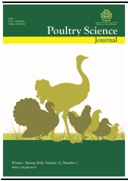

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Ascertaining the Effects of Grass and Leaf Meals on the Gut Health and Blood Indices of Broiler Chickens – A Systematic Review

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Abstract

As the broiler chicken industry continues to develop, antibiotic growth promoters in poultry feed are being phased out because of increasing country restrictions and consumer concerns about food safety. As a result of these bans and efforts to prevent antimicrobial resistance, research into antibiotic alternatives is accelerated to preserve or improve broilers' production performance. Due to the presence of beneficial compounds like tannins, saponins, flavonoids, and various others found in grass and leaf meals, they possess the potential to substitute antibiotics. This is because secondary metabolites in plant-derived phytobiotics have useful pharmacological qualities that may benefit broilers' overall production and health. Previous studies, including grass or leaf meals at a dosage of 0.025-20%, have shown several positive effects on gut histomorphology, gut microflora, and blood biochemistry. For instance, lipid profile and liver functions of broiler chickens improved through hypo-cholesterolaemic and hepatoprotective functions of phytocompounds. However, there are also contradictory data and a lack of information on the effect of these plant-based meals on broilers' blood biomarkers, such as acute phase proteins and heat shock proteins. Therefore, this review provides insight into the potential of grass or leaf meals and their effects on gut health, blood biochemistry, and biomarkers of broiler chickens.

Introduction

Antibiotics usage in the poultry industry

Presently, the broiler industry is the most intensive area of animal production. Throgh the years, the broiler industry has progressed from a backyard subsistence level to a highly commercialized and efficient production system with 'integrators' who manage all activities. These activities which include feed manufacturing, breeding, hatchery, broiler farming and meat processing are all integrated into a system known as the vertical integration system (Ferlito, 2020). During this transition to highly commercialized systems, antibiotic growth promoters (AGP) have been incorporated in birds' feed to enhance broiler traits such as daily weight gains and feed efficiency since the favorable benefits satisfy the demands of meat production (Hao *et al.*, 2014; Alghirani *et al.*, 2021a). This antibiotic use has been linked to higher rates of antimicrobial resistance (AMR) among bacteria isolated from animals, demonstrating that antibiotic resistance genes may be transmitted to human microbiota via the food chain (Dibner and Richards, 2005). Consequences of AMR may range from prolonged illness and side effects due to the use of alternative treatments to death following complete treatment failure (Hughes and Heritage, 2004). For that reason, improved control measures, public health interventions, and a monitoring system should be implemented to minimize the spread of antibiotic resistance in local chicken farms. More

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importantly, alternatives to antibiotics and their effects on broilers should be researched further to be effectively utilized in the poultry sector.

Grasses and leaves as phytobiotic in poultry

Because of prohibitions on the use of AGP in meat production in various countries, phytobiotics as an alternative has gained traction in the past years (EP, 2003; FAO, 2004). With increased knowledge and consumer awareness, intensified rejection of synthetic antibiotics makes way for consumers to accept the use of phytobiotic in animal feed since humans have taken herbal remedies for ages (Surai, 2014). 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 extracts (Galli *et al.*, 2020; Erwan *et al.*, 2021 a,b) and others (Reis *et al.*, 2019; Reda *et al.*, 2020; Bajagai *et al.*, 2022). Another form of phytobiotics such as grass or leaf meals, could also replace AGP as they contain a variety of bioactive molecules or phytocompounds such as flavonoids, saponins, tannins, alkaloids, and many more. These compounds are responsible for their antioxidant and antibacterial activities (Jimenez-Garcia *et al.*, 2018; Kuralkar and Kuralkar, 2021; Chung *et al.*, 2018; Ojo *et al.*, 2022). Certain phytocompounds such as tannins have a reputation for being an anti-nutritional factor when present in high doses, but supplementing broiler chicks with tannin diets at low doses has been shown to improve and promote immunological competence, intestinal microbial ecology, and gut health (Huang *et al.*, 2018). Similarly, multiple studies have demonstrated that appropriate levels of saponins in poultry diets may produce note-worthy results despite reports of affecting palatability due to bitter taste (Alghirani *et al.*, 2021b; Alghirani *et al.*, 2023). As a result, they might be utilized to enhance broiler diets to produce a profitable and sustainable production cycle, although additional research is needed. Therefore, this review aims to discuss the application of grass or leaf meals as a feed additive in poultry diets and the effects on gut histomorphology, gut microflora, serum lipid profile, blood biomarkers and biochemistry of broilers. This systematic research was finalized 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 keywords related to production. Keywords included were ‘broiler’, ‘grass meal’, ‘leaf meal’, ‘gut histomorphology’, ‘gut microflora’, ‘lipid profile’, ‘liver functions’, ‘acute phase proteins’, and ‘heat shock proteins’. Articles were then refined by restricting the publication year to 2012 to 2023, a range of 11 years, and restricted to English-only publications. Only full-text papers that featured at least one of the gut health or blood-related features were chosen to ensure that the information acquired was sufficient. A total of 31 full-text papers were reviewed for eligibility, and nine were eliminated for failing to match the appropriate criteria, resulting in 22 articles being included in this review.

PRISMA results

A comprehensive search of the Scopus database yielded 31 papers. There are no duplicates since they were removed during the article scanning process. Publications were filtered for irrelevant components before being examined for eligibility. A total of 22 articles were included in the information analysis. The publishing dates were restricted to 11 years, from 2012 to 2023, with the first in 2012 and the most recent in 2023. The year with the most articles published was 2020, followed by four in 2021 and 2022. Figure 1 shows that Asia accounted for the majority of the studies (55%), followed by Africa (32%), Oceania (9%), and Europe (4%). Furthermore, the plants researched in the selected studies can be classified as grasses, legumes, or non-legumes. Only one of the papers (4%) studied supplemental grass feed to broilers. Non-legume papers comprised 73% of those evaluated, whereas legumes comprised 23% (Figure 2). This review will go through the usage of grass or leaf meals as a feed supplement in broiler diets, as well as the effects on gut histomorphology, gut microbiota, serum lipid profile, blood biomarkers, and biochemistry.

The effect of grass or leaf meals on gut histomorphology of broilers

One of the hypothesized ways phytobiotic 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 intestinal morphology affect 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, stabilizing 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, thus activating 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 gut morphology, a rise in the production of enzymes such as proteases and amylases improves growth performance in the gastrointestinal tract by increasing absorptive cell development (Jamroz *et al.*, 2006). Several studies have found that incorporating phytobiotic 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). As a result, including phytobiotic in chicken feed has been proven to increase VH: CD ratio and promote digestive enzyme secretions.

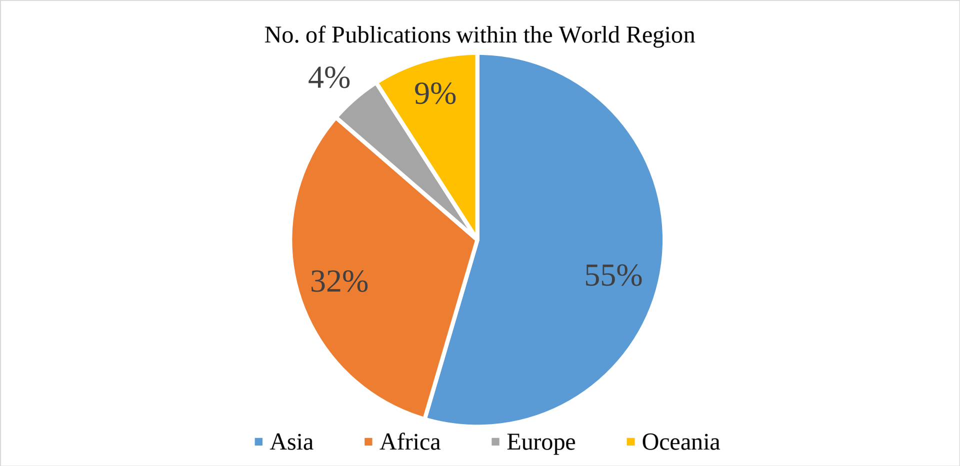

Figure 1. Number of included publications on grass or leaf meals as a feed additive in broiler diets and their effects on gut health and blood indices based on the world region.

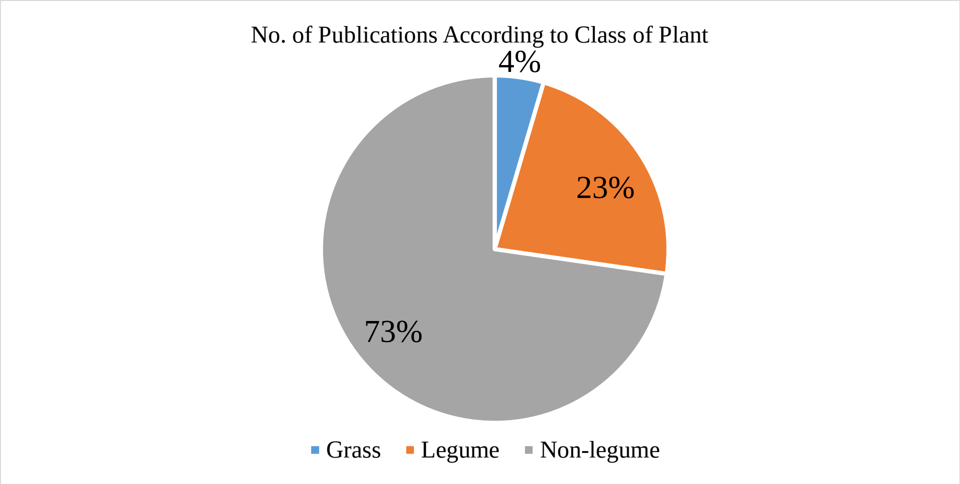

Figure 2. Number of included publications on grass or leaf meals as a feed additive in broiler diets and their effects on gut health and blood indices based on plant classification.

Supplementation of 25 mg/kg of *Brachiaria decumbens* powdered meal was 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 Cobb 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 morphology, enhancing lumen conditions such as number of pathogens, intestinal thickness, and mucus secretion (Tavangar *et al.*, 2021). Antioxidant properties 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 is 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 in comparison to the usage of tetracycline (1521.01-1723.00 µm vs 1407.15 µm) as antibiotics. There were no apparent differences (P > 0.05) between the villus width and crypt depth throughout all treatments supplemented with *Terminalia catappa* leaf extract and the antibiotic group. Therefore, VH:CD ratio of nanocapsulated (9.20) or non-nanocapsulated treatments (10.61) produced better results than the control (5.68) and antibiotic (6.15) group (Hidayati *et al.*, 2022). Similar results were discovered by Saharan *et al.* (2022) which studied the influence of supplementing 15, 25 and 35g of *Psidium guajava* leaf meal (PLGM) per kg of feed found that VH:CD ratio was significantly improved (P < 0.05). On top of that, the incorporation of *Azolla* leaf meal (ALM) into broiler diets at 50g and 100g per kg of baseline food resulted in an increase in villi length in the duodenum (P=0.09). Meanwhile, in the jejunum, treatment groups given 50 g 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 chickens, an increase in the villi length of the jejunum with the addition of 50 g 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 adding dietary phytobiotic with antibacterial action. These compounds may diminish pathogenic microbe growth and toxic chemicals. These chemicals inhibit intestinal mucous production by Goblet cells, minimizing intestinal damage and lumen repair (Hidayati *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 birds with 5.0 g 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 (Ahsan *et al.*, 2018). Basit *et al.* (2020b) also reported that with the supplementation of phytobiotics (4 g/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 in jejunum but improvements were significant (P < 0.0.5) in the duodenum and ileum of groups supplemented with *Piper betle*, *Pesicaria odorata*, halquinol and antibiotics. Therefore, significant (P < 0.05) positive results were seen in the VH: CD ratio of the 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 feeding fermented mixture of cassava pulp and *Moringa oleifera* leaf meal (FCPMO) through studying 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 favorable. 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 fiber content in FERM, since Saadatmand *et al.* (2019) showed that dietary fiber (rice hull) would result in a lower VH: CD. This negative effect would decrease absorptive capacity, resulting in poor development performance in grill chickens.

A summary of the effects of grass or leaf meals on the gut histomorphology of chickens can be found in Table 1 below. Based on previous experiments, there were more positive or non-significant results produced with the addition of plant-based phytobiotic in the diets of broilers. Negative findings could be explained by the high fiber content which is why it is crucial for thorough studies to be conducted on grass and leaf meals. This is necessary 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 fiber 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 phytobiotic as an antibiotic substitute.

Table 1. The different effects of various leaf meal supplementation on poultry's gut histomorphology.

Source	Inclusion levels	Effect on gut histomorphology	References
Brachiaria 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.	Hidayati *et al.* (2022)
Guava (*Psidium guajava*) leaf meal	15, 25 and 35g of PLGM	Improved VH:CD.	Saharan *et al.* (2022)
Azzola leaf meal	50 g and 100 g of *Azzola* leaf meal per kg of baseline food	Improved villi length in duodenum and jejunum. Depressed villi length in the ileum.	Abdelatty *et al.* (2021)
Rosemary leaf meal	5.0 g 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.* (2020a)
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)

The effect of grass or leaf meals on gut microflora of broilers

The chicken gastrointestinal system is home to diverse microorganisms living in symbiotic partnerships, ultimately impacting 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 indicates that plant-based feed additives positively impact the gut bacteria community when incorporated into the chicken diet. This is because bioactive substances present can break down the cellular membrane and interfere with the cytoplasm ecology of infections, thus acting as phytobiotic (Mohammadi Gheisar and Kim, 2018). When phytobiotics come into contact with the microbial cell membrane, they modify permeability for H+ and K+ cations, reducing the population of enteropathogens (Gilani *et al.*, 2021). For example, because microorganisms require iron for growth, tannins' antibacterial method of action is hypothesized to limit metal ions, impair microbe cell membrane permeability through complexes, and modify the morphology of cell walls (Suresh *et al.*, 2017; Basit *et al.*, 2020b). The tannin process of iron chelation reduces metal ion availability, limiting bacteria growth except for *Lactobacillus*, which does not require iron for development (Yilmaz and Li, 2018). Whereas saponins form interactions with membrane sterols, causing membrane damage, while alkaloids block topoisomerase and disrupt DNA synthesis (Suresh *et al.*, 2017). Moreover, with the incorporation of plant-origin active substances in the broiler diet, increased mucus production on the jejunum wall supports villi-related protective characteristics of phytocompounds, which might explain the lowered quantity of detrimental microbiomes to the epithelial wall (Jamroz *et al.*, 2006). These phytobiotic qualities will result in bacteriostatic and bacteriocidal actions, reducing pathogen populations and increasing the production and numbers of beneficial bacteria in the broiler's gut.

Alghirani *et al.* (2022) discovered that supplementing Ross308 broiler chicks with *B. decumbens* ground leaf meal suppressed the development of *Enterrococcus 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* (Zdunczyk *et al.*, 2010; Viveros *et al.*, 2011; Low, 2015; Hidayat *et al.*, 2021). Furthermore, compared to the other treatments, the group that received 25 mg/kg of *B. decumbens* had the highest standard plate count and coliform count throughout the experiment. These findings could be attributed to increased fecal coliforms and/or decreased lactobacilli, anaerobes, aerobes, and enterococci groups contributing to increased feed utilization. In another experiment, Mandal *et al.* (2014) discovered that adding MOLM, which also contains a wide variety of phytocompounds to day-old broiler chicks reduced microbial load compared to the other groups, including the control and antibiotic-supplemented groups. Incorporation of ALM into broiler diets was also demonstrated to linearly enhance L*actobaccili* species and reduce *Bacilli* as ALM inclusion levels rose, but decreased *Enterobacteriaceae* independent of ALM inclusion level. Abdelatty *et al.* (2021) could not determine if the alteration in intestinal mucus was driven by the change in microbiota with the addition of ALM or vice versa since ALM contains a large amount of silica, which might have affected the mucous contents. *Lactobacillus* count in the ileum and caecum was also higher ($P < 0.05$) in birds treated with rosemary leaf meal regardless of inclusion level in comparison to those fed with the negative control diet. Conversely, those fed the negative control diet had the highest ($P < 0.05$) count of *Escherichia 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* improve the intestinal microflora balance by reducing the number of hazardous microbes. This promotes the development of intestinal absorptive cells, which in turn encourages the growth of birds. Furthermore, it was reported that the supplementation of phytobiotics (*P. betle* and *P. odorata*) significantly increased ($P < 0.001$) the *Lactobacillus* population count compared to other groups (halquinol, antibiotics and negative control). Phytobiotics treated group also significantly decreased ($P < 0.05$) population count of *E. coli*, *Staphylococcus aureus*, *Clostridium*, and *Salmonella* (Basit *et al.*, 2020b). Thus, the inclusion of phytobiotic might result in a healthy gut microbiota population since they contain beneficial secondary metabolites such as flavonoids and phenolic compounds that favorably influence the microbiota population (Liaqat *et al.*, 2016; Rahman and Yang, 2018; Mustafa, 2019).

Table 2: The different effects of various leaf meal supplementation on poultry's' gut microbial population.

Source	Inclusion levels	Effect on gut microbial	References
Brachiaria 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)
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)
Rosemary leaf meal	2.5g and 5.0g of rosemary leaf meal	Higher *Lactobacillus* count in the ileum and caecum and lower *E. coli* and *Salmonella* count.	Ogwuegbu *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)
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)

Conversely, adding copra meal or cassava leaf meal to broiler diets had no effect ($P > 0.05$) on *E. coli* counts, even though the fermentation of dietary fibre in the ceca is thought to suppress pathogenic bacteria by lowering gut pH through the production of short-chain fatty acids (Khan and Iqbal, 2016; Jha and Mukku, 2019; Diarra and Anand, 2020). Diarra *et al.* (2023) found similar results ($P > 0.05$) on the *E. coli* count with the supplementation of copra meal, palm kernel meal and cassava leaf meal. They deduced that the supplementation doses did not provide substantial fibre content for relevant bacterial fermentation in the ceca. This theory is supported by Saharan *et al.* (2022), who found that PGLM 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 PGLM supplementation rate.

Despite some insignificant results with adding these leaf meals on the gut microbial population, it can also be said that including plant-based products has no deleterious effects on the gut microbial population in broiler birds. Table 2 elaborates on the different effects of various leaf meal supplementation on poultry's' gut microbial population.

The effect of grass or leaf meals on serum lipid profile, liver functions, and blood biomarkers of broilers

Tropical environments substantially influence animal well-being and impact animal output globally by encouraging animals to restrict heat generation by lowering feed intake. This leads to a detrimental impact on growth performance (Hansen, 2009). Besides, environmental, pathogenic, and nutritional issues, for example, can induce a state of stress, prompting a series of behavioral and physiological reactions that would reduce poultry performance (Tamzil *et al.*, 2013). Because of their antioxidant activity, phytobiotics could be utilized during heat stress and other stressor conditions to influence poultry serum biochemistry by lowering serum concentrations of cholesterol, triglycerides (TG), and low-density lipoproteins (LDL) while increasing high-density lipoproteins (HDL) levels in broilers (Gilani *et al.*, 2018). This may be due to the distinct features of phytobiotic , 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 be hepatotoxic, either owing to phytochemical ingredients or dose (Addy *et al.*, 2013) and blood parameters provide valuable insight for the health status of broiler birds (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 aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels would offer information on liver function and the occurrence of liver damage caused by toxins (Muazu and Aliyu-Paiko, 2020).

Additionally, blood biomarkers play a vital role in the poultry industry to estimate the broilers' welfare and health status (Attia *et al.*, 2011). 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 synthesized and accumulated as a result of cellular stress reaction, therefore may be used to identify stressful periods for the organism (Balakrishnan *et al.*, 2023). 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. APPs are a class of blood proteins primarily synthesized 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-defense mechanism that serves to trigger the acquired immune response (Janeway *et al.*, 2001). Therefore, in veterinary clinical pathology, APPs are helpful indicators for identifying infection, inflammation, trauma, and overall animal health (Gruys *et al.*, 2005). On the other hand, heat shock proteins (HSPs) are a group of proteins that are produced in response to various stressors such as physical, chemical, or biological stimuli such as heat and pathogenic infection (Ganter *et al.*, 2006; Staib *et al.*, 2007). Depending on their molecular weight, HSPs can be classified into several families (Basu *et al.*, 2002). While each HSP family has a unique purpose and function inside cells, the key role of HSPs is to protect and repair cells by promoting proper protein folding, which can occur under stressful situations (Sahin *et al.*, 2009). Research shows a substantial link between oxidation and HSP70 production (Ming *et al.*, 2010). Therefore, understanding blood biomarkers and biochemistry functions and reactions can aid in the development of better diagnostic procedures, treatment approaches, and diet formulations in the broiler industry.

Serum cholesterol reduced ($P < 0.05$) in birds supplemented with 10 and 15 g of mucuna leaf meal compared to birds 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 50 g 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 favorable results would prevent unwanted physiological conditions in broilers such as cardiovascular problems (Bueno *et al.*, 2017). Similarly, broilers supplemented with 10, 20 and 30 g 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 100 g/kg DM of diet of broiler birds discovered that the diet had a significant effect (P < 0.05) on AST, ALT, and ALP. Higher levels of MOLM resulted in lower AST and ALP levels, indicating 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 *P. 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 *P. odorata* leaf meal (Basit *et al.*, 2020a).

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 mobilization (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, all treatments did not affect serum cholesterol and triglyceride results (P > 0.05). The authors ascribed the reduced AST levels to the presence of tannins and saponins, which 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, TG, HDL, and LDL of broiler birds (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 metabolize 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 *A.annua* at 1g/kg of feed significantly decreased ($P < 0.05$) the mRNA expression of HSP70. *A. 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 *A. 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). Elevated DAO levels suggest necrosis of the intestinal mucosal cells, which ultimately impair intestinal function and permeability (Li *et al.*, 2002). More importantly, enzymatically treated *A. annua* supplementation was observed to significantly ($P < 0.05$) lower plasma DAO levels, indicating that enzymatically treated *A. annua* may enhance intestinal barrier function. These findings could be supported by another study that looked at the effects of pure rosemary extracts on heat stress in broilers and discovered that rosemary created a higher antioxidative condition (Tang *et al.*, 2018). Antioxidant activity of rosemary is 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 birds' 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.

Table 3: The different effects of various leaf or grass meal supplementation on serum lipid profile, liver functions, and blood biomarkers of poultry.

Source	Inclusion levels	Effect on serum lipid profile, blood biomarkers and biochemistry	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 *et al.* (2020a)
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)
Accacia 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 *Yucca 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)

While there were no known researches conducted that studied the effect of grass or leaf meals on the blood biomarkers such as APP, corticosterone and HSP, Alghirani *et al.* (2023) found that supplementing powdered *Yucca 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. Compared to the other treatment groups, broilers given 100mg/kg of Y. schidigera saponins had the lowest 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 serum amyloid A (SAA), alpha-1-acid glycoprotein (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 ceruloplasmin (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 100 mg/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 100 mg/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 (100 mg/kg) as saponins present could scavenge radicals, metal chelate, and synergize with other antioxidants, thus lowering HSP70 levels.

Table 3 summarizes 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 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, no previous research studied the effects of grass or leaf meals on blood biomarkers such as APP, corticosterone and HSP in broiler birds. Therefore, with the plethora of available plants, more viable options should be discovered and their effects studied to ensure a continuous supply should phytobiotics be adopted in the commercial poultry industry.

Summary

To summarize, based on the information provided and the conclusions of prior research, adequate inclusion levels of grass or leaf meals may positively influence overall gut health, serum lipid profile, blood biomarkers, and biochemistry. However, because phytocompound profiles and levels differ amongst plant species, additional study is needed to find the optimal amount in broiler feed to utilize them effectively. Furthermore, additional aspects like management, harvesting age, preparation procedures, and so on will all impact the phytocompound profiles of each plant meal and must be addressed. Additional research is also required to investigate the economic benefits of employing phytobiotics in broiler production, and the effects on end products such as meat quality. More data and information from studies will aid in filling research gaps on the use of grass and leaf meals and their effects on broilers’ physiology. As a result, both the broiler industry and consumers would profit from using phytobiotics as a viable, natural alternative to synthetic antibiotics, thereby improving food safety.

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