). This signifies that T6 had the best production efficiency.

4.1.2 Experiment B (*B. decumbens*)

The effect of *B. decumbens* leaf meal supplementation on the growth performance of Sasso broilers on days 28 and 56 is shown in Table 4.2. Only feed intake and cumulative FCR showed significant differences ($p<0.05$) during both the starter and finisher phases. Feed consumption of broilers treated with *B. decumbens* leaf meal, regardless of inclusion level was equivalent ($p>0.05$) to the T2 (antibiotic group) during both periods. Furthermore, during the starter phase, the FCR of T4 to T6 performed similarly ($p>0.05$) to T2. Whereas during the finisher phase, the FCR of T5 and T6 were comparable ($p>0.05$) to T2. Overall, this demonstrates that increasing the

amount of *B. decumbens* leaf meal added had a superior effect on broiler output, with T6 having comparable outcomes to T2.

4.1.3 Experiment C (*M. maximus*)

Table 4.3 shows the effect of *M. maximus* leaf meal supplementation on the growth performance of Sasso broilers on days 28 and 56. Treatments showed significant differences ($p<0.05$) in all growth performance indicators throughout the starter and finisher phases. Despite having a higher feed consumption throughout both stages, broiler chicks treated with *M. maximus* leaf meal exhibited a greater body weight increase and final body weight. Thus, higher levels of *M. maximus* supplementation also resulted in lower FCR. There was no significant different in the FCR between T6 and T2 broilers.

4.1.4 Comparison of the Best Treatments from Experiments A, B, and C

Table 4.4 compares the growth performance of the best treatments from trials A (T6), B (T6), and C (T6). Throughout the feeding trial, there was a considerable variation ($p<0.05$) in the feed intake and hence, a significant difference ($p<0.05$) in the FCR. Broilers fed 5.00g/kg *P. purpureum* exhibited the lowest FCR (1.82) compared to *B. decumbens* (1.92) and *M. maximus* (1.93), indicating greater growth performance.

Table 4.1 : Effect of *P. purpereum* Leaf Meal Supplementation on the Growth Performance of Sasso Broilers on Days 28 and 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
28-day-old (starter phase)							
Initial body weight (kg)	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.835
Final body weight (kg)	0.65±0.01	0.68±0.01	0.64±0.02	0.63±0.03	0.62±0.02	0.67±0.01	0.078
Body weight gain (kg)	0.61±0.01	0.63±0.01	0.60±0.02	0.58±0.03	0.57±0.02	0.63±0.01	0.078
Feed intake (kg)	0.97±0.02	0.92±0.01	0.93±0.02	0.89±0.04	0.88±0.02	0.88±0.01	0.067
Cumulative FCR	1.59±0.00^{a}	1.46±0.01^{b}	1.55±0.01^{a}	1.54±0.00^{a}	1.54±0.03^{a}	1.41±0.02^{b}	<0.000
Mortality bird	0	0	1	2	1	1	0.639
56-day-old (finisher phase)							
Final body weight (kg)	1.61±0.04	1.69±0.02	1.67±0.04	1.64±0.05	1.68±0.03	1.75±0.06	0.284
Body weight gain (kg)	1.57±0.04	1.65±0.02	1.63±0.04	1.59±0.05	1.64±0.03	1.71±0.06	0.284
Feed intake (kg)	3.36±0.08^{ab}	3.18±0.03^{ab}	3.46±0.08^{a}	3.20±0.10^{ab}	3.20±0.06^{ab}	3.11±0.10^{b}	0.037
Cumulative FCR	2.14±0.02^{a}	1.93±0.01^{c}	2.12±0.02^{a}	2.01±0.00^{b}	1.96±0.01^{bc}	1.82±0.00^{d}	<0.000
Mortality bird	0	0	1	0	0	0	0.416

All values were expressed as mean ± SE; superscripts $^{a, b, c, and d}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg; FCR: feed conversion ratio.

Table 4.2 : Effect of *B. decumbens* Leaf Meal Supplementation on the Growth Performance of Sasso Broilers on Days 28 and 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
28-day-old (starter phase)							
Initial body weight (kg)	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.835
Final body weight (kg)	0.69±0.01	0.65±0.02	0.66±0.02	0.63±0.01	0.66±0.02	0.65±0.01	0.232
Body weight gain (kg)	0.65±0.03	0.60±0.03	0.62±0.04	0.59±0.03	0.62±0.02	0.61±0.02	0.232
Feed intake (kg)	1.04±0.01^{a}	0.82±0.02^{b}	0.94±0.02^{ab}	0.86±0.01^{b}	0.87±0.02^{b}	0.84±0.01^{b}	<0.000
Cumulative FCR	1.60±0.04^{a}	1.35±0.01^{c}	1.52±0.04^{ab}	1.47±0.02^{abc}	1.41±0.05^{bc}	1.36±0.00^{c}	<0.000
Mortality bird	0	1	0	0	0	1	0.549
56-day-old (finisher phase)							
Final body weight (kg)	1.80±0.06	1.72±0.03	1.78±0.04	1.75±0.01	1.70±0.03	1.74±0.06	0.550
Body weight gain (kg)	1.76±0.06	1.68±0.03	1.73±0.04	1.71±0.01	1.66±0.03	1.70±0.06	0.550
Feed intake (kg)	3.62±0.12^{a}	3.21±0.07^{b}	3.54±0.08^{ab}	3.45±0.02^{ab}	3.30±0.06^{ab}	3.26±0.10^{b}	0.006
Cumulative FCR	2.06±0.02^{a}	1.91±0.02^{d}	2.04±0.01^{ab}	2.01±0.02^{ab}	1.99±0.01^{bc}	1.92±0.00^{cd}	<0.000
Mortality bird	1	0	0	1	1	1	0.849

All values were expressed as mean ± SE; superscripts $^{a, b, c, \text{and } d}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg; FCR: feed conversion ratio.

Table 4.3 : Effect of *M. maximus* Leaf Meal Supplementation on the Growth Performance of Sasso Broilers on Days 28 and 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
28-day-old (starter phase)							
Initial body weight (kg)	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.835
Final body weight (kg)	0.65±0.02^{ab}	0.62±0.02^{b}	0.60±0.01^{b}	0.65±0.02^{ab}	0.65±0.02^{ab}	0.71±0.02^{a}	0.011
Body weight gain (kg)	0.61±0.02^{ab}	0.57±0.02^{b}	0.56±0.01^{b}	0.60±0.02^{ab}	0.61±0.02^{ab}	0.67±0.02^{a}	0.011
Feed intake (kg)	1.00±0.04^{a}	0.78±0.03^{c}	0.88±0.02^{bc}	0.89±0.02^{ab}	0.86±0.01^{bc}	0.91±0.02^{ab}	<0.000
Cumulative FCR	1.63±0.00^{a}	1.35±0.01^{c}	1.42±0.01^{a}	1.48±0.02^{b}	1.42±0.04^{bc}	1.37±0.02^{c}	<0.000
Mortality bird	0	1	0	1	0	1	0.849
56-day-old (finisher phase)							
Final body weight (kg)	1.69±0.04^{ab}	1.62±0.05^{b}	1.63±0.05^{b}	1.70±0.04^{ab}	1.80±0.05^{ab}	1.87±0.04^{a}	0.002
Body weight gain (kg)	1.65±0.04^{ab}	1.58±0.05^{b}	1.59±0.05^{b}	1.67±0.04^{ab}	1.76±0.05^{ab}	1.83±0.04^{a}	0.002
Feed intake (kg)	3.39±0.09^{ab}	3.03±0.1^{b}	3.22±0.11^{ab}	3.36±0.09^{ab}	3.38±0.05^{ab}	3.54±0.07^{a}	0.006
Cumulative FCR	2.06±0.01^{a}	1.92±0.01^{b}	2.02±0.01^{a}	2.02±0.02^{a}	1.93±0.03^{b}	1.93±0.01^{b}	<0.000
Mortality bird	1	1	1	0	0	4	0.098

All values were expressed as mean ± SE; superscripts $^{a, b, \text{and } c}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg; FCR: feed conversion ratio.

Table 4.4 : Effects of *P. purpureum, B. decumbens,* and *M. maximus* Leaf Meal Supplementation on the Growth Performance of Sasso Broilers on Days 28 and 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
28-day-old (starter phase)				
Initial body weight (kg)	0.04±0.00	0.04±0.00	0.04±0.00	0.835
Final body weight (kg)	0.67±0.01^{ab}	0.65±0.01^{b}	0.71±0.02^{a}	0.0504
Body weight gain (kg)	0.63±0.01	0.61±0.02	0.67±0.02	0.0599
Feed intake (kg)	0.88±0.01^{ab}	0.84±0.01^{b}	0.91±0.02^{a}	0.0178
Cumulative FCR	1.41±0.02	1.36±0.00	1.37±0.02	0.0752
Mortality bird	1	1	1	1.00
56-day-old (finisher phase)				
Final body weight (kg)	1.75±0.06	1.74±0.06	1.87±0.04	0.1392
Body weight gain (kg)	1.71±0.06	1.70±0.06	1.83±0.04	0.1447
Feed intake (kg)	3.11±0.10^{b}	3.26±0.10^{ab}	3.54±0.07^{a}	0.0187
Cumulative FCR	1.82±0.00^{b}	1.92±0.00^{a}	1.93±0.01^{a}	<0.000
Mortality bird	0	1	4	0.0743

All values were expressed as mean ± SE; superscripts $^{a \text{ and } b}$ values within the row are significantly different at $p < 0.05$. FCR: feed conversion ratio.

Achieving optimal growth performance in broiler production is of the utmost importance since it has a direct impact on economic viability. A recent meta-analysis demonstrated that broilers given AGP-containing diets had better performance indices than those without (Cardinal et al., 2019). However, the excessive and persistent use of AGPs has resulted in the emergence of AMR, which poses a worldwide health concern to both human and animal health (Elmi et al., 2021). In order to combat this, countries have increasingly sought to prohibit or restrict the use of AGPs in livestock. This has spurred the interest in alternatives, such as phytobiotics, which encompasses bioactive compounds believed to have comparable properties to synthetic AGPs (Basit et al., 2020b). Phytobiotic supplementation may improve growth performance by improving FI, favourably modifying gastrointestinal dynamics, boosting feed efficiency through digestion and absorption, as well as activating endocrine and antioxidative defence mechanism (Valenzuela-Grijalva et al., 2017). As a result, there is a potential for their utilisation in enhancing broilers' growth performance.

The current study (experiment A) discovered that broilers fed with 5.00g/kg of *P. purpureum* grass meal had lower cumulative FCR in both the starter and finisher phases, with significantly lowered feed consumption during the finisher phase. The findings support the notion that despite decreased feed intake, active compounds in the grass have a good influence on the gastrointestinal tract because they encourage increased production of digestive enzymes, which aids in nutritional digestion and absorption (Rahimi et al., 2011). Qualitative analysis of phytocompounds present in *P. purpureum* grass used in experiment A displayed that it contained tannins, saponins, flavonoids, and phenolic flavonoids. These bioactive compounds stimulate a physiological reaction in the animals' bodies, acting as growth stimulants and nutrient

additives (Kuralkar and Kuralkar, 2021). Phenols are also thought to alter the gut microflora which enhances glycolysis and utilisation of glucose which could further explain the improved FCR despite lower FI (Mbikay, 2012; Nkukwana, 2012). To our knowledge, no prior research has been undertaken to investigate the effect of *P. purpureum* on growth performance of poultry. Therefore, the outcome of this study was novel. The findings of this study was in line with prior experiments with leaves from various plants. One such study by Xue et al. (2021) attributed improvements in the FCR to the use of a Kudzu-leaf treatment—a flavonoid-rich supplement. This association stems from an increase in gut flavonoid levels, which leads to a more balanced gut microbiota. As a result of this better balance, feed absorption is improved—resulting in increased feed efficiency. On top of that, flavonoid-rich guava-leaf meal demonstrated antibacterial and antioxidative activities, promoting gut health even further (Abang et al., 2023). Additionally, previous researches by Jang et al. (2004) and Czech et al. (2009) suggested that the combination of increased antimicrobial activity and production of digestive enzymes is thought to be the key to improved growth. Given that flavonoids were the most abundant phytocompound in *P. purpureum*, it is possible that they played a significant role in optimizing growth performance via changes in nutrient absorption physiology.

In the second experiment (experiment B), results showed that supplementation of 5.00g/kg of *B. decumbens* had the best growth performance which were comparable to the antibiotic-treated group. Quantitative analysis confirmed that saponins were the most abundant phytocompound present *B. decumbens* leaves, consistent with previous studies (Lima et al., 2012; Kassim et al., 2023). The inclusion of saponin-rich supplementation in broiler diets at appropriate levels has been proven to boost growth

performance and feed efficiency, which is consistent with the findings of this study (Chaudhary et al., 2018). The observed positive results in experiment B can be ascribed to the presence of steroidal saponins, which have beneficial effects on the digestive system, most notably via increasing nutritional absorption via an increase in villi height (Alghirani et al., 2022). In addition, the presence of other phytocompounds such as tannins, flavonoids, and alkaloids contributes to improved growth performance by boosting immunity, maintaining microflora balance, and exhibiting antimicrobial activity, thereby reducing pathogenic load and promoting intestinal health stability (Chaudhary et al., 2018a; Tonda et al., 2018; Redondo et al., 2022).While experiment B showed improved FCR with increasing inclusion levels, feed intake decreased as the inclusion level of *B. decumbens* increased. This could be due to saponins affecting the palatability of feed due to its bitter taste (Smithard, 2002). In an earlier study, Alghirani et al. (2022) reported enhanced broiler performance, as indicated by improved FCR, BWG, and final body weight, through the supplementation of 25mg/kg of *B. decumbens* across both starter and finisher phases. Although their findings supported the positive impact of *B. decumbens* on broilers' growth performance, our current experiment demonstrated divergent results. Notably, a higher inclusion level (5.00g/kg of *B. decumbens*) in the current study yielded superior outcomes compared to the earlier findings. The observed discrepancies could be explained by the variations between the two experiments, specifically the breed of broilers utilised and age at which they were slaughtered. Alghirani et al. (2022) employed fast-growing broilers (Ross308), slaughtered at 42-days old; while experiment B utilised slow-growing broilers (Sasso), harvested at 56-days old. Prior research suggests that indigenous broiler chickens supplemented can tolerate phytocompounds and fibre better in comparison to commercial chickens, potentially

elucidating why experiment B achieved enhanced growth performance despite a higher inclusion level—contrary to Alghirani et al.'s findings (Manyelo et al., 2022a; Manyelo et al., 2022b). Additionally, it is worth nothing that bitter taste sensitivity is reported to be more pronounced in younger chicks than in older ones (Dey et al., 2018; Kawabata and Tabata, 2022). This observation raises the possibility of feed rejection among Ross308 chickens at a younger age if supplied at higher inclusion levels, providing a theoretical explanation for the differing outcomes.

Experiment C produced results similar to experiment A and B, whereby T6 broilers supplemented with 5.00g/kg of *M. maximus* displayed a better growth performance overall. Quantitative analysis of *M. maximus* used in this experiment confirmed that alkaloids were the most prevalent phytocompound present in Guinea grass as per reported by previous studies (Kanife and Doherty, 2012; Abu Hafsa and Hassan, 2021). The results presented show that increasing inclusion levels of the grass meal increased feed intake of broilers possibly due to improved feed status, mainly flavour and palatability (Jamroz et al., 2002; Windisch et al., 2008; Valenzuela-Grijalva et al., 2017). Similar results were found in an experiment where dried guava leaf meals increased final body weight and average daily BWG correspondingly to those fed with higher levels of leaf meals. Rahimi et al. (2011) supports that notion by theorising that the addition of phyhtocompounds enhanced FI and FCR by stimulating appetite and increasing digestive secretion, thus improving nutrient digestion and easing absorption, as evidenced by improved BWG and final body weight in T6. Another possible method *M. maximus* might enhance broilers' growth performance is through gut fermentation modulation via antimicrobial effects (Valenzuela-Grijalva et al., 2017). As the presence of various physiologically active phytocompounds in *M.*

maximus such as alkaloids, tannins, saponins, and flavonoids may contribute to antifungal and antimicrobial actions against harmful pathogens in the gut which promote the growth status of broiler chickens in this experiment (Kanife and Doherty, 2012). Additionally, the improved broilers' growth performance of broilers could also be credited to the anti-inflammatory and antimicrobial qualities of *M. maximus* grass meal akin to dried guava leaf meal due to the presence of several phytocompounds (Abang et al., 2023). Sugiharto et al. (2019) even proposed that alkaloid-rich leaf meals might be used as antibiotic substitutes, which is reinforced by the findings of experiment C, which revealed no significant differences in cumulative FCR between the positive control (T2), T5, and T6.

While all three experiments demonstrated better growth performance with increasing inclusion levels, comparison of the best growth performance from all three experiments showed that *P. purpureum* produced better overall results. Although during the starter phase, the highest final body weight was from experiment C. This higher final body weight could be credited to a higher feed intake as shown in Table 4.4. However, it should be noted that both final body weight and feed intake were not significantly different from the results in experiment A. More insightful data can be found during the finisher phase whereby a lower feed intake in experiment A produced a significantly lower cumulative FCR in comparison to experiment B and C. As previously mentioned, these findings are attributed to the various phytocompounds present in *P. purpureum*. These active compounds could be responsible for the production of digestive enzymes, which promotes nutrition digestion and absorption in the gut despite a lower feed intake; hence improving feed efficiency (Rahimi et al., 2011). When comparing the phytocompound profiles of all three grasses used in the

experiments, *P. purpureum* demonstrated significantly higher tannin and flavonoid. This could possibly explain the better overall growth performance as leaf meals high in flavonoids and tannins such as *Moringa oleifera*, have shown improved weight gain and FCR (Fahey et al., 2001). This theory is further supported by the antioxidant activity results, demonstrating that *P. purpureum* had the highest DPPH radical scavenging activity. This is because phenolic groups, such as flavonoids, possess high redox potentials, enabling them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, thereby eradicating radicals. (Jack et al., 2020). Similarly, broilers fed with *Moringa oleifera* leaf meals at inclusion levels of 2.5-10g/kg of feed had lower feed intake during the first seven days but had significantly better FCR at the end of the experiment (Alshukri et al., 2018). They attributed these effects to alterations of the gut microflora due to phenolics found in Moringa leaves.

To summarise, coloured-broilers supplemented with 5.00g/kg of *P. purpureum* produced broilers with a better overall growth performance. At the end of the experiment, broilers from experiment A demonstrated a lower feed intake and cumulative FCR in comparison to the other two experiments. This might be related to its higher flavonoid and tannin content compared to *B. decumbens* and *M. maximus*, which may boost growth performance through antioxidative activity, enhanced digestion and absorption via digestive enzyme synthesis, and gut microbiota balance through antimicrobial action.

4.2 Apparent Ileal Digestibility

4.2.1 Experiment A (*P. purpereum*)

The impact of supplementing *P. purpereum* leaves on the apparent ileal nutritional digestibility of broilers on day 56 is shown in Table 4.5. Throughout the treatments, all parameters produced significant ($p<0.05$) results. In contrast to the other treatments, T6 had the best digestibility for CP, CF, and EE, whereas the lowest digestibility for DM and ash as compared to the other treatments. This suggests that T6 has greater ileal digestibility.

4.2.2 Experiment B (*B. decumbens*)

On day 56, broilers' apparent ileal nutritional digestibility was significantly affected ($p<0.05$) by the addition of *B. decumbens* leaves, as indicated in Table 4.6. All of the parameters showed outcomes that were significant ($p<0.05$) across the treatments. T6 exhibited higher digestibility for CP, CF, and EE compared to the other treatments, but had lower digestibility for DM and ash. This shows that the inclusion level of 5.00g/kg of *B. decumbens* allowed for easier nutrient absorption in the ileum.

4.2.3 Experiment C (*M. maximus*)

According to Table 4.7, the addition of *M. maximus* leaves considerably ($p<0.05$) impacted the apparent ileal nutritional digestibility of broilers on day 56. Across all of the treatments, all of the parameters revealed results that were significantly different ($p<0.05$). Comparing T6 to the other treatments, it was more digestible for CP, CF, and EE, but less so for DM and ash. This shows how the inclusion level of 5.00g/kg of *M. maximus* leaf meal enabled better nutritional absorption in the ileum.

4.2.4 Comparison of the Best Treatments from Experiments A, B, and C

The apparent ileal digestibility of the best treatments from experiments A (T6), B (T6), and C (T6) is compared in Table 4.8. Significant variations ($p<0.05$) were seen across all parameters. Overall, broilers supplemented with 5.00g/kg *P. purpureum* produced the greatest results, with the AID exhibiting the highest values for DM, CF, EE, and ash when compared to the other treatments. Although CP's AID was the second highest in experiment A (67.14%), it was not statistically different ($p>0.05$) from experiment C (67.68%). As a result, this study demonstrated that chickens fed with 5.00g/kg of *P. purpureum* had superior AID.

Table 4.5 : Effect of *P. purpereum* Leaf Supplementation on the Apparent Ileal Nutrient Digestibility of Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Dry matter (%)	71.12±0.06^{b}	73.63±0.31^{a}	69.05±0.33^{c}	67.15±0.81^{c}	62.58±0.43^{d}	60.52±0.15^{e}	<0.000
Crude protein (%)	51.91±0.36^{e}	65.75±0.20^{b}	60.72±0.32^{d}	62.75±0.38^{b}	66.44±0.16^{ab}	67.14±0.19^{a}	<0.000
Crude fibre (%)	26.63±0.35^{d}	27.71±0.29^{d}	32.26±0.20^{c}	33.26±0.13^{c}	34.67±0.06^{b}	36.55±0.40^{a}	<0.000
Ether extract (%)	65.63±0.14^{c}	72.53±0.12^{b}	73.08±0.37^{ab}	74.42±0.22^{ab}	73.94±0.35^{ab}	76.97±0.37^{a}	<0.000
Ash (%)	41.61±0.03^{b}	45.50±0.19^{a}	40.27±0.16^{c}	40.23±0.06^{c}	38.32±0.25^{d}	37.32±0.21^{d}	<0.000

All values were expressed as mean ± SE; superscripts $^{a, b, c, d, \text{and } e}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.6 : Effect of *B. decumbens* Leaf Meal Supplementation on the Digestibility of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Dry matter (%)	67.54±0.28^{b}	73.63±0.67^{a}	62.78±0.97^{c}	62.58±0.52^{c}	57.70±0.53^{d}	51.95±0.50^{e}	<0.000
Crude protein (%)	44.12±0.38^{f}	58.55±0.38^{b}	48.20±0.10^{e}	53.61±0.42^{d}	55.75±0.13^{c}	66.44±0.30^{a}	<0.000
Crude fibre (%)	26.63±0.27^{e}	32.68±0.11^{b}	27.71±0.29^{d}	29.78±0.37^{c}	30.77±0.12^{c}	33.79±0.22^{a}	<0.000
Ether extract (%)	65.63±0.32^{d}	76.01±0.24^{b}	72.53±0.53^{c}	73.42±0.43^{c}	74.90±0.51^{b}	78.12±0.39^{a}	<0.000
Ash (%)	56.83±0.37^{a}	58.06±0.30^{a}	51.42±0.56^{c}	42.08±0.27^{d}	37.07±0.24^{e}	34.48±0.59^{f}	<0.000

All values were expressed as mean ± SE; superscripts $^{a, b, c, d, e, \text{and } f}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.7 : Effect of *M. maximus* Leaf Meal Supplementation on the Apparent Ileal Nutrient Digestibility of Sasso Broilers on Day 56

Parameters	Treatments						p-value
	T1	T2	T3	T4	T5	T6	
Dry matter (%)	70.23±0.05a	71.49±0.10a	67.95±0.19b	66.25±0.79b	62.58±0.43c	60.60±0.27d	<0.000
Crude protein (%)	55.75±0.20e	64.44±0.16b	58.24±0.06d	60.54±0.3c	66.24±0.35b	67.68±0.34a	<0.000
Crude fibre (%)	26.63±0.35e	27.71±0.29d	28.39±0.03cd	29.23±0.14bc	29.45±0.23b	30.50±0.10a	<0.000
Ether extract (%)	65.63±0.14e	72.53±0.12b	69.40±0.19d	70.30±0.03c	72.68±0.28b	74.50±0.04a	<0.000
Ash (%)	37.64±0.33a	38.27±0.24a	37.40±0.21a	37.07±0.14a	35.05±0.37b	34.48±0.13b	<0.000

All values were expressed as mean ± SE; superscripts [a, b, c, d, and e] values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.8 : Effects of *P. purpureum*, *B. decumbens*, and *M. maximus* Leaf Meal Supplementation on the Apparent Ileal Nutrient Digestibility of Sasso Broilers on Day 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
Dry matter (%)	60.52±0.15[a]	51.95±0.50[b]	60.60±0.27[a]	<0.0000
Crude protein (%)	67.14±0.19[ab]	66.44±0.30[b]	67.68±0.34[a]	0.0299
Crude fibre (%)	36.55±0.40[a]	33.79±0.22[b]	30.50±0.10[c]	<0.0000
Ether extract (%)	76.97±0.37[a]	78.12±0.39[a]	74.50±0.04[b]	0.0005
Ash (%)	37.32±0.21[a]	34.48±0.59[b]	34.48±0.13[b]	<0.0000

All values were expressed as mean ± SE; superscripts [a, b, and c] values within the row are significantly different at $p < 0.05$.

Ileal digestibility refers to the ability of an animal to effectively absorb the nutrients from the feed they consume. This assessment is crucial in evaluating and estimating the nutritional value of feed ingredients according to the animals' requirements. Contradictory data has been observed in broilers with the usage of plant-based additives due to their fibre content and secondary metabolites, which may operate as anti-nutritive substances—affecting nutrient digestion (Nkukwana et al., 2014; Mateo et al., 2022). However, there is evidence that phytobiotic supplementation improve digestibility by increasing digestive enzyme release, modifying intestinal structure, and gut regulation by lowering pathogenic bacteria and inflammation in the gut (Frankic et al., 2009; Khan et al., 2012; Basit et al., 2020b).

Experiment A revealed that broilers supplemented with 5.00g/kg of *P. purpureum* grass meal had better digestibility for CP, CF, and EE. The data suggests that the addition of *P. purpureum*, which is high in flavonoids and tannins into broiler diets at appropriate levels could improve digestibility. Basit et al. (2020b) backed this belief by claiming that phytocompounds such as tannins, flavonoids, and polyphenols promote digestibility via gut control, hence boosting growth performance. However, limited researches on supplementing leaf meals to broilers presented contradictory data on digestibility. For example, *P. odorata*, which is high in flavonoids and polyphenols were found to improve CP digestibility when supplemented at 8g/kg of basal diet (Basit et al., 2020a). On the contrary, *P. betle* at 8g/kg significantly ($p<0.05$) reduced CP digestibility, possibly caused by the high concentration of tannins (Sav'on et al., 2006; Basit et al., 2020a). Tannins have a reputation of being an anti-nutritional factor due to its protein-binding abilities (Selle et al., 2010; Moyo et al., 2011). However, Huang et al. (2018) asserted that when presented at moderate doses, tannins

have the ability to enhance immunological competence, intestinal microbial ecology, and gut health in broiler chicks. Which in turn, could improve digestibility through improved nutrient uptake via gut function modifications (Valenzuela-Grijalva et al., 2017). Digestibility of EE and DM were also found to be improved in broilers supplemented with 4g/kg *P. betle* and 8g/kg *P. odorata* leaf meals (Basit et al., 2020a). This further supports the idea that the outcome of digestibility experiments on broilers with leaf meal supplementation is greatly reliant on the specific plant's phytocompound and nutritional profile to determine the optimal dosage.

Experiment B also indicated that T6 broilers had better CP, CF, and EE digestibility. The improved digestibility could be attributed to the presence of phytocompounds, particularly saponins. Numerous studies have demonstrated the potential benefits of supplementing poultry with dietary saponins as a feed additive at appropriate amounts (Smithard, 2002). Despite saponins having a notorious reputation as an anti-nutritional compound, research shows that their use as a feed additive may have beneficial effects. In vitro studies show that saponins can increase intestinal mucosal cell permeability, reduce active mucosal transport, and promote the intake of substances that are normally not absorbed owing to the stereo-structure of the saccharide chain (Johnson et al., 1986). Alghirani et al. (2021) supports this theory as they found that Ross308 broilers supplemented with saponin-rich *Yucca shidigera* had better ileal digestibility and gut health. Additionally, saponins could improve digestibility is through antibacterial effects which could improve the overall gut health (Kolawole et al., 2007). To the best of the author's knowledge, there was only one other study which supplemented *B. decumbens* to broilers. As such, Alghirani et al. (2022) found that CP, CF, and EE's digestibility were significantly improved with the supplementation

B. decumbens, similar to the findings of the current study. Alghirani et al. (2022) attributed these findings to *B. decumbens* stimulating the secretion of digestive enzymes. Nonetheless, it is crucial to note that Alghirani et al. (2022) discovered better digestibility results at a much lower dosage level, at 25mg/kg in comparison to the current study, at 5g/kg. Given that the previous experiment employed commercial Ross308, while the current experiment utilised Sasso broiler chickens, which are a coloured-broiler breed—the disparities in results might be explained by Sebola et al. (2018), who proposed that digestibility differed based on the bird's genetic capacity to absorb and utilise nutrients in a high-fibre diet.

All three trials revealed a decrease in DM and ash digestibility as grass inclusion amount increased. This might be related to the inclusion of insoluble fibres in broiler diets, which results in a shorter transit time of the digesta in the small intestine (Amerah et al., 2009). Backing up this theory is a review by Hetland et al. in 2004. These authors presented that insoluble fibre alters gut function and modulates nutrient digestion in the intestine. Thus, digestion of starch is higher and digesta passage rate is accelerated when a moderate amount of insoluble fibre is present in the diet. Due to this accelerated intestinal transit, there is less risk for colonisation of pathogenic bacteria in the gut. Aside from that, Experiment C also showed the highest digestibility for CP, CF, and EE. Several other studies backed these findings as feed intake and digestibility have been shown to improve with the addition of grass or leaf meals as it increases the endogenous secretion production in the small intestine, liver, and pancreas (Cross et al., 2007; Martens et al., 2012; Akande et al. 2010). Results from Experiment C supports this statement as feed intake increases with the addition of *M. maximus*. Alkaloids which are abundantly present in *M. maximus* may also be

responsible for the improvements in digestibility as they have antifungal and antimicrobial actions against pathogenic bacteria in the gut (Kanife and Doherty, 2012). Supporting that claim, *Sauropus androgynus* and *Polyalthia longifolia* are other examples of alkaloid-rich leaf meals may be utilised as antibiotic replacements due to their favourable effects to broilers' growth (Sugiharto et al., 2019). Onyiche et al. (2021) also ascribed the reduction of *Eimeria* oocyte counts to the alkaloids and saponins present in the extracts of *A. indica*. According to reports, the phytocompounds attach to key macromolecules in the parasite's membrane, causing instability and, ultimately, death. Therefore, the mode of mechanism for improvements in digestibility with the addition of *M. maximus* may be attributed to the antimicrobial effects that alter gut fermentation functions (Valenzuela-Grijalva et al., 2017).

The use of fibre in chicken diets is fraught with controversy since it has been shown to be a nutrient diluent and limits digestibility. Despite the reduction in nutrient content, modest supplementation has little detrimental effects on production performances (Walugembe et al, 2014). Dietary fibre has been known to improve nutritional digestibility by increasing pancreatic enzymatic activity and reversing peristalsis (Hetland et al., 2003; Amerah et al., 2009; Mateos et al., 2012). This enables bile salts to be transported to the gizzard by reverse peristalsis and combined with gastric secretions. Thus, fat emulsification is improved by lowering the ability of fat droplets to coat nutrients, allowing nutrients to be more easily digested and absorbed (Hetland et al., 2004). A comparison of the best treatments from all three experiments showed that *P. purpureum* produced the best overall digestibility, possibly due to synergistic effects of possessing relatively lower saponins, tannins,

and alkaloids with higher flavonoid content, in comparison to the other two grasses. Though, CP and EE did not significantly differ between *P. purpureum* and *B. decumbens*. While DM and CP did not significantly differ between *P. purpureum* and *M. maximus*. A lower DM and CP digestibility in *B. decumbens* could be due to the higher saponin concentration in comparison to the other two grasses. This is because saponins can form saponin-protein complexes which damage the intestinal membrane—causing an increase of permeability which enables the uptake of substances that are unabsorbed (e.g. toxins, microbes, allergens), and finally obstructs the active mucosal nutrient transport (Dei et al., 2007). Furthermore, the higher DM and EE digestibility in *P. purpureum* is consistent with the findings of Basit et al. (2020a), who attributed the findings to flavonoids and polyphenols, which alters circulatory and endocrine indicators, boost cellular and mucosal immunity, thereby improving broiler health and digestibility.

At the end of the experiment, broilers from experiment A demonstrated a higher CF and ash digestibility. Although, digestibility for DM, CP, EE, and CF were not the highest numerically, it did not significantly differ from one or the other two grasses. In short, those supplemented with 5.00g/kg of *P. purpureum* produced broilers with an overall better apparent ileal digestibility. The results may be due to the various phytocompounds profile and its respective concentrations, as well as different modes of actions on the digestive system—mainly though gut modifications such as stimulation of enzyme production, permeability and transport or antimicrobial effects.

4.3 Gut Histomorphology

4.3.1 Experiment A (*P. purpereum*)

Table 4.9 shows the effect of *P. purpureum* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56. Compared to the negative and positive control (except jejunum), supplementation of *P. purpureum* leaf meal increased ($p<0.05$) the villi height in the duodenum, jejunum, and ileum with the highest supplementation level at 5.00g/kg of feed contributing to a higher villi height. Supplementation of *P. purpureum* leaf meal also decreased ($p<0.05$) the crypt depth in duodenum, jejunum, and ileum when compared to the negative control, except for T3 in the duodenum. Broilers in T4 to T6 showed a lower ($p<0.05$) crypt depth when compared to the positive control in the duodenum and jejunum, whereas T3 to T6 broilers showed similar results ($p>0.05$) when compared to the positive control group. Overall, T6 showed best results as it had the highest ($p<0.05$) villi height to crypt depth ratio in duodenum and ileum; while in the jejunum, T2 had the highest villi height to crypt depth ratio, but had no significant differences ($p>0.05$) when compared to T6.

4.3.2 Experiment B (*B. decumbens*)

The effect of *B. decumbens* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56 is displayed in Table 4.10. Supplementation of *B. decumbens* leaf meal showed an increased ($p<0.05$) villi height in the duodenum and ileum when compared to the negative and positive control group. In the jejunum, only T5 and T6 showed comparable ($p>0.05$) villi height to the positive control group. All inclusion levels of *B. decumbens* leaf meal supplementation showed a lower ($p<0.05$) crypt depth compared to the negative control group. On top of that,

in the duodenum, supplementation of *B. decumbens* displayed lower ($p<0.05$) crypt depth compared to the negative control group, with comparable results ($p>0.05$) when compared to the positive control group. Overall, T6 showed the best effects on the small intestine histology as it exhibited the highest ($p<0.05$) villi height: crypt depth ratio in the duodenum, jejunum, and ileum.

4.3.3 Experiment C (*M. maximus*)

Table 4.11 illustrates the effect of *M. maximus* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56. In the duodenum, T3 to T6 broilers showed no significant differences ($p>0.05$) when compared to T2, the positive control group. Whereas in the jejunum, only T6 showed comparable ($p>0.05$) in comparison to T2. On the other hand, T5 and T6 broilers exhibited better ($p<0.05$) villi height in the ileum when compared to T2. Additionally, T6 showed the lowest ($p<0.05$) crypt depth in the duodenum, jejunum, and ileum. Therefore, T6 had the best ($p<0.05$) small intestine histomorphology results as T6 showed the highest villi height: crypt depth ratio across the duodenum, jejunum, and ileum.

4.3.4 Comparison of the Best Treatments from Experiments A, B, and C

Table 4.12 illustrates the comparison of the best treatment groups of trials A (T6), B (T6), and C (T6) on the small intestine histomorphology of Sasso broilers on day 56. Supplementation of 5.00g of *M. maximus* per kg of feed had a higher ($p<0.05$) villi height in the jejunum and ileum whereas in *B. decumbens* had a higher ($p<0.05$) villi height in the duodenum. On the other hand, 5.00g of *P. purpureum* per kg of feed resulted in a lower crypt depth in the duodenum and jejunum. However, in the jejunum, the crypt depth of *M. maximus* supplemented group did not significantly

($p>0.05$) differ from the *P. purpureum* supplemented group. Additionally, *M. maximus* supplemented group had the lowest crypt depth in the ileum. Because of this, *M. maximus* supplemented group had the best overall effect on the small intestine histomorphology as it displayed a higher ($p<0.05$) villi height to crypt depth ratio in the jejunum and ileum.

Table 4.9 : Effect of *P. purpureum* Leaf Meal Supplementation on the Small Intestine Histomorphology of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Villi height (µm)							
Duodenum	481.95±152.41^{e}	626.25±198.04^{b}	560.70±177.31^{c}	502.07±158.77^{de}	535.69±169.40^{cd}	675.19±213.51^{a}	<0.0000
Jejunum	572.01±180.88^{a}	593.34±187.63^{a}	471.42±149.08^{b}	446.94±141.34^{b}	452.05±140.95^{b}	463.86±146.69^{b}	<0.0000
Ileum	313.19±99.07^{c}	362.12±114.54^{b}	382.52±120.96^{ab}	409.42±129.47^{a}	416.79±131.80^{a}	423.50±133.92^{a}	<0.0000
Crypt depth (µm)							
Duodenum	118.48±37.47^{b}	117.95±37.30^{b}	131.57±41.61^{a}	99.42±31.44^{c}	82.44±26.07^{d}	78.15±24.71^{d}	<0.0000
Jejunum	126.98±40.16^{a}	78.81±24.92^{b}	80.22±25.37^{b}	70.64±22.34^{c}	68.75±21.74^{c}	67.58±21.37^{c}	<0.0000
Ileum	130.25±41.19^{a}	73.40±23.21^{b}	73.03±23.09^{b}	68.60±21.69^{b}	68.49±21.57^{b}	68.47±21.65^{b}	<0.0000
Villi height: Crypt depth							
Duodenum	4.07±1.29^{d}	5.32±1.68^{c}	4.27±1.35^{d}	5.06±1.60^{c}	6.53±2.06^{b}	8.66±2.74^{a}	<0.0000
Jejunum	4.51±1.43^{d}	7.56±2.39^{a}	5.92±1.87^{c}	6.35±2.01^{bc}	6.59±2.08^{bc}	6.90±2.18^{ab}	<0.0000
Ileum	2.41±0.76^{d}	4.96±1.57^{c}	5.32±1.6^{bc}	5.97±1.89^{ab}	6.13±1.94^{a}	6.21±1.96^{a}	<0.0000

All values were expressed as mean ± SE; superscripts $^{a, b, c, d \text{ and } e}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.10 : Effect of *B. decumbens* Leaf Meal Supplementation on the Small Intestine Histomorphology of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Villi height (µm)							
Duodenum	481.95±152.41^{c}	626.25±198.04^{b}	705.33±233.05^{a}	705.35±223.05^{a}	715.87±226.38^{a}	718.00±227.05^{a}	<0.0000
Jejunum	572.01±180.88^{ab}	593.34±187.63^{a}	539.21±170.51^{b}	540.60±170.95^{b}	566.01±179.11^{ab}	581.56±183.84^{a}	0.0002
Ileum	313.19±99.07^{d}	362.20±114.54^{c}	425.46±137.54^{b}	437.29±138.28^{ab}	452.62±143.13^{ab}	471.54±149.11^{a}	<0.0000
Crypt depth (µm)							
Duodenum	118.48±37.47^{a}	117.95±37.30^{a}	93.21±29.47^{b}	90.74±28.39^{b}	89.62±28.34^{b}	86.42±27.33^{b}	<0.0000
Jejunum	126.98±40.16^{a}	78.81±24.92^{bc}	80.00±25.30^{b}	75.27±25.80^{bcd}	73.87±23.36^{cd}	72.60±22.96^{d}	<0.0000
Ileum	130.25±41.19^{a}	73.40±23.21^{b}	80.37±25.41^{b}	79.50±25.14^{b}	77.84±24.61^{b}	75.20±23.78^{b}	<0.0000
Villi height: Crypt depth							
Duodenum	4.07±1.29^{c}	5.32±1.68^{b}	7.58±2.40^{a}	7.80±2.47^{a}	8.03±2.54^{a}	8.32±2.63^{a}	<0.0000
Jejunum	4.51±1.43^{d}	7.56±2.39^{ab}	6.76±2.14^{c}	7.18±2.27^{bc}	7.68±2.43^{ab}	8.03±2.54^{a}	<0.0000
Ileum	2.41±0.76^{d}	4.96±1.57^{c}	5.38±1.70^{bc}	5.52±1.75^{bc}	5.84±1.85^{ab}	6.29±1.99^{a}	<0.0000

All values were expressed as mean ± SE; superscripts $^{a, b, c, and\ d}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.11 : Effect of *M. maximus* Leaf Meal Supplementation on the Small Intestine Histomorphology of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Villi height (µm)							
Duodenum	481.95±152.41^{b}	626.25±198.04^{a}	587.13±185.67^{a}	584.82±184.94^{a}	598.08±189.13^{a}	619.03±195.76^{a}	<0.0000
Jejunum	572.01±180.88^{b}	593.43±187.63^{ab}	501.21±158.50^{c}	510.16±161.33^{c}	588.66±186.15^{b}	628.19±198.65^{a}	<0.0000
Ileum	313.19±99.07^{c}	362.20±114.54^{b}	330.49±104.51^{bc}	357.79±113.14^{b}	459.02±145.16^{a}	487.71±154.23^{a}	<0.0000
Crypt depth (µm)							
Duodenum	118.48±37.47^{a}	117.95±37.30^{a}	112.50±35.58^{ab}	109.96±34.77^{ab}	111.75±35.34^{ab}	101.64±32.14^{b}	0.0004
Jejunum	126.98±40.16^{a}	78.81±24.92^{b}	77.24±24.42^{b}	70.33±22.24^{c}	67.01±21.19^{c}	65.76±20.80^{c}	<0.0000
Ileum	130.25±41.19^{a}	73.40±23.21^{bc}	80.01±25.30^{b}	73.23±23.16^{c}	65.82±20.82^{d}	57.18±18.08^{e}	<0.0000
Villi height: Crypt depth							
Duodenum	4.07±1.29^{c}	5.32±1.68^{b}	5.22±1.65^{b}	5.35±1.69^{b}	5.36±1.69^{b}	6.09±1.93^{a}	<0.0000
Jejunum	4.51±1.43^{e}	7.56±2.39^{c}	6.50±2.05^{d}	7.26±2.30^{c}	8.80±2.78^{b}	9.59±3.03^{a}	<0.0000
Ileum	2.41±0.76^{e}	4.96±1.57^{c}	4.14±1.31^{d}	4.91±1.55^{c}	6.99±2.21^{b}	8.55±2.70^{a}	<0.0000

All values were expressed as mean ± SE; superscripts $^{a, b, c, d \text{ and } e}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.12 : Effects *of P. purpureum, B. decumbens,* and *M. maximus* Leaf Meal Supplementation on the Small Intestine Histomorphology of Sasso Broilers on Day 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
Villi height (µm)				
Duodenum	675.19±213.51^{b}	718.00±227.05^{a}	619.03±195.76^{c}	<0.0000
Jejunum	463.86±146.69^{c}	581.56±183.84^{b}	628.19±198.65^{a}	<0.0000
Ileum	423.50±133.92^{b}	471.54±149.11^{a}	487.71±154.23^{a}	<0.0000
Crypt depth (µm)				
Duodenum	78.15±24.71^{c}	86.42±27.33^{b}	101.64±32.14^{a}	<0.0000
Jejunum	67.58±21.37^{b}	72.60±22.96^{a}	65.76±20.80^{b}	0.0021
Ileum	68.47±21.65^{b}	75.20±23.78^{a}	57.18±18.08^{c}	<0.0000
Villi height: Crypt depth				
Duodenum	8.66±2.74^{a}	8.32±2.63^{a}	6.09±1.93^{b}	<0.0000
Jejunum	6.90±2.18^{c}	8.03±2.54^{b}	9.59±3.03^{a}	<0.0000
Ileum	6.21±1.96^{b}	6.29±1.99^{b}	8.55±2.70^{a}	<0.0000

All values were expressed as mean ± SE; superscripts $^{a, b, \text{and } c}$ values within the row are significantly different at $p < 0.05$.

The incorporation of grass or leaf meal in poultry diets may impact gut function by altering the gastrointestinal system. Broiler intestinal health may be measured by examining changes in gut structure and appearance. A higher villi height to crypt depth ratio indicates that the broiler has more effective digesting and absorbing ability owing to a greater absorptive surface area, which allows for more nutritional uptake (Pohl et al., 2012). Increased villus height and VH:CD were also linked to enhanced epithelial cell turnover, which in turn stimulated cell mitosis (Khan et al, 2017). Furthermore, broilers with shorter crypt depth have a lower turnover rate of intestinal mucosal cells. Consequently, excelling in growth performance due to decreased maintenance demands, allowing more energy to be diverted towards development (Alghirani et al., 2021). Several theories suggest phytocompounds are capable of altering the intestinal structure, thus affecting broilers' production performances. Though, its exact mechanism has yet to be completely understood.

Previous studies found that phytobiotics in poultry diets have the potential to enhance intestinal morphology, which translates to better production performance (Jamroz et al., 2006; Ferdous et al. 2021; Gilani et al., 2021). Results from Experiment A corroborated this, since data demonstrated that adding *P. purpureum* at 5.00g/kg enhanced gut structure. T6 showed the greatest villi height to crypt depth ratio in the duodenum and ileum. While T6 did not have the greatest numerical value for VH:CD in the jejunum, it was comparable to T2 (positive control). This improvement might be attributed to flavonoids found in *P. purpureum*, which have antioxidative characteristics. This is due to the fact that antioxidants may boost digestive tract growth and development by increasing villus height, which improves absorptive surface area, enzyme secretions, and nutrient transfer (Olukosi and Dono, 2014).

Supporting this is Ogwuegbu et al. (2021), who found that gut histological traits were improved in broilers with the addition of 5.0g/kg rosemary leaves which contain several phytocompounds including flavonoids. Additionally, they claimed that rosemary leaves have antioxidant, antimicrobial, and antifungal properties due to these bioactive compounds, therefore improving gut morphology and microflora. On the contrary, Yang et al. (2019) discovered that supplementing cinnamon essential oil or a combination with bamboo leaf flavonoid extracts had no effects on the small intestine. The differences in results could possibly be due to the varying amounts of flavonoids presented to the broilers.

Experiment B displayed results similar to Experiment A, whereby T6 showed the best VH:CD. The findings here might be attributed to the higher content of saponins in *B. decumbens* in addition to the presence of other phytocompounds. Saponins contain antioxidative characteristics that may have altered intestine structure and improved lumen conditions (Tavangar et al., 2021). On the same note, these antioxidative qualities may encourage digestive tract growth and development, resulting in greater villus height, enhanced absorptive surface area, enzyme secretions, and nutrient transfer. (Olukosi and Dono, 2014). A comparable experiment by Alghirani et al. (2022) discovered that 25mg/kg of *B. decumbens* supplemented to commercial broilers improved intestinal morphology by villus height increments and reduced crypt depth. While the current and earlier study both found positive effects of *B. decumbens* on the intestinal histology, it is important to note that different strains of broilers were used—possibly affecting the appropriate dosage requirements. Furthermore, saponin extracts have been demonstrated to have phytotoxic and antibacterial properties, notably against Gram-negative bacteria (Kolawole et al., 2007). Hadayati et al. (2022)

corroborates this statement as they claim that incorporating phytobiotics in poultry diets with antibacterial properties will reduce pathogenic microbe growth. As such, it is possible to speculate that improvements in gut morphology of Experiment B may be linked to a more balanced gut microbiota and the suppression of harmful bacteria.

Experiment C showed the highest VH:CD throughout the small intestine. Previous studies utilising various leaf meals of differing inclusion levels have also shown overall positive effects on the VH:CD ratio (Basit et al., 2020b; Ogwuegbu et al., 2021; Abdelatty et al., 2021; Saharan et al., 2022). On the other hand, there were some other studies which found insignificant or detrimental effects on the gut with the inclusion of plant-based meals in broiler diets (Saadatmand et al., 2019; Sugiharto et al., 2020). Nonetheless, due to the various plants utilised in each experiment, it is important to determine the fibre content and type as well as the appropriate inclusion levels to fully reap its benefits. The action of several phytocompounds present in *M. maximus*, notably alkaloids (which are the most prevalent) may be related to the increased villus height and decreased crypt depth. Phytocompounds such as alkaloids, tannins, saponins and flavonoids have antimicrobial effects that are beneficial to the gut of broilers. Thus, making alkaloid-rich plants a suitable candidate for antibiotic replacements (Kanife and Doherty, 2012; Sugiharto et al., 2019). Earlier experiments utilising extracts from *A. indica* which contain alkaloids and saponins have also been shown to reduce the oocyte counts of *Eimeria*, a common enteric parasite that causes coccidiosis in broilers (Onyiche et al., 2021). A reduction of pathogenic bacteria in the intestine reduces the risk of intestine-related diseases and, as a result, foster a healthier gut (Chrubasik et al., 2005; Pohl et al., 2012). Furthermore, less pathogenic microorganisms mean less intestine damage and the need of energy for lumen

restoration (Hadayati et al., 2022). This translates to greater nutrient absorption and, as a result, better growth performance.

All three grasses showed improvements in gut morphology when added to the broilers' diets. But, when comparing the best treatments from Experiment A, B, and C, it was found that 5.00g/kg of *M. maximus* had the best overall effect on gut histomorphology. *M. maximus* showed the highest VH:CD ratio in the jejunum and ileum. The jejunum is the principal site of nutrition absorption in chickens. Therefore, an increase in the villi length of the jejunum is advantageous to the growth of the poultry because it enhances the intestine's absorptive capacity (Abdelatty et al., 2021). *M. maximus* used in Experiment C had alkaloids as the most prevalent phytocompound present, followed by tannins. Alkaloids antibacterial mode of mechanism has been known to block topoisomerase and disrupt DNA synthesis (Suresh et al., 2017), reducing the number of pathogenic bacteria in the gut which directly or indirectly affects digestion as gut health is optimally maintained, allowing for nutrient uptake. Sanguinarine, a subclass of alkaloid, inhibits nuclear factor Kappa-B (NF-κB) pathway which plays a crucial role in pro-inflammatory cytokine expression, thus reducing inflammation in the gut (González-Castejón and Rodriguez-Casado, 2011). Therefore, the data suggests that alkaloids in *M. maximus* could be responsible for improving intestinal health and morphology in the broiler chickens.

In conclusion, the addition of grass meals may have benefits on the intestinal health of broiler chickens due to the synergistic effect of various phytocompounds. Here, 5.00g/kg of *M. maximus* in Experiment C showed the most beneficial effects on gut histomorphology, likely relating to its higher concentration of alkaloids and tannins.

However, the exact mechanism has yet to be completely understood as there are many modes of actions through which phytocompounds may affect the intestinal health. A more in-depth study would be beneficial to further understand the role of phytocompounds and its effects on the gut in order to fully utilise grass meals as an antibiotic replacement.

4.4 Cecal Microbial Population

4.4.1 Experiment A (*P. purpereum*)

Table 4.13 shows the cecal microorganisms' population of broilers supplemented with *P. purpureum* on day 56. *Escherichia coli* was detected in all treatment groups whereas non-lactose fermenting *E. coli* was detected in T1 and T2. *Bacillus* sp. were detected in T3 to T6 with *Bacillus cereus* in T1. No *Salmonella* were identified throughout all treatments. The coliform and standard plate count were almost similar but it can be noted that the highest coliform and standard plate count was found in T6.

4.4.2 Experiment B (*B. decumbens*)

On day 56, the population of cecal microorganisms in broilers treated with *B. decumbens* is shown in Table 4.14. *E. coli* was found in every treatment group and T1 to T3 broilers had non-lactose fermenting *E. coli*. *Bacillus* sp. were discovered in T5 and T6, and *Bacillus cereus* in T1. Throughout all treatments, no *Salmonella* were found. The coliform and standard plate counts were almost identical, although T6 had the highest coliform and standard plate numbers.

4.4.3 Experiment C (*M. maximus*)

On day 56, the cecal microorganism population of broilers treated with *M. maximus* is shown in Table 4.15. *E. coli* was found in every treatment group. Non-lactose fermenting *E. coli* was found in T1 and T3 to T5 broilers. Throughout all treatments, no *Bacillus* sp. or *Salmonella* were found. The coliform and standard plate counts were almost identical, while T6 demonstrated the highest coliform and standard plate numbers.

4.4.4 Comparison of the Best Treatments from Experiments A, B, and C

The best treatments of *P. purpureum, B. decumbens,* and *M. maximus* leaf meal supplementation were compared for their effects on the cecal microorganisms of Sasso broilers. *E. coli* was identified in all three leaf meal groups but *Salmonella* was not identified. *Bacillus* sp. was isolated and identified in *P. purpureum* and *B. decumbens*. The coliform count and standard plate count were identical for *P. purpureum, B. decumbens,* and *M. maximus*. It can be concluded that all three leaf meals at 5.00g/kg had similar properties on the cecal microorganisms of Sasso broilers on day 56 with *M. maximus* having a slight upper hand.

Table 4.13 : Effect of *P. purpureum* SUPPLEMENTATION on the Cecal Microorganisms of Sasso Broilers on Day 56

Parameters	Treatment					
	T1	T2	T3	T4	T5	T6
Isolation and Identification	*E. coli* (3+) NFL *E. coli* *Bacillus cereus* (3+)	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (3+) *Bacillus* sp. (3+)	*E. coli* (4+) *Bacillus* sp. (3+)	*E. coli* (3+) *Bacillus* sp. (3+)	*E. coli* (3+) *Bacillus* sp. (3+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Coliform count (cfu/g)	3.5×10^6	2.5×10^7	1.0×10^6	2.3×10^6	6.6×10^6	1.6×10^8
Standard plate count (cfu/g)	2.2×10^7	1.2×10^8	2.5×10^7	2.7×10^7	1.1×10^8	1.4×10^9

Note: cfu: Colony-forming unit. NLF *E. coli*: Non-lactose fermenting *E. coli*. T1: Negative control; T2: Positive control; T3: 1.25g/kg; T4: 2.50g/kg; T5: 3.75g/kg; T6: 5.00g/kg.

Table 4.14 : Effect of *B. decumbens* Supplementation on the Cecal Microorganisms of Sasso Broilers on Day 56

Parameters	Treatment					
	T1	T2	T3	T4	T5	T6
Isolation and Identification	*E. coli* (3+) NFL *E. coli* *Bacillus cereus* (3+)	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (3+) NFL *E. coli* (2+)	*E. coli* (3+)	*E. coli* (3+) Bacillus sp. (2+)	*E. coli* (3+) Bacillus sp. (3+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Coliform count (cfu/g)	7.2×10^5	2.5×10^7	1.8×10^7	6.5×10^7	1.4×10^8	1.6×10^8
Standard plate count (cfu/g)	7.4×10^6	1.2×10^8	6.0×10^7	1.3×10^8	1.8×10^8	1.4×10^9

Note: cfu: Colony-forming unit. NLF *E. coli*: Non-lactose fermenting *E. coli*. T1: Negative control; T2: Positive control; T3: 1.25g/kg; T4: 2.50g/kg; T5: 3.75g/kg; T6: 5.00g/kg.

Table 4.15 : Effect of *M. maximus* Supplementation on the Cecal Microorganisms of Sasso Broilers on Day 56

Parameters	Treatment					
	T1	T2	T3	T4	T5	T6
Isolation and Identification	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (4+)	*E. coli* (3+) NFL *E. coli* (2+)	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (4+) NFL *E. coli* (2+)	*E. coli* (3+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Coliform count (cfu/g)	8.3×10^6	2.5×10^7	1.6×10^7	3.2×10^7	7.6×10^7	1.6×10^8
Standard plate count (cfu/g)	4.3×10^6	1.2×10^8	2.1×10^7	5.1×10^7	3.6×10^7	1.4×10^9

Note: cfu: Colony-forming unit. NLF *E. coli*: Non-lactose fermenting *E. coli*. T1: Negative control; T2: Positive control; T3: 1.25g/kg; T4: 2.50g/kg; T5: 3.75g/kg; T6: 5.00g/kg.

Table 4.16 : Effects of *P. purpureum*, *B. decumbens*, and *M. maximus* Leaf Meal Supplementation on the Cecal Microorganisms of Sasso Broilers on Day 56

Parameters	Treatment		
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)
Isolation and Identification	*E. coli* (3+) *Bacillus* sp. (3+)	*E. coli* (3+) Bacillus sp. (3+)	*E. coli* (3+)
Salmonella identification	Negative	Negative	Negative
Coliform count (cfu/g)	1.6×10^8	1.6×10^8	1.6×10^8
Standard plate count (cfu/g)	1.4×10^9	1.4×10^9	1.4×10^9

Note: cfu: Colony-forming unit.

The cecal microbial population in broilers play an important role in the overall health and production performance (Anadón et al., 2019). Understanding the influence of these microorganism and the relationship they play in a broiler's gut is pivotal for optimising production performances as they play a role in nutrient absorption, metabolism and immune function. Therefore, in order to improve broiler performance and health, the pathogenic microbe population must be kept in check. Otherwise, the microbial equilibrium may be thrown off balance, weakening the intestinal barrier and enabling harmful chemicals or pathogenic bacteria to penetrate the intestinal lumen (Gilani et al., 2021). The addition of phytobiotics which react with pathogenic bacteria's cell membranes and disturb the cytoplasm ecology, may have some positive impacts on the cecal microbial population (Gheisar and Kim, 2018). There are different theories on how these phytobiotics react with microbial cell membranes through their various bioactive compounds (Jamroz et al., 2006; Suresh et al., 2017; Gilani et al., 2021). Nevertheless, past research has revealed that the inclusion of leaf meals and/or their extracts has demonstrated to have either favourable or inconsequential impacts on the gut bacteria community.

In Experiment A, *E. coli* was isolated and identified but no *Salmonella* were identified in all treatment groups. While, *Bacillus* species was identified in all treatments of Experiment A except for the antibiotic control and T6; whereas *Bacillus cereus* was only isolated in T1. Based on the results, it can be suggested that the addition of *P. purpureum* into the broiler diets managed to suppress the growth of pathogenic *B. cereus*, similar with the usage of antibiotic (T2) as it was only identified in T1 in both experiments. *Bacillus* sp. refers to a member of the genus *Bacillus*, which is a diverse group of aerobic, rod-shaped, gram-positive bacteria (Henriques and Moran Jr., 2000).

But, because the specific *Bacillus* species identified isn't mentioned in the grass meal treated group, it could refer to the beneficial or pathogenic member of the *Bacillus* genus. However, improved growth performance data from Experiment A implies that the highest coliform and standard plate count recorded in T6 were due to multiplication of beneficial bacteria. This further indicates that the *Bacillus* sp. in T3-T6 of experiment A were of the advantageous kind such as *B. subtilis* or *B. coagulans*. Previous experiments which provided leaf meals to broilers also found promising results in reducing the growth of pathogenic bacteria in the cecal microbiota (Mandal et al., 2014; Abdelatty et al., 2021; Hidayati et al., 2022). Hidayati et al. (2022) reported that the addition of *Terminalia catappa* leaf extract which are rich in phytochemicals such as flavonoids, tannins, saponins, alkaloids, and many more have the potential to suppress the growth of pathogenic microbes such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella thypii*, and *Bacillus cereus*. Flavonoids found in *P. purpureum* may be responsible for the findings of Experiment A, given their antibacterial properties are one of the most researched pharmacological effects. The key element that permits bacteria to be penetrated and antibacterial activity to be exerted is flavonoid-protein interactions and their amphiphilic characteristics (Shamsudin et al., 2022). This suggests that the introduction of *P. purpureum* at an appropriate dose could create an optimal environment for the proliferation of beneficial bacteria within the cecum (Vidanarachchi et al., 2006).

Similar to Experiment A, *B. cereus* was isolated in only T1 of Experiment B. On the other hand, *Bacillus* sp. was only identified in T5 and T6. It is possible that the absence of antibiotics or *B. decumbens* in the negative control (T1) permitted the development of pathogenic *B. cereus* due to the lack of antibacterial action. Evidently, the

identification of *B. cereus* found in the cecum of T1 broilers poses a threat to consumers as cross contamination to the meat and/or poultry products may occur during manufacturing. Human illnesses such as diarrhoea and emetic-type reaction could occur due to toxins produced by *B. cereus* (Smith et al., 2004). Therefore, it is critical to restrict the proliferation of pathogenic bacteria in the cecal microbial balance of chickens to reduce the risk of a food-borne disease epidemic. However, as previously mentioned, the *Bacillus* sp. found in T5 and T6 of Experiment B are not identified. Thus, based on the other parameters such as the improved growth performance, higher coliform and standard plate count, it can be speculated that the *Bacillus* sp. isolated in T5 and T6 are of beneficial kind. In general, greater standard plate and coliform counts indicate an increase in total bacteria, beneficial bacteria, and a decrease in harmful bacteria (Alghirani et al., 2021). The cecum provides an ideal habitat for microorganisms to appropriately use nutrients while also increasing animal health and production performance through continuous replication (Muhammad et al., 2021). These findings may lead to increased feed utilisation, as seen by the growth performance of these treatment groups. The results of Experiment B differed from the findings of a comparable experiment by Alghirani et al., (2022). Whereby no *Bacillus* sp. were discovered in those treatment groups with the supplementation of *B. decumbens* above 50mg/kg. Alghirani et al. (2022) hypothesised that the concentration of phytochemical substances at lower dosages was insufficient to prevent the proliferation of *Bacillus* sp. But, since the exact species were no identified, we are unable to explain the differences in findings of Experiment B and from the previous experiment. It can be theorised that the different breeds used in each experiment would have different tolerance to fibre, thus altering the gut microbial population in the cecum (Mendes et al., 2003). Saponins' physiological role in plants is not entirely

understood, although it is known that saponins have antibacterial properties, inhibit mould growth, and is a part of the plants' defence system (Francis et al., 2002). According to Ndomou and Mube (2023), saponins' mode of action is to disrupt bacterial membrane permeabilisation, making saponins an effective phytobiotic. Therefore, the high saponin concentration in *B. decumbens* implies its potential role in enhancing the microbial population within the cecum.

Similar to the other two experiments, Experiment C did not identify any *Salmonella* in the cecal microbiota. Though, unlike Experiment A and B, there were no *Bacillus* sp. identification in all treatment groups Experiment C. Alkaloids are naturally present and found in plants as water-soluble salts of organic acids. Because of the presence of one or more nitrogen atoms in their chemical structure, they have alkaline characteristics. Alkaloids can be further classified based on their chemical structures, biological activity, biosynthesis pathway and complexity into heterocyclic and non-heterocyclic alkaloids. Beneficial effects to the growth of broilers have also been observed when broiler chickens are fed with alkaloid-rich leaf meals such as *Sauropus androgynus* and *Polyalthia longifolia*, which may be employed as antibiotic replacements (Sugiharto et al., 2019). The antibacterial action of alkaloids is known to inhibit topoisomerase and disrupt DNA synthesis, lowering the quantity of harmful bacteria in the gut (Suresh et al., 2017). Additionally, chelerythrine—a class of alkaloids, inhibits protein kinase C, which is implicated in the Toll-like receptor 2 pathway, which recognises microorganisms and controls immune responses. Therefore, various subclasses of isoquinoline alkaloids have been shown to enhance the intestinal environment, subsequently improving nutrition utilisation through various pathways (Rundle et al., 2023). By reducing the number of pathogenic

microbes in the gut, intestinal absorptive cells are able to grow, promoting bird development. This is reflected in the results presented in Experiment C, whereby increasing levels of *M. maximus* grass meal increased the coliform and standard plate count. Additionally, T6 which had the best growth performance in Experiment C also had no NFL *E. coli* detected, similar to T2 (antibiotic-treated group). Hence, it can be inferred that the impact of *M. maximus* grass meal on the cecal microbial population is likely linked to the elevated alkaloid content found in the grass.

Moreover, when the best treatments from all three tests were compared, the coliform count and standard plate count did not vary considerably. Many phytobiotics have been proved to limit bacterial growth, and the strength of their action is dependent on the target bacteria, the phytobiotics, and the amount utilised. Indeed, phytocompounds from experimental grasses have demonstrated substantial effects on repressing the growth of pathogenic bacteria in the gut. But, phytobiotics' health benefits and growth-promoting effects may differ depending on a variety of pathways based on their different biological activities. Comparisons of the best treatments from all three tests revealed little variations except from the presence of *Bacillus* sp., albeit it was not determined if it was a favourable *Bacillus* genus member such as *B. subtilis*. As a result, a more in-depth analysis of the bacteria of the cecal population might give additional information about which grass meal would be most helpful to the microbial community of the broilers. Increasing concerns of antimicrobial resistance as well as the restrictions on antibiotics spur the need for rapid discovery of alternatives to antibiotic growth boosters. Thus, using phytobiotics in the form of grass meal may provide effective protection against dangerous microbes while also stimulating helpful bacteria to aid in the creation of an optimum gut microflora balance.

4.5 Carcass Traits

4.5.1 Experiment A (*P. purpereum*)

Table 4.17 shows the effect of *P. purpereum* leaf meal supplementation on the carcass characteristics of Sasso broilers on day 56. There were no significant differences ($p>0.05$) found in the carcass characteristics with or without the supplementation of *P. purpureum*. Numerically, the dressing percentage and important cuts such as breast, drumstick and wings of T6 broilers fed with 5.00g/kg were higher as compared to the other broiler groups.

4.5.2 Experiment B (*B. decumbens*)

The effect of *B. decumbens* meal supplementation on the carcass traits of Sasso broilers on day 56 is shown in Table 4.18. In the full gizzard and GIT, there were significant variations ($p<0.05$) among the treatments. Increasing levels of leaf meal supplementation resulted in increasing values of full gizzard and GIT with T6 chickens showing a significantly ($p<0.05$) higher percentage. However, there were no appreciable differences ($p>0.05$) between the other treatment groups supplemented with *B. decumbens* and the positive and negative control groups for the full gizzard as well as the positive control group for the GIT.

4.5.3 Experiment C (*M. maximus*)

The effect of *M. maximus* supplementation on the carcass traits of Sasso broilers on day 56 is shown in Table 4.19. In kill-out weight and liver, significant differences ($p<0.05$) were discovered. While T3 displayed the lowest kill-out weight percentage, T6 displayed the highest. For the liver parameter, T2 showed the lowest percentage

and T3 showed the greatest, although T4, T5, and T6 did not vary significantly ($p>0.05$) from T2 in this regard.

4.5.4 Comparison of the Best Treatments

4.5.5 from Experiments A, B, and C

Table 4.20 compares the effects of the best treatment of experiments A, B and C on the carcass characteristics. Significant differences ($p<0.05$) were only found in broiler chickens supplemented with 5.00g/kg of *B. decumbens* whereby they had the highest percentage (2.55%) of full gizzard and those supplemented with 5.00g/kg of *M. maximus* had the lowest full gizzard percentage (1.94%). It can be established that supplementing 5.00g/kg of the three different leaf meals did not significantly affect the carcass traits except for the gizzard of Sasso broilers on day 56.

Table 4.17 : Effect of *P. purpereum* Leaf Meal Supplementation on the Carcass Characteristics of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Final live weight (kg)	1.66±0.05	1.78±0.02	1.68±0.04	1.74±0.05	1.74±0.04	1.80±0.05	0.143
Kill-out weight (%)	93.91±0.54	95.32±0.21	94.15±0.0.33	94.39±0.23	94.51±0.35	94.96±0.50	0.134
De-feathered weight (%)	84.32±0.68	90.56±0.94	88.60±0.62	88.62±0.53	88.91±0.32	89.32±0.75	0.529
Dressing percentage (%)	77.94±0.16	77.93±0.54	77.11±0.93	77.50±0.93	77.23±0.60	78.55±0.80	0.759
Breast (%)	16.80±0.73	17.79±0.45	17.39±0.46	17.41±0.64	18.02±0.40	18.04±0.60	0.617
Drumstick (%)	10.10±0.26	10.49±0.38	10.42±0.50	10.40±0.24	10.47±0.23	10.78±0.27	0.813
Wings (%)	8.79±0.22	8.93±0.12	9.06±0.28	9.12±0.12	9.17±0.17	9.34±0.16	0.422
Head (%)	3.21±0.51	3.71±0.23	3.45±0.13	3.64±0.17	3.74±0.18	3.77±0.15	0.648
Neck (%)	2.04±0.14	2.06±0.09	2.09±0.13	2.11±0.12	2.21±0.06	2.40±0.07	0.166
Shanks (%)	4.52±0.27	4.72±0.21	4.58±0.25	4.61±0.22	4.63±0.23	4.71±0.31	0.992
Full gizzard (%)	1.79±0.12	1.68±0.10	1.76±0.13	1.92±0.13	2.02±0.23	2.12±0.13	0.280
Empty gizzard (%)	1.51±0.08	1.43±0.06	1.48±0.07	1.50±0.10	1.54±0.06	1.60±0.13	0.806
GIT (%)	4.10±0.90	6.34±0.29	6.06±0.26	6.64±0.26	7.02±0.44	7.12±0.32	0.131
Heart (%)	0.48±0.02	0.51±0.02	0.50±0.03	0.52±0.02	0.56±0.06	0.58±0.06	0.446
Liver (%)	2.11±0.14	2.07±0.10	2.01±0.08	1.99±0.16	1.88±0.10	1.77±0.07	0.307

All values were expressed as mean ± SE. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.18 : Effect of *B. decumbens* Leaf Meal Supplementation on the Carcass Characteristics of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Final live weight (kg)	1.66±0.05	1.78±0.02	1.67±0.04	1.70±0.04	1.70±0.02	1.71±0.08	0.545
Kill-out weight (%)	94.96±0.05	95.32±0.21	95.12±0.26	95.80±0.29	95.76±0.24	95.14±0.32	0.319
De-feathered weight (%)	89.10±0.34	90.75±0.44	90.42±0.46	90.40±0.47	90.45±0.60	90.79±0.40	0.136
Dressing percentage (%)	76.08±1.41	77.94±0.16	77.93±0.54	77.64±1.13	78.17±0.77	79.40±0.62	0.221
Breast (%)	17.42±0.47	17.79±0.45	17.29±0.58	18.41±0.61	17.46±0.52	18.23±0.21	0.490
Drumstick (%)	10.78±0.27	10.49±0.38	11.36±0.78	10.41±0.39	10.52±0.32	10.79±0.20	0.666
Wings (%)	9.11±0.12	8.79±0.22	9.41±0.29	9.81±0.48	9.355±0.23	9.16±0.21	0.203
Head (%)	3.21±0.51	3.71±0.23	3.87±0.13	3.86±0.24	3.62±0.13	3.67±0.13	0.553
Neck (%)	2.25±0.05	2.22±0.05	2.22±0.04	2.32±0.05	2.37±0.08	2.35±0.07	0.244
Shanks (%)	4.52±0.27	4.72±0.21	4.38±0.12	4.80±0.32	4.09±0.29	4.42±0.22	0.378
Full gizzard (%)	1.79±0.12^{b}	1.68±0.10^{b}	1.79±0.12^{b}	1.74±0.14^{b}	1.96±0.09^{b}	2.55±0.14^{a}	0.000
Empty gizzard (%)	1.51±0.08	1.43±0.06	1.50±0.08	1.63±0.05	1.53±0.06	1.42±0.09	0.346
GIT (%)	6.06±0.26^{b}	6.34±0.29^{ab}	6.60±0.27^{ab}	6.62±0.52^{ab}	7.30±0.34^{ab}	7.51±0.28^{a}	0.037
Heart (%)	0.50±0.03	0.51±0.02	0.54±0.04	0.62±0.02	0.54±0.04	0.54±0.03	0.106
Liver (%)	2.01±0.08	2.11±0.14	2.39±0.31	2.16±0.12	2.08±0.18	1.88±0.11	0.464

All values were expressed as mean ± SE; superscripts $^{a \text{ and } b}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.7 g/kg; T6: 5.00 g/kg.

Table 4.19 : Effect of *M. maximus* Supplementation on the Carcass Characteristics of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Final live weight (kg)	1.67±38.51	1.69±8.48	1.72±49.69	1.67±9.39	1.76±89.24	1.79±25.93	0.487
Kill-out weight (%)	95.00±0.44ab	95.29±0.30ab	93.92±0.57b	95.37±0.22ab	95.18±0.28ab	96.08±0.39a	0.006
De-feathered (%)	90.73±0.2	90.73±0.29	89.60±0.48	90.27±0.34	90.37±1.42	90.44±0.29	0.919
Dressing (%)	78.47±1.09	79.75±0.59	77.53±1.17	78.85±1.62	79.12±1.26	81.37±1.13	0.329
Breast (%)	16.91±0.42	17.70±0.87	16.69±0.19	17.90±0.16	17.53±0.57	17.44±0.91	0.693
Drumstick (%)	10.92±0.59	10.52±0.47	10.32±0.32	10.61±0.25	11.04±0.34	11.13±0.48	0.723
Wing (%)	9.00±0.46	9.55±0.17	9.35±0.40	9.38±0.16	9.28±0.47	9.68±0.31	0.805
Head (%)	3.46±0.13	3.73±0.16	3.69±0.16	3.86±0.20	3.82±0.26	4.12±0.18	0.264
Neck (%)	2.53±0.10	2.34±0.12	2.10±0.11	2.42±0.10	2.43±0.07	2.21±0.07	0.743
Shank (%)	4.52±0.22	4.82±0.21	4.75±0.19	4.65±0.24	4.55±0.19	4.87±0.24	0.809
Full gizzard (%)	1.58±0.09	1.85±0.15	2.05±0.20	2.03±0.14	1.88±0.20	1.94±0.14	0.349
Empty gizzard (%)	1.27±0.05	1.47±0.06	1.47±0.07	1.54±0.09	1.51±0.09	1.55±0.11	0.19
GIT (%)	6.70±0.24	7.06±0.34	7.00±0.31	7.34±0.17	6.90±0.25	7.76±0.55	0.294
Heart (%)	0.58±0.02	0.51±0.02	0.52±0.01	0.55±0.04	0.56±0.03	0.60±0.04	0.267
Liver (%)	2.34±0.1ab	1.68±0.03c	2.67±0.27a	2.09±0.12bc	1.93±0.08bc	2.09±0.08bc	<0.000

All values were expressed as mean ± SE; superscripts [a, b, and c] values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.20 : Effects of *P. purpureum*, *B. decumbens,* and *M. maximus* Leaf Meal Supplementation on the Carcass Characteristics of Sasso Broilers on Day 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
Final live weight (kg)	1.80±0.05	1.71±0.08	1.79±25.93	0.1113
Kill-out weight (%)	94.96±0.50	95.14±0.32	96.08±0.39	0.6037
De-feathered (%)	89.32±0.75	90.79±0.40	90.44±0.29	0.2211
Dressing (%)	78.55±0.80	79.40±0.62	81.37±1.13	0.4207
Breast (%)	18.04±0.60	18.23±0.21	17.44±0.91	0.7559
Drumstick (%)	10.78±0.27	10.79±0.20	11.13±0.48	0.6976
Wing (%)	9.34±0.16	9.16±0.21	9.68±0.31	0.4939
Head (%)	3.77±0.15	3.67±0.13	4.12±0.18	0.3422
Neck (%)	2.40±0.07	2.35±0.07	2.21±0.07	0.0867
Shank (%)	4.71±0.31	4.42±0.22	4.87±0.24	0.5173
Full gizzard (%)	2.12±0.13^{ab}	2.55±0.14^{a}	1.94±0.14^{b}	0.0189
Empty gizzard (%)	1.60±0.13	1.42±0.09	1.55±0.11	0.6631
Gastrointestinal tract (%)	7.12±0.32	7.51±0.28	7.76±0.55	0.9465
Heart (%)	0.58±0.06	0.54±0.03	0.60±0.04	0.5477
Liver (%)	1.77±0.07	1.88±0.11	2.09±0.08	0.1936

All values were expressed as mean ± SE; superscripts $^{a \text{ and } b}$ values within the row are significantly different at $p < 0.05$.

Carcass traits are a key factor in broiler production as they impact the entire economic worth of the bird. Poor body weight results in low carcass yield and a lower dressing percentage. Furthermore, final live weight has a direct influence on breast muscle yield, which is one of the most important financial criteria for both producer and retailer. Therefore, a producer's main focus would be to improve these traits through interventions such as management, nutrition, or genetics in order to meet consumer demands. The application of phytocompounds into diets of broilers could be one of the ways to improve the quality of carcass due to their antibacterial and antioxidant properties, while adding product value (Puvača et al., 2011; Adriani et al., 2019).

Previous studies reported that the addition of kudzu leaf meal—which is said to be high in flavonoids, lowered carcass weights and correspondingly, breast yield, in comparison to the control group (Zaefarian et al., 2019). But, the application of *P. purpureum* grass meal in Experiment A had no significant effects on the carcass characteristics of Sasso broilers on day 56. Nonetheless, it can be observed that increasing levels of supplementation from T3 to T6 numerically improved some carcass traits such as final live weight, kill-out weight, dressing percentage, several of the carcass cut-out parts and giblet percentage. A meta-analysis of dietary flavonoids in broilers revealed that flavonoids had only a minimal influence on carcass and organ composition, as higher dosages (up to 60,000mg/kg) of flavonoids increased liver weight, likely due to detoxification process (Prihambodo et al., 2021). The primary action of flavonoids, which are a class of polyphenols, is mainly associated with antioxidant properties. Because of their delocalized electron structure, flavonoids would serve as buffers by absorbing free radicals and converting them into less reactive flavin radicals (Ndomou and Mube, 2023). Flavonoids present in *P.*

purpureum grass meal could be responsible for the slight improvements of carcass characteristics as indicated by previous studies. Despite no significant enhancements in the carcass traits of treated broilers, more importantly, this indicates that the addition of *P. purpureum* grass meal was not detrimental to the body and organ development of Sasso broilers.

Experiment B revealed that 5.00g/kg of *B. decumbens* significantly increased the levels of full gizzard and GIT percentage, with no other significant improvements in the other measured carcass traits. Conversely, a previous study that incorporated *Gliricidia sepium* leaf meal in broiler meals also found the weight of the gizzard and liver to be increased, but the authors ascribed it to the presence of tannins, alkaloids, and nitrates that function as anti-nutritional agents (Kagya-Agyemang et al., 2007). Likewise, Alghirani et al. (2022), also observed significant changes in the carcass characteristics of Ross308 broilers supplemented with *B. decumbens*. They ascribed it to the presence of saponins and other phytochemicals, despite the fact that other comparable trials revealed no significant alterations, presumably due to variations in variables across the studies. In the current experiment, empty gizzard did not reveal any significant changes amongst all treatments. For that reason, the data indicates that the higher weight of full gizzard and GIT is due to feed still present and retained. A possibility that may explain the current findings is that a larger weight of the full gizzard and GIT suggests that this is due to the presence of more insoluble fibre in T6. This enhances digestive capacity and allows for faster transit through the digestive system (Hetland and Svihus, 2001). The theory is further supported by improved digestibility of ether extract in the current experiment as enzyme-substrate interactions is enhanced. This is because the amount of bile acids in the gizzard rises according to

the amount of gizzard contents, indicating gastroduodenal reflux and better digestibility (Hetland et al.,2003). The situation occurs simply because an empty gizzard, which lacks feed cues, is unable to regulate downstream digestion processes (Duke, 1992). Hence, based on the data presented, the addition of 5.00g/kg of *B. decumbens* could improve digestibility despite not showing significant differences in the cut-out percentages.

Similarly, the application of *M. maximus* in broiler feed did not show much effects on the carcass traits of Sasso broilers either. Significant differences were only found in the kill-out weight % and liver %, with T6 displaying the highest kill-out weight and T3 with the highest liver relative weight. In a previous experiment using Arbour Acres broilers and Mucuna leaf meals, Oloruntola et al. (2022) found that increasing the dose of Mucuna leaf meal supplementation resulted in a greater dressing percentage. Mucuna leaf meals are reported to be high in alkaloids, flavonoids, saponins, tannins, terpenoids, and other phytochemicals. Additionally, Faria et al. (2011) also found better carcass yield with the addition of cassava leaf meal. Phytocompounds can have anabolic effects via modifying animal metabolism, mimicking β-adrenergic agonist chemicals, or boosting plasma norepinephrine levels by inhibiting catechol-o-methyltransferase. In summary, these two approaches alter the animal's metabolism by increasing protein synthesis and lipolysis while lowering lipogenesis, hence promoting muscle tissue growth (Valenzuela-Grijalva et al., 2017; Oloruntola et al., 2022). This could explain the significantly lower relative liver weight in T4-T6 in comparison to the negative control as reduced fatty acid storage in the liver may lower liver weight to some extent, though not definite (Zaefarian et al., 2019). Nevertheless, the liver weight % in this study was still within the normal weight range as an extreme

increase in liver weight would indicate a surge of detoxification activity in the hepatocytes (Prihambodo et al., 2020). On the other hand, several other studies found opposing effects, in which the addition of various leaf meals lowered carcass quality, which reflected a decline in production performances (Donkoh et al., 2002; Kagya-Agyemang et al., 2007; Zaefarian et al., 2019). In short, the data indicates that the inclusion of *M. maximus* into broiler diets does not adversely affect broiler development. Furthermore, it may potentially enhance lipid metabolism, a topic that will be explored in a subsequent sub-section.

Discrepancies in the obtained results from previous studies may stem from variations in supplementation levels and other experimental factors, such as the type of plant-based material employed. Upon comprehensive analysis, comparing the outcomes of Experiments A, B, and C highlighted a lack of significant differences in carcass characteristics, except for the notable variance in full gizzard results. This suggests that each grass meal at 5.00g/kg exhibits distinct advantages, potentially contributing to varied production performances. Consequently, it can be asserted that exploring the benefits of each phytobiotic is crucial. The applicability of these findings extends to potential economic benefits for the industry, emphasizing the need for further research to delve into the individual phytobiotics and their economic implications.

4.6 Meat Quality

4.6.1 Experiment A (*P. purpereum*)

Table 4.21 displays how adding *P. purpereum* leaf supplements affected the meat quality of broilers on day 56. There were significant differences ($p<0.05$) found among treatments in drip loss at 24h and 48h, colour (L*, a*, b*) and tenderness for both

breast and drumstick muscle, with the addition of pH in the drumstick muscle during meat quality analysis. The T6 group's breast muscle produced the maximum b* and tenderness (lowest shear force) while having the lowest drip loss% at 48 hours, L*, and a*. The drumstick's meat quality analysis showed that, although T6 did not significantly differ ($p>0.05$) from T2, T4, or T5, drip loss% after 24 and 48 hours was lowest in T6. The colours a* had the lowest T6 values and the colours b* had the greatest values. Muscle pH of the drumstick also showed slight differences ($p=0.0197$) between treatment groups as T1 showed the lowest pH value (6.32). Overall, positive results can be seen in supplementing *P. purpureum* to Sasso broilers.

4.6.2 Experiment B (*B. decumbens*)

Table 4.22 illustrates the effect of *B. decumbens* leaf meal supplementation on Sasso broiler meat quality on day 56. Meat quality examination of the breast muscle revealed significant ($p<0.05$) changes in colour (L*, a*, b*) and tenderness across treatment groups, whereas drumstick revealed significant ($p<0.05$) differences in pH, drip loss at 24h, colour (L*, a*, b*), and shear force values. While T2 breast muscle had the lowest L* reading of 54.65, it did not differ substantially ($p>0.05$) from T4, T5, and T6, which had values of 57.34, 56.01, and 55.72, respectively. T2 had the lowest shear force in both the breast and the drumstick muscle, followed by T6 with no significant differences ($p>0.05$). T6 showed the lowest redness (a*) of the breast muscle, whereas T5 and T6 had the greatest yellowness (b*) readings. Drumstick muscle pH exhibited significant variations ($p<0.05$), with T1 (5.44) having the lowest pH value and T2 (6.73) having the highest. T4-T6 did not, however, differ substantially ($p>0.05$) from T1 and T2. Drip loss was lowest in T2 (1.75%), followed by T6 (2.29%), and the *B. decumbens*-treated groups (T3-T6) were not significantly different ($p>0.05$) from T2.

Colour analysis revealed that a* was lowest in T6 (9.76) but did not change substantially ($p>0.05$) from T2 (10.07) or T5 (10.44), but b* was greatest in T6 (24.79). Shear force was also assessed, and T2 generated the most tender drumstick (382.78) but did not vary significantly ($p>0.05$) from the *B. decumbens* fed group, T3-T6 (397.95-432.96), showing that addition of *B. decumbens* leaf meal could enhance Sasso broiler meat quality.

4.6.3 Experiment C (*M. maximus*)

Table 4.23 illustrates the effect of *M. maximus* leaf meal supplementation on Sasso broiler meat quality on day 56. There were significant differences ($p<0.05$) between treatment groups in drip loss percentages at 24 and 48 hours, L*, and shear force of breast muscle, whereas drumstick revealed significant differences ($p<0.05$) in cooking loss percentage, drip loss percentage at 48 hours, and b*. Group T2 had the lowest drip loss % in both the 24h and 48h evaluations, but their results were not substantially different ($p>0.05$) from the other groups supplemented with *M. maximus* leaf meal. Shear force was lowest in T6 breast meat, indicating that T6 possessed greater softness. While T2 showed the lowest drip loss at 48h, it did not significantly differ ($p>0.05$) to T3-T6. Besides, drumstick meat analysis showed that T6 had the best results as it showed the lowest cooking loss (20.93%) and the highest yellowness (b*) (17.92) value which are desired.

4.6.4 Comparison of the Best Treatments from Experiments A, B, and C

Table 4.24 compares the meat quality of Sasso broilers on day 56 to the optimal treatment of trials A, B, and C. Cooking loss%, drip loss at 48h, colour (a* and b*), and softness of the breast muscle all showed significant variations ($p<0.05$).

Furthermore, significant variations ($p<0.05$) were identified in cooking loss%, drip loss at 24h and 48h, colour (a* and b*), and drumstick muscle tenderness. Breast muscle of broilers supplemented with 5.00g/kg of *P. purpureum* had the lowest drip loss at 48h, redness and tenderness in comparison to the other treatments. Although experiment B (5.00g/kg of *B. decumbens*) had a lower cooking loss percentage (25.11%), it did not significantly vary ($p>0.05$) from experiment A (5.00g/kg of *P. purpureum*) (25.39%). Besides, breast meat from broilers of experiment A also recorded the highest value for yellowness which is a desired trait for customers when selecting coloured-broilers meat. Similarly, meat analysis of the drumstick revealed that experiment A had the lowest drip loss at 24h, colour L*, and tenderness. While drip loss at 48h and redness (a*) did not record the lowest value, they did not vary substantially ($p>0.05$) from the other trial that did. On top of that, tenderness was the best in experiment A and thus generally, it can be concluded that experiment A produced meat with the best quality.

Table 4.21 : Effect of *P. purpereum* Leaf Supplementation on the Meat Quality of Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Breast							
Cooking loss (%)	22.97±0.77	21.95±1.04	22.14±1.12	22.17±0.70	23.32±0.79	25.39±1.02	0.1504
Muscle pH	6.27±0.15	6.02±0.01	5.99±0.04	6.02±0.05	6.22±0.10	6.19±0.12	0.149
Drip loss at 24 h (%)	5.87±0.51^{a}	2.83±0.44^{b}	4.25±0.58^{ab}	4.62±0.87^{ab}	4.60±0.54^{ab}	2.63±0.51^{b}	0.0050
Drip loss at 48 h (%)	6.75±0.48^{a}	6.14±0.28^{ab}	5.55±0.90^{ab}	5.51±0.61^{ab}	3.88±0.52^{bc}	2.83±0.64^{c}	0.0006
Colour L* (lightness)	65.21±1.17^{a}	57.75±0.35^{cd}	63.68±1.87^{ab}	62.04±0.83^{abc}	60.75±0.30^{bcd}	56.81±0.72^{d}	<0.000
Colour a* (redness)	11.52±0.19^{a}	7.66±0.25^{d}	10.26±0.35^{b}	9.18±0.24^{c}	7.44±0.06^{d}	5.27±0.07^{e}	<0.000
Colour b* (yellowness)	17.93±0.20^{d}	20.12±0.37^{c}	18.35±0.12^{d}	21.62±0.28^{c}	24.30±0.37^{b}	28.33±0.57^{a}	<0.000
Shear force	436.62±12.14^{a}	387.04±23.37^{ab}	378.35±9.67^{ab}	361.38±5.46^{b}	351.37±11.53^{b}	272.61±17.41^{c}	<0.000
Drumstick							
Cooking loss (%)	26..46±1.15	23.46±0.49	26.56±1.20	28.16±1.22	26.51±2.02	28.11±0.54	0.4191
Muscle pH	6.32±0.08^{b}	6.76±0.01^{a}	6.42±0.11^{ab}	6.43±0.04^{ab}	6.49±0.14^{ab}	6.60±0.07^{ab}	0.0197
Drip loss at 24 h (%)	4.07±0.30^{a}	1.65±0.11^{b}	3.65±0.66^{a}	1.25±0.29^{b}	1.11±0.24^{b}	0.62±0.19^{b}	<0.000
Drip loss at 48 h (%)	5.48±0.37^{a}	2.56±0.15^{b}	5.77±1.16^{a}	1.84±0.43^{b}	1.68±0.33^{b}	1.28±0.32^{b}	<0.000
Colour L* (lightness)	44.63±0.32^{c}	50.71±0.50^{b}	60.71±0.53^{a}	59.45±0.27^{a}	50.18±0.45^{b}	50.32±0.66^{b}	<0.000
Colour a* (redness)	12.15±0.15^{a}	10.61±0.25^{bc}	11.25±0.23^{abc}	11.29±0.20^{ab}	10.49±0.16^{bc}	10.34±0.27^{c}	<0.000
Colour b* (yellowness)	13.82±0.17^{c}	13.85±0.06^{c}	18.35±0.15^{b}	18.60±0.19^{b}	19.16±0.45^{b}	21.58±0.56^{a}	<0.000
Shear force	460.26±10.34^{a}	443.57±13.17^{ab}	440.65±9.14^{ab}	429.07±12.03^{ab}	425.66±23.02^{ab}	388.91±10.17^{b}	0.0482

All values were expressed as mean ± SE; superscripts $^{a, b, c, d, and e}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.22 : Effect of *B. decumbens* Leaf Meal Supplementation on the Meat Quality of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Breast							
Cooking loss (%)	24.72±1.34	22.70±1.37	23.10±0.99	23.43±0.94	24.05±1.09	25.11±0.96	0.606
Muscle pH	5.96±0.02	6.17±0.10	5.98±0.04	6.01±0.01	6.06±0.08	6.13±0.11	0.227
Drip loss at 24 h (%)	5.24±0.91	2.15±0.49	4.87±1.62	4.74±0.66	4.15±0.76	3.00±0.46	0.148
Drip loss at 48 h (%)	7.38±0.84	4.6±0.99	7.28±1.51	6.19±0.82	5.95±1.02	4.84±0.66	0.269
Colour L* (lightness)	60.93±0.37^{a}	54.65±0.47^{c}	59.51±0.76^{ab}	57.34±0.43^{bc}	56.01±1.19^{c}	55.72±0.86^{c}	<0.000
Colour a* (redness)	18.10±0.30^{a}	9.30±0.28^{d}	12.06±0.53^{b}	11.23±0.27^{bc}	10.34±0.27^{cd}	7.39±0.09^{e}	<0.000
Colour b* (yellowness)	18.10±0.03^{c}	21.56±0.45^{b}	21.62±0.28^{b}	21.66±0.21^{b}	24.34±0.32^{a}	24.34±0.32^{a}	<0.000
Shear force	505.22±19.22^{a}	279.49±17.84^{c}	486.97±25.28^{a}	373.90±7.56^{b}	358.59±14.35^{bc}	283.88±21.94^{c}	<0.000
Drumstick							
Cooking loss (%)	29.76±1.89	25.24±0.67	29.76±1.89	27.18±0. 76	27.05±1.34	26.61±0.37	0.180
Muscle pH	5.44±0.02^{b}	6.73±0.01^{a}	6.44±0.08^{b}	5.63±0.02^{ab}	6.47±0.08^{ab}	6.56±0.10^{ab}	0.010
Drip loss at 24 h (%)	4.44±0.53^{a}	1.75±0.15^{b}	3.28±0.87^{ab}	3.25±0.42^{ab}	2.56±0.56^{ab}	2.29±0.25^{ab}	0.017
Drip loss at 48 h (%)	5.60±0.40	2.66±0.19	5.38±0.92	4.27±1.44	4.04±0.88	3.78±0.92	0.221
Colour L* (lightness)	44.80±0.49^{e}	58.13±0.27^{a}	48.55±0.30^{d}	51.21±0.78^{c}	54.75±0.92^{b}	56.09±0.40^{ab}	<0.000
Colour a* (redness)	11.61±0.58^{ab}	10.07±0.23^{cd}	11.25±0.23^{abc}	11.25±0.23^{abc}	10.44±0.30^{bcd}	9.76±0.18^{d}	<0.000
Colour b* (yellowness)	14.14±0.31^{c}	15.81±0.38^{bc}	16.26±0.17^{bc}	16.61±0.58^{bc}	19.11±0.19^{b}	24.79±0.26^{a}	<0.000
Shear force	514.13±20.62^{a}	382.78±19.81^{b}	432.96±18.20^{ab}	414.65±20.06^{b}	410.52±19.21^{b}	397.95±14.75^{b}	0.001

All values were expressed as mean ± SE; superscripts $^{a, b, c, d \text{ and } e}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.23 : Effect of *M. maximus* Leaf Meal Supplementation on the Meat Quality of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
Breast							
Cooking loss (%)	21.40±0.69	22.24±0.97	22.65±1.01	22.85±0.53	21.44±0.41	20.16±0.67	0.146
Muscle pH	6.08±0.09	5.92±0.05	6.04±0.02	6.02±0.09	6.08±0.04	6.11±0.07	0.418
Drip loss at 24 h (%)	5.90±0.39^{a}	1.94±0.31^{c}	5.04±1.16^{ab}	2.98±0.58^{abc}	2.40±0.36^{bc}	2.34±0.28^{c}	0.001
Drip loss at 48 h (%)	6.94±0.57^{a}	3.32±0.36^{b}	5.71±0.92^{ab}	4.36±0.41^{ab}	3.85±0.61^{ab}	3.55±0.60^{b}	0.007
Colour L* (lightness)	61.03±0.78^{a}	56.03±0.88^{b}	59.62±0.49^{a}	58.06±1.09^{ab}	56.55±0.80^{b}	56.44±0.49^{b}	<0.000
Colour a* (redness)	9.48±0.15	9.30±0.22	9.71±0.11	9.24±0.68	9.26±0.00	9.12±0.24	0.430
Colour b* (yellowness)	21.41±0.23	21.34±0.38	22.53±0.40	20.91±0.68	21.17±0.45	21.24±0.40	0.179
Shear force	504.27±18.37^{a}	455.42±13.68^{a}	544.41±50.61^{a}	483.44±10.70^{a}	449.73±10.15^{ab}	349.15±1.37^{b}	<0.000
Drumstick							
Cooking loss (%)	23.04±1.01^{bc}	22.94±0.82^{bc}	26.81±0.64^{a}	24.74±0.8^{ab}	24.61±0.53^{ab}	20.93±0.96^{c}	<0.000
Muscle pH	6.53±0.09	5.60±0.14	6.53±0.09	6.62±0.06	6.56±0.12	6.71±0.09	0.828
Drip loss at 24 h (%)	1.33±0.27	0.55±0.12	1.07±0.24	1.04±0.19	0.95±0.36	0.76±0.17	0.577
Drip loss at 48 h (%)	2.26±0.41a	1.03±0.17b	1.90±0.22^{ab}	1.72±0.26^{ab}	1.70±0.07^{ab}	1.06±0.11^{ab}	0.023
Colour L* (lightness)	52.97±0.73	51.91±0.96	53.32±0.95	53.64±1.14	52.33±0.52	52.54±0.80	0.797
Colour a* (redness)	11.10±0.43	12.10±0.54	11.71±0.3	11.32±0.27	11.46±0.37	11.34±0.36	0.594
Colour b* (yellowness)	16.41±0.37^{b}	17.22±0.26^{ab}	17.36±0.56^{ab}	17.49±0.41^{ab}	17.50±0.23^{ab}	17.92±0.18^{a}	0.082
Shear force	531.71±24.71	573.84±26.21	642.79±13.78	558.39±21.08	475.06±13.61	415.00±11.35	0.207

All values were expressed as mean ± SE; superscripts $^{a, b, \text{and } c}$ values within the row are significantly different at $p < 0.05$. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.24 : Effects of *P. purpureum*, *B. decumbens*, and *M. maximus* Leaf Meal Supplementation on the Meat Quality of Sasso Broilers on Day 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
Breast				
Cooking loss (%)	25.39±1.02^{a}	25.11±0.96^{a}	20.16±0.67^{b}	0.0005
Muscle pH	6.19±0.12	6.13±0.11	6.11±0.07	0.8397
Drip loss at 24 h (%)	2.63±0.51	3.00±0.46	2.34±0.28	0.5146
Drip loss at 48 h (%)	2.83±0.64^{b}	4.84±0.66^{a}	3.55±0.60^{ab}	0.0491
Colour L* (lightness)	56.81±0.72	55.72±0.86	56.44±0.49	0.2022
Colour a* (redness)	5.27±0.07^{c}	7.39±0.09^{b}	9.12±0.24^{a}	<0.0000
Colour b* (yellowness)	28.33±0.57^{a}	24.34±0.32^{b}	21.24±0.40^{c}	<0.0000
Shear force	272.61±17.41^{b}	283.88±21.94^{b}	349.15±1.37^{a}	0.0001
Drumstick				
Cooking loss (%)	28.11±0.54^{a}	26.61±0.37^{b}	20.93±0.96^{c}	<0.0000
Muscle pH	6.60±0.07	6.56±0.10	6.71±0.09	0.3387
Drip loss at 24 h (%)	0.62±0.19^{b}	2.29±0.25^{a}	0.76±0.17^{b}	<0.0000
Drip loss at 48 h (%)	1.28±0.32^{b}	3.78±0.92^{a}	1.06±0.11^{b}	0.0065
Colour L* (lightness)	50.32±0.66^{c}	56.09±0.40^{a}	52.54±0.80^{b}	<0.0000
Colour a* (redness)	10.34±0.27^{b}	9.76±0.18^{c}	11.34±0.36^{a}	<0.0000
Colour b* (yellowness)	21.58±0.56^{b}	24.79±0.26^{a}	17.92±0.18^{c}	<0.0000
Shear force	388.91±10.17^{b}	397.95±14.75^{ab}	415.00±11.35^{a}	0.02045

All values were expressed as mean ± SE; superscripts $^{a, b, \text{and } c}$ values within the row are significantly different at $p < 0.05$.

Incorporation of pre-slaughter dietary interventions, such as phytobiotics, can modify the physiological mechanisms within the chicken's body without altering the nutritional composition of the diet. Since dietary intervention occurs before slaughter, effects are standardised across the flock producing more consistent results. This approach is deemed more natural, ultimately influencing the quality of the resulting meat product. In addition, feeding is an important aspect that alters meat quality as it can also alter the fatty acid profile of the meat (Brunel et al., 2006). On top of that, the visual appeal of the product typically influences a consumer's buying decisions since it the first indicator of the quality, notably the colour of meat (Mancini and Hunt, 2005). Other than that, pH value, tenderness, and cumulative drip loss are all essential elements in determining meat quality (Janisch et al., 2012). Apart from microbial-related quality loss in meat, oxidation is the principal cause of meat degradation. Therefore, the application of phytobiotics as an antioxidant is hypothesised to improve meat quality. However, it is crucial to highlight that the efficacy of phytobiotics varies depending on the specific bioactive compounds present, their quantities, and results differs according to the breed, sex, and production management of the chickens (Grashorn and Clostermann, 2002; Mendes et al., 2003).

Rapid post-mortem metabolism leads to a quick decline in post-mortem pH as carcass temperatures remain high after slaughtering. The combination of these factors may cause protein denaturation in the muscle, resulting in pale, soft, and exudative meat (PSE) (Woelfel et al., 2002). Drip loss is the loss of muscle contents, which causes meat to lose its natural texture and flavour. Meat quality is closely associated with drip loss as this will affect the juiciness, taste, and acceptability of meat (Ponsuksili et al., 2008). Not only does this predicament affect the product quality, but it will impact the

economic value as well. Poor product quality, characterized as meat with low pH and high drip loss, stems from increased glycolysis and impairment of mitochondrial function due to reactive oxygen species (ROS) production (Estévez, 2015). The generation of ROS will have severe consequences in broiler's meat as it affects skeletal muscle development and lipid peroxidation (Altan et al., 2003; Kumar et al., 2012). ROS impairs meat quality through a series of events affects the operation of the electron transport chain, ATP synthesis, produces calcium imbalance, and oxidises many proteins within mitochondria, as well as disrupting the membrane (Cadenas and Davies, 2000; Zissimopoulos and Lai, 2006; Slimen et al., 2014). This relates to acidosis of the chicken meat which occurs when anaerobic glycolysis in the muscle is activated, causing more H+ and lactic acid to build, resulting in a decrease of muscle pH. A reduction in muscle pH causes limited water holding capacity, which leads to PSE (Van Laack et al, 2000; Strasburg and Chiang, 2009).

In the current research, broilers supplemented with 5.00g/kg of grass meal from Experiment A yielded superior meat quality in both breast and drumstick meat. The improvements in drip loss and shear force values breast and drumstick meat of the experiments could be attributed the less acidic meat of muscle pH. Previous experiments which utilised flavonoids have established that flavonoids may improve meat quality and alleviate pre-slaughter stressors (Rajput et al., 2014). This is because they have high antioxidant abilities that are able to remove free radicals (ROS), superoxide and hydroxyl radicals by single-electron transfer (RiceEvans et al. 1996; Choi et al. 2002). A prior research which included *Moringa oleifera* leaf meals into broiler diets found that there were improved meat quality traits such as decreased drip loss, attributed to the array of phytocompounds, including flavonoids which possess

antioxidant activity (Nkukwana et al., 2015). The antioxidant activity of flavonoids is said to be safely removing oxidants like superoxide and converting them into water and oxygen as final products, thus reduces the need for the body to produce antioxidant enzymes (Surai et al., 2019). Furthermore, flavonoids have been discovered to improve meats' shelf-life by lowering lipid oxidation and the development of spoilage microbes. The oxidation of unsaturated fatty acids in phospholipids and triacylglycerols, which leads to off-flavours, is decreased through the application of dietary flavonoids (Kamboh et al, 2019).

In Experiment B, broilers supplemented with 5.00g/kg of *B. decumbens* showed improvements in the colour and shear force values of both breast and drumstick muscle. Additionally, the pH and drip loss at 24 hours for the drumstick muscle of T6 showed improvements compared to the other treatment groups. The current study suggests that the saponins abundantly present in *B. decumbens* contributed to the improved meat quality of treated broilers. Dietary saponins are believed to decrease lipid oxidation by inhibiting the activity of cholesterol and lipids, in addition to their antioxidant effects, as these are major components required for lipid oxidation in meat. This theory is supported by previous studies showing that the application of saponins in broiler diets enhances lipid oxidative stability due to their strong antioxidant activity (Tippel et al., 2017; Bera et al., 2019). This safeguarding prevents the oxidation of low-density lipoproteins, thereby reducing protein degradation and delaying the oxidation of myoglobin and haemoglobin. This impacts the overall appearance of the product as myoglobin and haemoglobin pigments influence meat discoloration based on their oxidation level (Mancini and Hunt, 2005). Additionally, previous studies have shown that incorporating saponin-rich alfalfa meal (4%) in chicken diets significantly

lowers the peroxide value of chicken fat (Tkáčová et al., 2015). In a more recent study, Alghirani et al. (2022) discovered that the addition of 25mg/kg of *B. decumbens* improved meat quality of commercial broilers and attributed the findings to the secondary compounds found in *B. decumbens*, which protected the muscles from oxidative damage. Therefore, the use of phytobiotics as antioxidants, particularly *B. decumbens*, is hypothesised to enhance meat quality. However, it's important to note that the effectiveness of phytobiotics can vary depending on the specific bioactive compounds present, their concentrations, and the breed, sex, and management practices of the chickens. These factors may explain the discrepancies observed between the current study and that of Alghirani et al. (2022) (Grashorn and Clostermann, 2002; Ponte et al., 2004).

Based on the results from Experiment C, it is hypothesised that alkaloids are responsible for the lower drip loss and cooking loss as incremental levels of *M. maximus* leaf supplementation produced better results. As previously stated, oxidative damage to the skeletal muscle tissues diminishes meat quality. A study on isoquinoline alkaloids and its effects on oxidative damage and protein catabolism were shown to improve muscle conditions (Kikusato et al., 2021; Rundle et al., 2023). Muscle degradation may be a metabolic reaction to recruit amino acids from the skeletal muscle to supply energy substrates or to synthesise peptides and proteins, such as acute phase proteins. In response to physiological and pathological stimuli, corticosterone is released and it induces proteolysis via the intracellular signalling system. Findings from this study showed that corticosterone secretions were suppressed and plasma total protein content were restored in broilers supplemented with isoquinoline alkaloids, subjected to stressful conditions (Kikusato et al., 2021). Additionally,

plasma concentration of uric acid (an endogenous antioxidant) was increased with the administration of corticosterone (Liu et al., 2012). The study discovered that isoquinoline alkaloids may prevent oxidative damage since plasma uric acid levels were lowered after administration. Thus, the improved meat quality in *M. maximus* supplemented chickens could be attributed to high levels of alkaloids present in the grass.

A comparison of the best treatments of all three grass meals revealed that 5.00g/kg of *P. purpureum* produced the overall best meat quality. A lower drip loss in both the breast and drumstick muscle allowed for a more tender and lower shear force reading. This is because meat with higher water losses produces cooked meat that is more dry and brittle due to the inadequacy of WHC and protein extractability (Barbut et al., 2008; Owens et al., 2009). Additionally, T6 of Experiment A produced broiler with breast meat that are more yellow in colour. This is a typical trait that is usually sought after by consumers who prefer slow-growing broilers (Aini, 1990). Additionally, redness in the breast meat of this group were also lower and this is representative of breast meat, as it is almost entirely composed of white fibres. White fibres in are lower in myoglobin content in comparison to red fibres which are more evident in the thigh muscle (Wideman et al., 2016). Thus, grass meals could be studied further to optimise its usage as a plant-based additive in broiler feed as the meat quality has been shown to improve with the usage of *P. purpureum* possibly due to their stronger antioxidant effects.

4.7 Blood Biomarkers

4.7.1 Experiment A (*P. purpereum*)

The impact of *P. purpureum* leaf meal supplementation on blood biomarkers in Sasso broilers on day 56 is shown in Table 4.25. There are substantial variations in every parameter ($p<0.05$) across treatment groups. T6 group had the lowest values for all blood biomarker indicators evaluated, including SAA, AGP, CP, corticosterone, and HSP70. It should be emphasised, however, that there were no significant changes ($p>0.05$) between T4 and T6 for SAA. Similarly, no significant variations ($p>0.05$) in AGP were identified between treatment groups T4 to T6 and T2. Additionally, there were no significant variations ($p>0.05$) in CP between T5 and T6, whereas there were no significant differences in HSP70 between T2, T5, and T6. When T1 (the negative control) was compared to T6, T6 reduced SAA levels by 24.88%, AGP levels by 23.36%, CP levels by 35.71%, corticosterone levels by 43.96%, and HSP70 levels by 24.64%. Thus, this indicates that T6 had less stress leading to better performances in comparison to the other treatment groups.

4.7.2 Experiment B (*B. decumbens*)

Table 4.26 shows the effect of *B. decumbens* leaf meal supplementation on blood biomarkers in Sasso broilers on day 56. Each parameter varied significantly ($p<0.05$) among treatment groups. All blood biomarker parameters assessed, including SAA, CP, corticosterone, and HSP70, were also the lowest in the T6 group. It should be noted that there were no significant differences ($p>0.05$) in SAA between T2 and T4 to T6 broilers. While T2 had the lowest numerical value for AGP, no significant differences ($p>0.05$) in AGP were found between T2 (17.44 l/ml) and T6 (20.86 l/ml).

Furthermore, there were no significant differences ($p>0.05$) in CP and corticosterone levels between T5 and T6, although there were no significant differences in HSP70 between T2 and T4 to T6 treatment groups. Overall, T6 decreased SAA, AGP, CP, corticosterone, and HSP 70 levels by 27.33%, 35.32%, 46.40%, 56.12%, and 21.41% respectively in contrast to T1. As a result, T6 exhibited less stress thus performed better in contrast to the other treatment groups.

4.7.3 Experiment C (*M. maximus*)

Table 4.27 shows the effect of *M. maximus* leaf meal supplementation on blood biomarkers in Sasso broilers on day 56. There were significant differences in all parameters examined ($p<0.05$) across treatment groups, with the T6 group having the lowest values for all blood biomarker markers assessed. However, there were no statistically significant variations ($p>0.05$) between T2 to T6 for SAA, AGP, and HSP70. Furthermore, there were no significant differences ($p>0.05$) in CP between T2 and T3 to T6, while T4 to T6 differed considerably ($p<0.05$) from T3. Corticosterone levels were the lowest in T6 (135.13 pg/ml) and substantially different from T1 to T5 (168.33-195.73 pg/ml) broilers. T6 reduced SAA, AGP, CP, corticosterone, and HSP 70 levels by 20.32%, 28.12%, 23.47%, 30.96%, and 29.87%, respectively, as compared to T1. Therefore, it can be concluded that T6 broilers were the less stressful in which performed better than the other treatment groups.

4.7.4 Comparison of the Best Treatments from Experiments A, B, and C

Table 4.28 shows the comparison between the best treatments of *P. purpureum, B. decumbens,* and *M. maximus* leaf meal supplementation on the blood biomarkers of Sasso broilers on day 56. Only AGP and CP showed significant differences ($p<0.05$)

when comparing the best treatment groups of *P. purpureum, B. decumbens* and *M. maximus* leaf meal supplementation. *B. decumbens* showed the lowest numerical value for both AGP (20.86 µl/ml) and CP (5.66 µl/ml), but did not significantly differ ($p>0.05$) from *P. purpureum* (22.77 µl/ml and 5.94 µl/ml resepectively). This shows that *P. purpureum* or *B. decumbens* supplementation at 5.00 g/kg of feed could potentially be beneficial to reduce the stress effects in Sasso broilers.

Table 4.25 : Effect of *P. purpureum* Leaf Meal Supplementation on the Blood Biomarkers of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
SAA (ng/ml)	8.28±0.40^{a}	8.22±0.13^{ab}	8.11±0.43^{ab}	7.53±0.39^{abc}	6.82±0.20^{bc}	6.22±0.22^{c}	0.0007
AGP ((µl/ml)	29.71±0.84^{a}	24.92±0.93^{bc}	27.60±0.81^{ab}	25.62±0.39^{bc}	25.01±0.20^{bc}	22.77±0.22^{c}	0.0003
CP (µl/ml)	9.24±0.15^{a}	8.60±0.17^{ab}	9.40±0.22^{a}	7.98±0.31^{b}	6.28±0.32^{c}	5.94±0.21^{c}	<0.0000
Corticosterone (pg/ml)	235.79±6.86^{a}	227.72±7.25^{a}	228.80±7.65^{a}	161.78±2.85^{b}	160.51±3.40^{b}	132.14±3.45^{c}	<0.0000
HSP 70 (pg/ml)	156.72±4.80^{a}	126.48±5.48^{bc}	150.08±4.67^{a}	132.08±2.17^{b}	122.58±3.16^{bc}	118.10±4.39^{c}	<0.0000

Note: All values were expressed as mean ± SE; $^{a, b, \text{and } c}$ values with superscript within row are significantly different at $p < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70.

Table 4.26 : Effect of *B. decumbens* Leaf Meal Supplementation on the Blood Biomarkers of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
SAA (ng/ml)	8.60±0.33^{a}	6.83±0.13^{b}	8.30±0.24^{a}	7.12±0.29^{b}	6.81±0.22^{b}	6.25±0.23^{b}	<0.0000
AGP ((µl/ml)	32.25±1.43^{a}	17.44±0.59^{d}	29.18±1.09^{b}	27.81±0.54^{bc}	25.27±0.30^{c}	20.86±0.45^{d}	<0.0000
CP (µl/ml)	10.56±0.39^{a}	8.03±0.21^{b}	10.04±0.39^{a}	8.17±0.17^{b}	6.24±0.28^{c}	5.66±0.19^{c}	<0.0000
Corticosterone (pg/ml)	301.74±10.4^{a}	207.14±5.48^{b}	190.60±5.30^{bc}	164.67±3.65^{cd}	140.43±5.28^{de}	130.73±3.60^{e}	<0.0000
HSP 70 (pg/ml)	146.08±1.62^{a}	116.38±0.32^{bc}	130.73±3.74^{ab}	119.35±0.86^{bc}	115.38±5.06^{bc}	114.83±5.26^{c}	<0.0000

Note: All values were expressed as mean ± SE; $^{a, b, c, d, and e}$ values with superscript within row are significantly different at $p < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70.

Table 4.27 : Effect of *M. maximus* Leaf Meal Supplementation on the Blood Biomarkers of Sasso Broilers on Day 56

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
SAA (ng/ml)	8.66±0.47^{a}	7.46±0.24^{ab}	8.05±0.28^{ab}	7.93±0.23^{ab}	7.08±0.12^{b}	6.90±0.24^{b}	0.0030
AGP ((µl/ml)	35.81±0.77^{a}	29.09±1.22^{b}	29.27±1.11^{b}	28.91±0.79^{b}	26.46±0.47^{b}	25.74±0.51^{b}	<0.0000
CP (µl/ml)	10.44±0.21^{a}	9.12±0.25^{bc}	10.32±0.46^{ab}	9.18±0.21^{abc}	8.22±0.29^{c}	7.99±0.23^{c}	<0.0000
Corticosterone (pg/ml)	195.73±5.70^{a}	193.45±6.84^{a}	190.57±6.95^{a}	168.93±5.28^{a}	168.33±10.28^{a}	135.15±5.10^{b}	<0.0000
HSP 70 (pg/ml)	191.13±4.28^{a}	151.03±4.50^{b}	157.00±8.26^{b}	151.00±3.88^{b}	145.03±6.59^{b}	134.03±7.24^{b}	<0.0000

Note: All values were expressed as mean ± SE; $^{a, b, \text{and } c}$ values with superscript within row are significantly different at $p < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70

Table 4.28 : Effects of *P. purpureum*, *B. decumbens* and *M. maximus* Leaf Meal Supplementation on the Blood Biomarkers of Sasso Broilers on Day 56

Parameters	Treatments			p-value
	P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
SAA (ng/ml)	6.22±0.22	6.25±0.23	6.90±0.24	0.1079
AGP ((µl/ml)	22.77±0.22^{b}	20.86±0.45^{b}	25.74±0.51^{a}	0.0013
CP (µl/ml)	5.94±0.21^{b}	5.66±0.19^{b}	7.99±0.23^{a}	<0.0000
Corticosterone (pg/ml)	132.14±3.45	130.73±3.60	135.15±5.10	0.7479
HSP 70 (pg/ml)	118.10±4.39	114.83±5.26	134.03±7.24	0.090

Note: All values were expressed as mean ± SE; $^{a \text{ and } b}$ values with superscript within row are significantly different at $p < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70.

Blood biomarkers play a vital role in the poultry industry as a method of estimating the broilers' welfare and health status (Attia et al., 2011). APP are a class of blood proteins primarily synthesised by hepatocytes, though they can be produced in other tissues and organs, whose concentrations either increase (positive APP) or decrease (negative APP) in order to maintain homeostasis balance as part of the acute phase response (APR). APR is a part of the early-defence mechanism that serves to trigger the acquired immune response (Janeway et al., 2001). Therefore, acute phase proteins (APPs) are thought to have diagnostic and prognostic potential due to the link between blood biomarker levels and host response to inflammation and/or infection (Ceron et al., 2005). Whereas heat shock proteins (HSPs) are synthesised and accumulated as a result of cellular stress reaction, therefore may be used to identify stressful periods for the organism (Balakrishnan et al., 2023). According to research, there is a substantial link between oxidation and HSP70 production (Ming et al., 2010). Plus, it is one of major HSP that responds to heat-stressed broilers because it has a cytoprotective role in the gastrointestinal system following damage or stress (Gu et al., 2012; Liu et al., 2014; Hajati et al., 2015). Both biomarkers are significant components and indications of the immunological response and well-being of broiler chickens, notably in infections and non-infectious illnesses, as well as in nutrition studies.

Serum amyloid A, alpha-1 acid glycoprotein, and ceruloplasmin are positive APPs whereby they are raised in infectious disorders such as bacterial or viral infections (Johnson et al., 1999; Murata et al., 2004). Therefore, lower levels of these APPs indicate better health. Serum amyloid A (SAA) is considered to be a major and most sensitive APP in the APR in most animal species (Cray et al., 2009). SAA functions as chemotaxis to immune cells such as monocytes, polymorphonuclear cells, and T

cells, prevents oxidative tissue damage, inhibits pyrexia, and downregulates the inflammatory process (Uhlar and Whitehead, 1999). Furthermore, SAAs' main function during an APR is to modulate lipoprotein transport and metabolism, allowing cholesterol to remain in damaged tissues where it is needed for membrane repair and regeneration, as well as to transport cholesterol and clear lipid debris from bacteria and damaged areas of tissue. (Humam et al., 2021). Another important class of positive moderate APPs in chickens are alpha-1 acid glycoprotein (AGP), a highly glycosylated protein, produced and secreted by hepatocytes (O'Reilly and Eckersall, 2014). Its natural anti-inflammatory agent and protein binding properties aids in the clearance of lipopolysaccharide (LPS) by binding to LPS and neutralising its toxicity, playing a crucial role in the early stages of inflammation and infection. Several researches have studied changes in AGP levels during trauma, stress, neoplasia, and viral and bacterial infections with or without vaccinations (Murata et al., 2004).

Ceruloplasmin (CP), on the other hand, is a multifunctional protein found in vertebrate plasma that is responsible for storing and distributing copper throughout the body (Jarosz et al., 2018). Its antioxidative role is supported by evidence of CP donating copper to superoxide dismutase (SOD) enzyme production, thus, eliminating reactive oxygen species (ROS) (Song et al., 2009). CP is also involved in signalling multifunctional enzymes as an anti-inflammatory response of cells by donating copper, preventing them from cellular damage through many different metabolic pathways (Floris et al., 2000). Additionally, corticosterone is the primary stress hormone in avian species, is often employed to measure physiological responses to stressors (Najafi et al., 2015). HSP is highly expressed in broilers as it responds to an infection. HSPs act as a molecular chaperone that assists proper protein folding and

presentation of antigens to immune cells, which helps to trigger an immune response (Liew et al., 2003). Furthermore, HSP70 is shown to be significantly expressed in virus infections because it is implicated in virus entry, virus genome replication, virus polymerase activity modulation, virus packaging, and innate immune response (Meng et al., 2021). In short, APPs play a role in stress adaptation and cellular defence, whereas HSPs are function as an inflammatory response and disease development. Understanding their functions and reactions can aid in the development of better diagnostic procedures, treatment approaches, and diet formulations in the broiler industry.

Experiment A demonstrated that the addition of *P. purpureum* leaf meal into broiler diets at optimum levels reduced acute phase protein markers and HSP70 levels. This suggests that the chickens were healthier or more resilient to stressors. To the best of the author's knowledge, there were no other studies done that observed the effects of grass or leaf meals on broilers' acute phase proteins; but a few studies looked into the expression of HSP70 with supplementation of plant-based additives. T4- T6 of *P. purpureum* treated groups displayed lower SAA, AGP, CP and corticosterone levels compared to the negative and positive control. This suggests that broilers supplemented with 5.00g/kg of *P. purpureum* had best immunity as they had the lowest concentration of plasma APP and corticosterone. In a previous study, dietary flavonoids were confirmed to have immunomodulatory effects in chickens (Kamboh et al., 2018). A possible mechanism for the immunomodulatory effect might be through NF-κB pathways to alleviate immune damage (Esmaeil et al., 2017). Additionally, the inclusion of *Artemisia annua* into broiler diets reduced mRNA expression of HSP70, similar to Experiment A. The lower levels of HSP70 were

attributed to high antioxidant activity of flavonoids and phenolics present in *A. annua* (Song et al., 2017). Another study by Tang et al. (2018) revealed that rosemary extracts, which are high in antioxidants elevated HSP70 production before and during heat stress induction, protecting the chicken heart against heat stress. Generally, reduced expression of HSP70 in long term stressors, such as heat stress shows beneficial effects on thermal conditioning of broilers (Balakrishnan et al., 2023). Flavonoids have been shown to inhibit HSP70 expression, as it modulates heat shock transcription factor activity. This occurs due to their antioxidant properties, which contribute to the restoration of mitochondrial function and the inhibition of enzymes implicated in reactive oxygen species (ROS) formation (D'Andrea, 2015). Therefore, the lower levels of HSP70 in *P. purpurerum* treated broilers could be attributed to the high content of flavonoids present in the grass meal.

In Experiment B, the diminished levels of SAA, CP, and corticosterone observed in T6 may be ascribed to the heightened saponin levels present in the diet. Similar to flavonoids, saponins have been linked to the inhibition of the production through the regulation of toll-like receptor 4 (TLR4), nuclear factor kappa beta (NF-κB), and phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB)-NF-κB pathway (Zhang et al., 2016; Xie et al., 2018). Saponin's antioxidant role is also associated with their capacity to downregulate the production of ROS, alleviating oxidative stress by modulating NF-E2-related factor (Nrf-2)-antioxidant response element (ARE) pathway (Chen et al., 2014). This theory is substantiated by a study conducted by Alghirani et al. (2023), wherein Ross308 broiler chickens were supplemented with saponin-rich *Y. schidigera*. The research revealed that administering 100mg/kg of *Y. schidigera* saponins resulted in the lowest levels of SAA, AGP, and corticosterone. In

the same line, a study by Li et al. (2022), focusing on the impact of dietary soy saponins on layer hens, demonstrated enhancements in the antioxidant and immune functions of the laying hens. Furthermore, mRNA levels of NF-κB and interleukin-6 in the ileum were downregulated, indicating the downregulation of intestinal inflammation. This affirms the findings of the present experiment, as evidenced by the lower HSP70 levels observed in T5 and T6 during Experiment B, suggesting reduced inflammation in the broilers of that group.

Various studies have reported that alkaloid derivatives and/or plants high in alkaloids have been utilised as an AGP substitute in poultry and livestock production due to their positive impacts on production performances (Vacek et al., 2010; Kantas et al., 2015). Liu et al. (2022) suggested that supplementation of *Macleaya cordata* extract which are high in isoquinoline alkaloids reduced inflammation marked by decreased levels of ALT, interleukin-1β, and interleukin-6. This shows hepatoprotective effects of alkaloids through inhibition of TLR4, NF-κB, myeloid differentiation factor 88 (MyD88), Nrf-2, and other related inflammatory signaling pathways (Hu et al., 2020). Heat-stressed broilers treated with isoquinoline alkaloids also showed improved growth performance, which was linked to reduced oxidative damage, protein catabolism, intestinal barrier function, and inflammation (Kikusato et al., 2021). These findings align with the results from Experiment C, wherein Sasso broilers supplemented with *M. maximus* exhibited blood biomarker levels that were either similar to or significantly lower than those of the group treated with antibiotics. Earlier models have suggested a significant role for elevated HSP70 levels in the chaperone machinery, influencing the selection of proteins affected by oxidative or other toxic damage. Consequently, reduced levels of HSP70 imply a lesser extent of

damage (Surai and Kochish, 2017). As observed, T6 broilers from Experiment C revealed the lowest values for the measured blood biomarkers, indicating that the observed positive effects could be attributed to elevated levels of alkaloids.

Upon comparing the optimal treatments from all three experiments, it became evident that only AGP and CP exhibited significant differences across all blood biomarkers parameters measured. *P. purpureum* and *B. decumbens* had lower AGP and CP levels measured in comparison to *M. maximus*. These results suggest that higher concentration of flavonoids and saponins present in *P. purpureum* and *B. decumbens* respectively may have contributed to greater antioxidative effects compared to *M. maximus*, consequently leading to a more pronounced reduction in inflammatory markers. In conclusion, acute phase proteins (APPs) and heat shock proteins (HSPs) are useful tools in studying infectious and non-infectious disorders, as well as nutrition research. APPs play a role in stress adaptation and cellular defence, whereas HSPs are function as an inflammatory response and disease development. Understanding their functions and reactions with the addition of grass meals or phytobiotics can aid in the development of better diet formulations in the broiler industry.

When evaluating the most effective treatments across all three experiments, it becomes evident that *B. decumbens* exhibits slightly superior positive effects on liver enzyme functions, whereas *P. purpureum* demonstrates a slightly better performance in lipid profiling. These results can be attributed to the varying predominant phytocompounds, with *B. decumbens* containing more saponins than *P. purpureum* and *P. purpureum* having a higher concentration of flavonoids than *B. decumbens*, leading to differences in antioxidative strength. Comparatively, *P. purpureum* demonstrates an overall

superior lipid profile compared to *B. decumbens* and *M. maximus*. In summary, measuring liver enzyme production serves as a valuable indicator of broiler stress levels, while lipid profiling offers insights into broiler health and the quality of the end product. Given the scarcity of information on blood biomarkers and broiler biochemistry concerning phytobiotic use, further studies are essential to enhance our understanding and optimise the utilization of phytobiotics in broiler production.

4.8 Blood Biochemistry

4.8.1 Experiment A (*P. purpereum*)

Table 4.29 shows the effect of *P. purpureum* leaf meal supplementation on the blood biochemistry of Sasso broilers on day 56. There were significant differences ($p<0.05$) found in all liver functions tested which are ALP, AST, GGT, and ALT. ALP, AST, GGT, and ALT were found to be the lowest in T6 at 131.32 U/L, 96.46 U/L, 10.02 U/L, and 2.17 U/L respectively. GGT values for T5 (10.37 U/L) was not significantly different ($p>0.05$) from T6. Significant differences ($p<0.05$) were also found in cholesterol, TG, LDL, and VLDL levels, but no significant differences were found in HDL when assessing the lipid profile. T6 showed the lowest values for cholesterol, TG, LDL, and VLDL indicating better blood biochemistry results.

4.8.2 Experiment B (*B. decumbens*)

Table 4.30 shows the effect of *B. decumbens* leaf meal supplementation on the blood biochemistry of Sasso broilers on day 56. There were significant differences ($p<0.05$) found in all liver functions tested which are ALP, AST, GGT, and ALT. ALP, AST, GGT, and ALT were found to be the lowest in T6 at 129.54 U/L, 159.10 U/L, 6.38

U/L, and 1.55 U/L respectively. ALP and ALT values for T5 was not significantly different ($p>0.05$) from T6 and AST values did not significantly differ ($p>0.05$) across all treatments when compared to the positive control (T2). Significant differences ($p<0.05$) were also found in TG, LDL, HDL and VLDL levels, but no significant differences were found in cholesterol when assessing the lipid profile. T6 showed the lowest values for TG, LDL, HDL and VLDL. Therefore, T6 treatment group outperformed the other treatment groups when comparing blood biochemistry results.

4.8.3 Experiment C (*M. maximus*)

The impact of *M. maximus* leaf meal supplementation on the blood biochemistry of Sasso broilers on day 56 is shown in Table 12. There were significant changes ($p<0.05$) in all liver functions examined, including ALP, AST, GGT, and ALT. T6 had the lowest levels of ALP, AST, GGT, and ALT, with values of 129.89 U/L, 162.21 U/L, 8.86 U/L, and 1.24 U/L, respectively. However, AST levels between T6 (162.21 U/L) and T2 (172.65 U/L) did not differ substantially ($p>0.05$). When the lipid profile was examined, significant variations ($p<0.05$) were detected across all treatments. Despite T2 having the lowest cholesterol levels among all treatments, the cholesterol levels of T6 (4.01 mmol/L) and T2 (3.94 mmol/L) did not significantly different ($p>0.05$) from each other. Similarly, triglyceride levels in T6 and T2 were 0.46 mmol/L and 0.50 mmol/L, respectively with no significant differences ($p>0.05$). HDL levels were the highest in T6 (3.64 mmol/L), though not significantly different ($p>0.05$) from T2 (3.13 mmol/L). LDL and VLDL were lowest in T2 at 0.37 mmol/L and 0.09 mmol/L respectively, but VLDL did not significantly differ ($p>0.05$) from T6. Overall, this indicates that the supplementation of *M. maximus* at 5.00g/kg of feed had similar effects to antibiotic treated group.

4.8.4 Comparison of the Best Treatments from Experiments A, B, and C

Table 4.32 compares the effects of supplementing *P. purpureum*, *B. decumbens*, and *M. maximus* leaf meal on the blood biochemistry of Sasso broilers on day 56. Significant variations ($p<0.05$) were identified for AST, GGT, and ALT in the liver function test among the three grass meals fed at the maximum inclusion level (T6 at 5.00g/kg of feed), whereas cholesterol, TG, LDL, and VLDL exhibited significant differences ($p<0.05$) in lipid profile. When evaluating the best treatment from all three grass meals, mixed findings were discovered, with the lowest AST levels reported in T6 of *P. purpureum* at 96.46 U/L in comparison to *B. decumbens* (159.10 U/L) or *M. maximus* (162.21 U/L). *M. maximus*, on the other hand, had lower GGT and ALT levels (8.86 U/L and 1.21 U/L) than *P. purpureum* (10.02 U/L and 2.17 U/L) and *B. decumbens* (6.38 U/L and 1.55 U/L). The lipid profile data revealed that the T6 of *P. purpureum* treatment group had the lowest cholesterol, LDL, and VLDL levels, with values of 3.06 mmol/L, 0.27 mmol/L, and 0.09 mmol/L, respectively. TG levels were the lowest in *B. decumbens* group at 0.39 mmol/L although this value did not significantly differ from *P. purpureum* group at 0.44 mmol/L. Overall, all three leaf meals at 5.00g/kg had different influences on the liver and lipid functions of Sasso broilers on day 56.

Table 4.29 : Effect of *P. purpureum* Leaf Meal Supplementation on the Blood Biochemistry of Sasso Broilers on Day 56

Parameters	Normal range	Treatments						
		T1	T2	T3	T4	T5	T6	p-value
Liver functions								
ALP (U/L)	55-2345	174.19±3.20^{b}	187.87±5.27^{a}	179.94±3.51^{ab}	140.05±1.09^{c}	136.47±1.35^{c}	131.32±0.64^{c}	<0.0000
AST (U/L)	98-294	282.66±13.19^{a}	172.65±5.15^{b}	166.19±0.52^{b}	157.74±6.08^{b}	145.17±2.44^{b}	99.46±0.97^{c}	<0.0000
GGT (U/L)	8-48	14.61±0.32^{ab}	12.88±0.35^{c}	14.61±0.43^{ab}	13.92±0.28^{bc}	10.37±0.24^{d}	10.02±0.52^{d}	<0.0000
ALT (U/L)	<11	3.47±0.07^{a}	2.50±0.09^{bc}	2.72±0.05^{bc}	2.76±0.06^{bc}	2.47±0.05^{c}	2.17±0.06^{d}	<0.0000
Lipid profile								
Cholesterol (mmol/L)	1.09-5.65	4.01±0.16^{ab}	3.94±0.09^{b}	4.50±0.11^{a}	3.90±0.15^{b}	3.87±0.07^{b}	3.06±0.12^{c}	<0.0000
TG (mmol/L)	N/A	0.51±0.02^{ab}	0.46±0.01^{bc}	0.57±0.02^{a}	0.55±0.02^{a}	0.53±0.01^{a}	0.44±0.01^{c}	<0.0000
LDL (mmol/L)	N/A	0.55±0.02^{b}	0.37±0.01^{c}	0.69±0.01^{a}	0.51±0.02^{b}	0.39±0.02^{c}	0.27±0.01^{d}	<0.0000
HDL (mmol/L)	N/A	3.00±0.16	3.13±0.05	3.11±0.08	3.12±0.14	3.29±0.05	3.46±0.15	0.1152
VLDL (mmol/L)	N/A	0.11±0.00^{ab}	0.09±0.00^{bc}	0.11±0.00^{a}	0.11±0.00^{a}	0.11±0.00^{a}	0.09±0.00^{c}	<0.0000

Note: All values were expressed as mean ± SE; $^{a, b, c, \text{and } d}$ values with superscript within row are significantly different at $p < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase; N/A: Not Available. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.30 : Effect of *B. decumbens* Leaf Meal Supplementation on the Blood Biochemistry of Sasso Broilers on Day 56

Parameters	Normal range	Treatments						
		T1	T2	T3	T4	T5	T6	p-value
Liver functions								
ALP (U/L)	55-2345	174.19±3.20^{ab}	187.87±5.27^{a}	160.06±2.00^{bc}	155.82±3.31^{c}	143.53±5.05^{cd}	129.54±4.49^{d}	<0.0000
AST (U/L)	98-294	282.66±13.19^{a}	172.65±5.15^{b}	182.52±0.31^{b}	168.74±6.18^{b}	174.96±5.88^{b}	159.10±4.13^{b}	<0.0000
GGT (U/L)	8-48	14.61±0.32^{b}	12.89±0.35^{c}	17.01±0.31^{a}	16.01±0.45^{ab}	11.93±0.51^{c}	6.38±0.30^{d}	<0.0000
ALT (U/L)	<11	3.47±0.07^{a}	2.50±0.09^{b}	2.32±0.08^{b}	1.90±0.04^{c}	1.63±0.07^{cd}	1.55±0.05^{d}	<0.0000
Lipid profile								
Cholesterol (mmol/L)	1.09-5.65	4.01±0.16	3.94±0.09	4.16±0.14	3.72±0.09	3.71±0.18	3.62±0.09	0.0641
TG (mmol/L)	N/A	0.51±0.02^{a}	0.47±0.01^{abc}	0.47±0.02^{ab}	0.44±0.01^{abc}	0.43±0.02^{bc}	0.39±0.01^{c}	0.0009
LDL (mmol/L)	N/A	0.55±0.02^{bc}	0.37±0.01^{d}	0.76±0.03^{a}	0.56±0.03^{b}	0.46±0.02^{cd}	0.43±0.02^{d}	<0.0000
HDL (mmol/L)	N/A	3.00±0.16^{b}	3.13±0.05^{ab}	3.07±0.09^{ab}	3.16±0.10^{ab}	3.34±0.08^{ab}	3.51±0.13^{a}	0.0368
VLDL (mmol/L)	N/A	0.10±0.00^{a}	0.09±0.00^{abc}	0.09±0.00^{ab}	0.09±0.00^{abc}	0.09±0.00^{bc}	0.08±0.00^{c}	0.0009

Note: All values were expressed as mean ± SE; $^{a, b, c, and d}$ values with superscript within row are significantly different at $p < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase; N/A: Not Available. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.31 : Effect of *M. maximus* Leaf Meal Supplementation on the Blood Biochemistry of Sasso Broilers on Day 56

Parameters	Normal range	Treatments T1	T2	T3	T4	T5	T6	p-value
Liver functions								
ALP (U/L)	55-2345	174.19±3.20^{b}	187.87±5.27^{ab}	204.29±5.63^{a}	174.05±2.55^{b}	140.85±4.53^{c}	129.89±1.14^{c}	<0.0000
AST (U/L)	98-294	282.66±13.19^{a}	172.65±5.15^{cd}	223.93±3.28^{b}	215.54±5.92^{b}	201.38±4.41^{bc}	162.21±3.21^{d}	<0.0000
GGT (U/L)	8-48	14.61±0.32^{bc}	12.88±0.35c^{d}	22.7±0.59^{a}	14.90±0.15^{b}	12.33±0.53^{d}	8.86±0.27^{e}	<0.0000
ALT (U/L)	<11	3.47±0.07^{b}	2.50±0.09^{c}	3.95±0.04^{a}	3.67±0.09^{b}	1.60±0.06^{d}	1.24±0.05^{e}	<0.0000
Lipid profile								
Cholesterol (mmol/L)	1.09-5.65	4.56±0.15^{a}	3.94±0.09^{b}	4.13±0.09^{ab}	4.04±0.07^{ab}	4.03±0.11^{ab}	4.01±0.16^{b}	0.0169
TG (mmol/L)	N/A	0.51±0.02^{bc}	0.46±0.01^{c}	0.59±0.02^{a}	0.53±0.02^{ab}	0.51±0.01^{bc}	0.50±0.02^{bc}	0.0006
LDL (mmol/L)	N/A	0.55±0.02^{b}	0.37±0.01^{d}	0.68±0.02^{a}	0.57±0.01^{b}	0.51±0.01^{bc}	0.48±0.02^{c}	<0.0000
HDL (mmol/L)	N/A	3.00±0.16^{b}	3.13±0.05^{ab}	3.50±0.10^{ab}	3.59±0.06^{ab}	3.61±0.22^{a}	3.64±0.12^{a}	0.0083
VLDL (mmol/L)	N/A	0.10±0.00^{bc}	0.09±0.00^{c}	0.12±0.00^{a}	0.11±0.00^{ab}	0.10±0.00^{bc}	0.10±0.00^{bc}	0.0006

Note: All values were expressed as mean ± SE; $^{a, b, c, d \text{ and } e}$ values with superscript within row are significantly different at $p < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase; N/A: Not Available. T1: negative control; T2: positive control; T3: 1.25 g/kg; T4: 2.5 g/kg; T5: 3.75 g/kg; T6: 5.00 g/kg.

Table 4.32 : Effects of *P. purpureum*, *B. decumbens*, and *M. maximus* Leaf Meal Supplementation on the Blood Biochemistry of Sasso Broilers on Day 56

Parameters	Normal range	Treatments			p-value
		P. purpureum (5.00g/kg)	*B. decumbens* (5.00g/kg)	*M. maximus* (5.00g/kg)	
Liver functions					
ALP (U/L)	55-2345	131.32±0.64	129.54±4.49	129.89±1.14	0.8869
AST (U/L)	98-294	99.46±0.97^{b}	159.10±4.13^{a}	162.21±3.21^{a}	<0.0000
GGT (U/L)	8-48	10.02±0.52^{a}	6.38±0.30^{b}	8.86±0.27^{a}	0.0003
ALT (U/L)	<11	2.17±0.06^{a}	1.55±0.05^{b}	1.24±0.05^{c}	<0.0000
Lipid profile					
Cholesterol (mmol/L)	1.09-5.65	3.06±0.12^{b}	3.62±0.09^{a}	4.01±0.16^{a}	0.0005
TG (mmol/L)	N/A	0.44±0.01^{ab}	0.39±0.01^{b}	0.50±0.02^{a}	0.0020
LDL (mmol/L)	N/A	0.27±0.01^{b}	0.43±0.02^{a}	0.48±0.02^{a}	<0.0000
HDL (mmol/L)	N/A	3.46±0.15	3.51±0.13	3.64±0.12	0.6189
VLDL (mmol/L)	N/A	0.09±0.00^{ab}	0.08±0.00^{b}	0.10±0.00^{a}	0.0020

Note: All values were expressed as mean ± SE; $^{a, b, \text{and } c}$ values with superscript within row are significantly different at $p < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase; N/A: Not Available.

Serum biochemical and haematological parameters serve as dependable indicators of the health condition of animals. For instance, assessing levels of AST, ALT, and ALP provides insights into liver function and the presence of liver damage resulting from exposure to toxins (Muazu and Aliyu-Paiko, 2020). On top of that, lipids play a crucial role in the regular growth and development of broilers in addition to sensory attributes. Cholesterol is utilised in the construction of cell membranes and the synthesis of hormones. Lipoproteins, which consist of cholesterol and proteins, are categorized as either high density or low density based on their protein-to-fat ratio. Triglycerides, present in blood plasma, form part of the plasma lipids in conjunction with cholesterol. However, an excess of circulating cholesterol in the bloodstream poses a risk of clogging blood vessels, leading to an increased susceptibility to heart disease and stroke. In recent years, growing health concerns in relation to animal protein sources in human diet has driven the livestock industry to develop products of lower cholesterol and saturated fatty acid contents (Ponte et al., 2004). Phytobiotics have been linked to lipid metabolism modification due to their hypocholesterolaemic effects (Fujioka et al., 2003; Gilani et al., 2018). Additionally, the addition of plants and/or their extracts into diets have been observed to have hepatoprotective potential due to the presence of bioactive compounds (Venmathi Maran et al., 2022).

In Experiment A, increasing levels of *P. purpureum* leaf meal into Sasso broiler diets demonstrated decreasing levels of liver enzymes, suggesting better liver function. Therefore, based on the current experiment, it can be inferred that supplementing the diet with *P. purpureum* leaf meal may be beneficial for maintaining liver health. A previous experiment incorporating *P. odorata* leaf meal into broiler diets also showed reduction of AST, ALT and ALP levels similar to results from the current experiment

(Basit et al., 2020c). In essence, liver enzymes are typically more present within cells than in plasma (serum), only entering the blood stream due to cell damage (Tavangar et al., 2021). Therefore, the decline in liver enzyme levels in Experiment A may be ascribed to the presence of antioxidant compounds in *P. purpureum* leaf meal, such as flavonoids. Antioxidant compounds mitigate oxidative damage to the liver and consequently reduces the release of these enzymes into the bloodstream (Vitaglione et al., 2004). The addition of *P. odorata* leaf meal also reduced levels of serum cholesterol and triglycerides (Basit et al., 2020c). Improved findings with the addition of phytobiotics were attributed to the presence of flavonoids and secondary bioactive compounds in the leaves (Kamboh and Zhu, 2013). Lower levels of *P. purpureum* inclusion did not lead to improvements in lipid profile levels compared to the control group, likely due to inadequate levels of bioactive compounds capable of influencing fat metabolism. This is because flavonoids are recognized for their ability to modify fat metabolism, influencing the composition of fatty acids and the omega-3 to omega-6 ratio, ultimately leading to a reduction in both cholesterol and triglyceride levels (Kamboh and Zhu, 2013). Moreover, flavonoids, known for their antioxidant properties, can also impact lipid metabolism by inhibiting the formation of LDL (Habibian et al., 2019). Therefore, the decreased levels of cholesterol, triglycerides, and LDL observed in T6 of Experiment A could be attributed to the higher flavonoid content present in the diet.

Likewise, incorporating *B. decumbens* as a phytobiotic feed additive has the potential to enhance both liver function and the lipid profile in broilers. In a prior study conducted by Alghirani et al. (2023), it was found that supplementing broilers with *Y. schidigera* saponins resulted in decreased stress biomarkers. This suggests a

diminished inflammatory response and stress reaction, potentially contributing to improved growth performance. Therefore, the positive effect from the present study is likely attributable to the presence of saponins as it is the highest phytocompound present in *B. decumbens*. Saponins are known to possess hepatoprotective effects, leading to a reduction in the levels of liver enzymes (Lala et al., 2018). Considering that ALT is a highly sensitive marker for hepatocyte damage, this hepatoprotective theory of saponins is further substantiated as all groups treated with *B. decumbens* exhibited comparable ALT results to the antibiotic group (Hassan et al., 2016). While no significant differences were noted in cholesterol levels, it is clear that T6 exhibited lower cholesterol values, and comparable HDL and LDL readings relative to the antibiotic group. This observation implies a potential reduction in triglyceride synthesis and an enhanced redistribution of cholesterol among lipoprotein molecules (Tokofai et al., 2020). The favourable impact observed on lipid profiles in this experiment is also associated with the cholesterol-lowering effects of saponins. The first mechanism for saponins are through inhibition of pancreatic cholesterol esterase and bile acid binding, leading to a decrease in cholesterol solubility in micelles. Consequently, this may potentially delay cholesterol absorption in the gastrointestinal tract (Ngamukote et al., 2011). The second mode of action suggests that saponins form large aggregates with bile salts in the colon, hence limiting ileal bile salt reabsorption. This inhibition prompts the liver to produce more bile salts from cholesterol, leading to a reduction in blood cholesterol levels (Mahmoodzadeh et al., 2017).

Experiment C demonstrated that supplementing *M. maximus* leaf meal at the highest level (5.00g/kg of feed) improved liver function by reducing liver enzyme levels in the bloodstream. The outcomes of this investigation could be linked to the elevated

concentration of alkaloids found in *M. maximus* leaves, aligning with prior studies that have indicated positive effects with the use of alkaloid-rich supplementation in broilers. For example, Liu et al. (2022) observed a decrease in serum ALT with dietary *Macleaya cordata* extract, indicating reduced hepatocyte damage; similar to the results shown in Experiment C. However, in contrast to the present study, *M. cordata* extract notably increased serum levels of TG, HDL, and LDL. The authors attributed this to heightened metabolism during the growth and development of broiler chickens, contributing to enhanced growth performance (Khadem et al., 2014). Nevertheless, results from Experiment C were in line with Tokofai et al. (2020) which showed lowered total cholesterol and LDL levels with the supplementation of *V. amygdalina* leaf meal. A plausible explanation for the mechanism of phytobiotics with hypocholesterolaemic effects lies in the volatile oils present in plants. These oils may hinder the function of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), a liver enzyme that regulates cholesterol production, consequently leading to a reduction in blood cholesterol levels (Fujioka et al., 2003). Thus, the mixed results of lipid profile effects from Experiment C to prior experiments could be due to varying levels and interaction of bioavailable compounds of volatile oil in each plant source.

When evaluating the most effective treatments across all three experiments, it becomes evident that *B. decumbens* exhibits slightly superior positive effects on liver enzyme functions, whereas *P. purpureum* demonstrates a slightly better performance in lipid profiling. These results can be attributed to the varying predominant phytocompounds, with *B. decumbens* containing more saponins than *P. purpureum* and *P. purpureum* having a higher concentration of flavonoids than *B. decumbens*, leading to differences

in antioxidative strength. Correspondingly, *P. purpureum* demonstrates an overall superior lipid profile compared to *B. decumbens* and *M. maximus* likely due to the action of flavonoids. In summary, measuring liver enzyme production serves as a valuable indicator of broiler stress levels, while lipid profiling offers insights into broiler health and the quality of the end product. Given the scarcity of information on blood biomarkers and broiler biochemistry concerning phytobiotic use, further studies are essential to enhance our understanding and optimize the utilisation of phytobiotics in broiler production.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, the study demonstrated that under tropical conditions, coloured broilers supplemented with 5.00 g/kg of *P. purpureum*, *B. decumbens*, or *M. maximus* improved growth performance, nutrient digestibility, gut health, cecal microflora, meat quality, and blood parameters. Among these, *P. purpureum* emerged as the most effective supplement, attributed to its high flavonoid and tanning content, leading to better feed efficiency, enhanced nutrient digestibility, and improved meat quality with reduced drip loss and desirable yellow coloration. Additionally, *P. purpureum* showed superior lipid profiles and lower inflammatory markers, highlighting its antioxidative and gut-health-promoting properties.

Overall, this study investigating the effects of local Malaysian grass and its phytocompounds on production performances of coloured-broilers have the potential to yield several benefits across academia, industry, community, and the country, in line with the principles of a quadruple helix model. To further elaborate in the context of the quadruple helix model, this research can provide new insights on the viability of grasses as an innovative feed additive or as an antibiotic replacer. With this new understanding, new market opportunities could arise within the agriculture sector benefiting several stakeholders, including forage farmers, nutraceutical producers, and poultry farmers alike. Moreover, the community and country may benefit from this innovation as natural remedies reduces the reliance on synthetic materials, thus promoting environmental conservation and sustainability. Furthermore, public health concerns relating to antimicrobial resistance associated with antibiotic growth

promoters are addressed through the discovery and use of alternative therapies in poultry feed. Lastly, the contribution of research and innovation in agricultural studies would accelerate economic growth and development by creating new opportunities.

5.1 Recommendations for Future Research

Amid increased worries about antibiotic resistance, there is a growing drive to restrict antibiotics in animal feed. Grass meals show potential as a novel addition for the poultry market due to their varied variety of phytocompounds, which vary in composition across different grass kinds. Therefore, as a result of the insights gained in this study, the following recommendations are offered to further the development of grass meals as a feed additive:

1. Undertake a similar study in a controlled, closed-house environment.
2. Conduct comprehensive profiling of additional phytocompounds present in each grass variant and evaluate their antimicrobial susceptibility.
3. Conduct a study to evaluate the effects of higher inclusion rates of grass meals on broiler performance and investigate any potential correlation.

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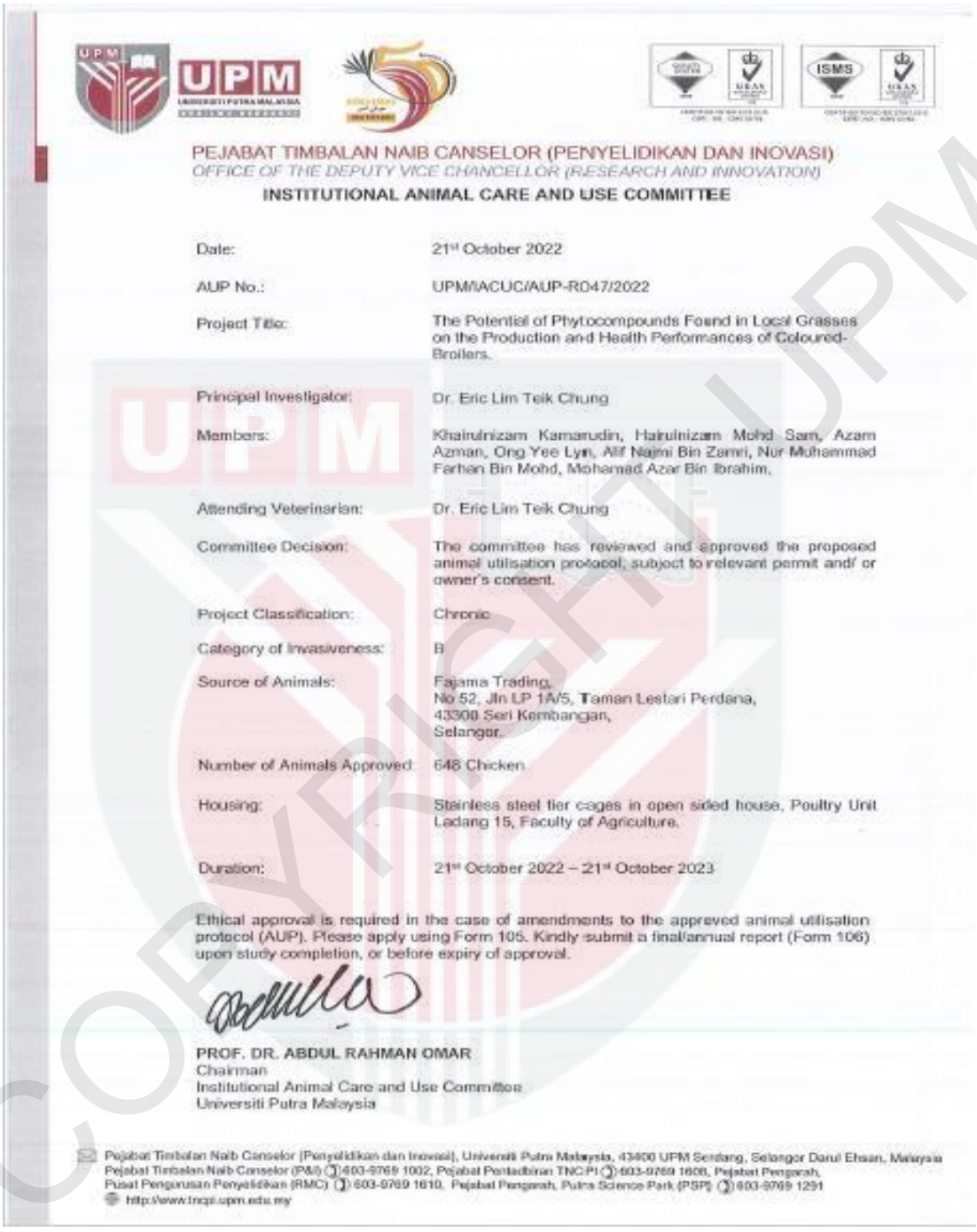
APPENDICES



PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	21st October 2022
AUP No.:	UPM/IACUC/AUP-R047/2022
Project Title:	The Potential of Phytocompounds Found in Local Grasses on the Production and Health Performances of Coloured-Broilers.
Principal Investigator:	Dr. Eric Lim Teik Chung
Members:	Khairulnizam Kamarudin, Hairulnizam Mohd Sam, Azam Azman, Ong Yee Lyn, Alif Najmi Bin Zamri, Nur Muhammad Farhan Bin Mohd, Mohamad Azar Bin Ibrahim.
Attending Veterinarian:	Dr. Eric Lim Teik Chung
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
Project Classification:	Chronic
Category of Invasiveness:	B
Source of Animals:	Fajama Trading, No 52, Jln UP 1A/5, Taman Lestari Perdana, 43300 Seri Kembangan, Selangor.
Number of Animals Approved:	648 Chicken
Housing:	Stainless steel tier cages in open sided house, Poultry Unit Ladang 15, Faculty of Agriculture.
Duration:	21st October 2022 – 21st October 2023

Ethical approval is required in the case of amendments to the approved animal utilisation protocol (AUP). Please apply using Form 105. Kindly submit a final/annual report (Form 106) upon study completion, or before expiry of approval.

PROF. DR. ABDUL RAHMAN OMAR
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) (603-9769 1002, Pejabat Pentadbiran TNC:PI (603-9769 1608, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) (603-9769 1610, Pejabat Pengarah, Putra Science Park (PSP) (603-9769 1291
http://www.inqui.upm.edu.my

Figure 1 : IACUC Approval Sheet

Figure 2 : Preparation of Grass Meal (Drying)

Figure 5 : Preparation of Grass Meal (Grinding)

Figure 3 : Housing of Coloured-Broilers

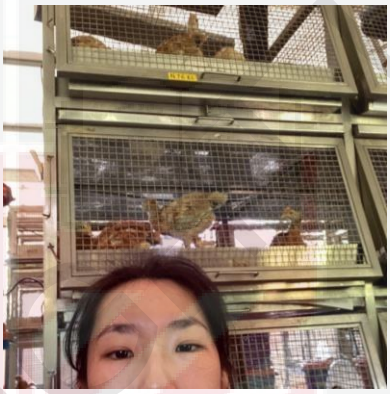


Figure 6 : Rearing of Coloured-Broilers

Figure 4 : Post-Mortem for Dead Broiler

Figure 7 : EE Analysis

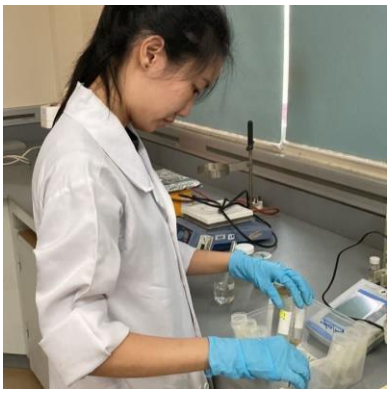


Figure 8 : Qualitative Analysis of Phytocompounds

Figure 9 : Slides of Intestine Samples for Analysis

Figure 10 : Acute Phase Protein Analysis Reading

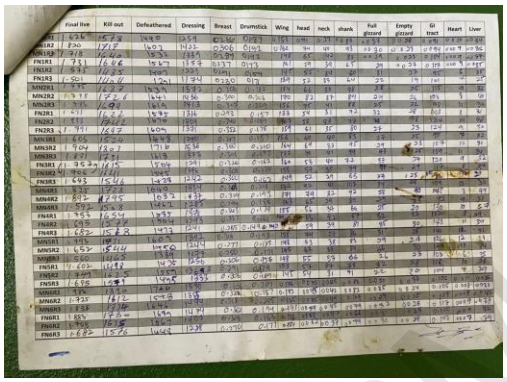


Figure 11 : Data Collection for Carcass Characteristics

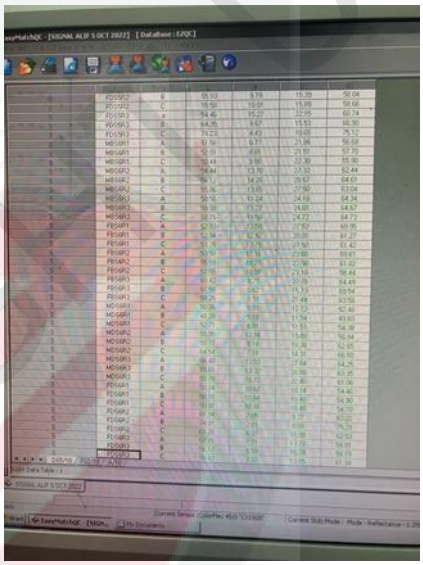


Figure 12 : Data Collection for Meat Quality Analysis

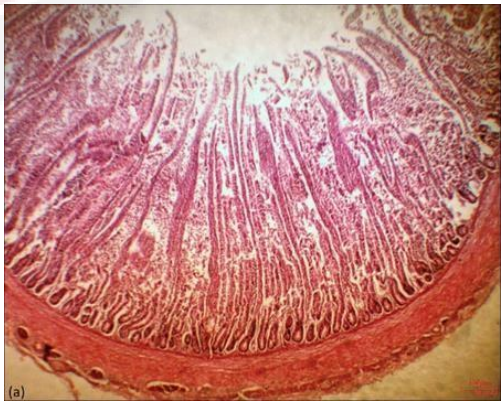


Figure 13 : Micrograph of Duodenum

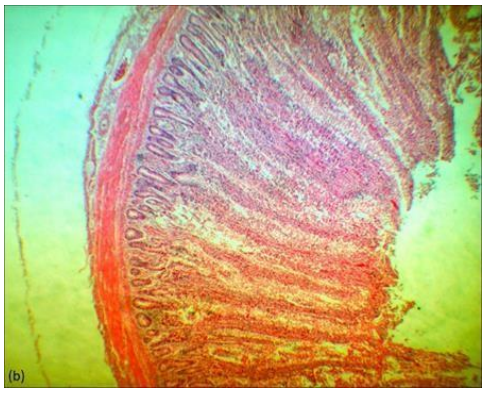


Figure 14 : Micrograph of Jejunum

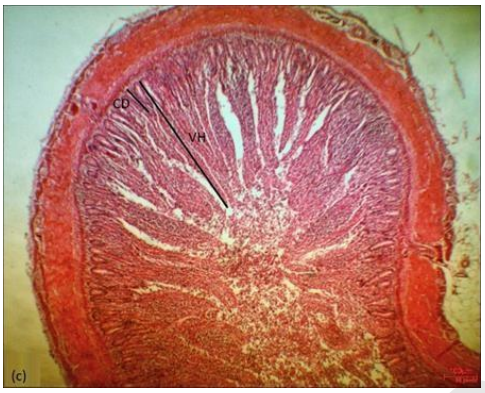


Figure 15 : Micrograph of Ileum with Villi Height (VH) and Crypt Depth (CD) Measurement Indicator

BIODATA OF STUDENT

The student was born in October 1996 in Ipoh, Perak, Malaysia. She earned her Bachelor of Agriculture (Animal Science) degree from Universiti Putra Malaysia in November 2020. Following graduation, she worked for a year with a integrated poultry company in Malaysia. In March 2022, she began her PhD studies at the Institute of Tropical Agriculture and Food Security.

LIST OF PUBLICATIONS

Journal Articles

Ong, Y. L., Chung, T., Lim, E., Nayan, N., Tan, N. P., Jesse, F. F. A., & Sazili, A. Q. (2024a). Ascertaining the Effects of Grass and Leaf Meals on the Gut Health and Blood Indices of Broiler Chickens-A Systematic Review. *Poultry Science Journal*, *12*(1), 1-17.

Ong, Y. L., Chung, T., Lim, E., Nayan, N., Tan, N. P., Jesse, F. F. A., & Sazili, A. Q. (2024b). Are Locally Sourced Grass or Leaf Meals a Double-Edged Sword in Poultry Broiler Production? A Comprehensive Review. *Malaysian Applied Biology*, 53(6), 1-19.

Ong, Y. L., Chung, T., Lim, E., Nayan, N., Ab Aziz, M. F., Jesse, F. F. A., & Sazili, A. Q. (2024d). Enhancing Growth Performance and Health of Colored-Broiler Chickens with Signal Grass Meal Supplementation. *Livestock Science*, 289, p.105585

Ong, Y. L., Chung, T., Lim, E., Nayan, N., Ab Aziz, M. F., Jesse, F. F. A., & Sazili, A. Q. (2025). Phytobiotic Effects of Guinea Grass Supplementation on Production and Health Performances of Coloured-broilers. *Italian Journal of Animal Science*, 24(1), 149-163

Under Review

Ong, Y. L., Chung, T., Lim, E., Nayan, N., Ab Aziz, M. F., Jesse, F. F. A., & Sazili, A. Q. (2024c). Evaluating the role of Napier grass meal and its phytocompounds as a dietary supplement for coloured-broiler chickens. *Annals of Agricultural Sciences*, Submission ID: AOAS-S-24-00488.