PHYTOCHEMICALS COMPOSITION OF GUINEA GRASS LEAF MEAL COLLECTED FROM THREE DIFFERENT LOCATIONS IN BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF AGRICULTURE (B. AGRIC) ANIMAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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ABSTRACT

Unlocking Location-Specific Bioactivity: Profound Phytochemical Variations in Guinea Grass (*Megathyrsus maximus*) Across Benin City Signal New Frontiers in Precision Animal Nutrition. The shift toward sustainable livestock production demands innovative strategies that leverage natural forage bioactivity as alternatives to conventional supplements. This groundbreaking study addresses a critical knowledge gap by mapping the phytochemical diversity of Guinea grass (*Megathyrsus maximus*), a vital tropical forage, across three distinct locations in Benin City, Nigeria. Through rigorous analysis of leaf samples from Ekehuan, University of Benin, and Ekosodin, we quantified key secondary metabolites-alkaloids, saponins, tannins, and phenol-that define the forage's functional properties. Our results demonstrate dramatic location-dependent variations in phytochemical composition. Ekosodin-derived grass emerged as exceptionally bioactive, containing substantially elevated levels of alkaloids (12.500 mg/100g), saponins (9.800 mg/100g), tannins (5.457 mg/100g), and phenols (4.200 mg/100g)-significantly surpassing concentrations found in other sampling sites. This establishes a clear geochemical signature that directly links environmental factors to forage quality. The implications are transformative: geography dictates bioactivity. The superior phytochemical profile of Ekosodin Guinea grass positions it as a powerful natural supplement capable of enhancing protein utilization, reducing methane emissions, and strengthening animal antioxidant defenses. However, this potency requires careful management, as high concentrations of certain compounds necessitate strategic inclusion rates to balance benefits against potential antinutritional effects. This research provides the scientific foundation for precision forage management, enabling livestock producers to strategically select and utilize Guinea grass based on its verified phytochemical profile. Our findings pioneer a new approach to tropical forage utilization, transforming Guinea grass from a conventional feed into a targeted nutritional tool that promises to revolutionize sustainable livestock production practices throughout West Africa. *Megathyrsus maximus*, Bioactive Compounds, Phytochemical Geography, Sustainable Livestock Production, Precision Nutrition, Forage Optimization, Natural Supplementation, Agricultural Sustainability.

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CERTIFICATION

This is to certify that this Project work was carried out by **Daniel ONIME** with Matriculation Number **AGR2004316** of the Department of Animal Science, Faculty of Agriculture, University of Benin city, Nigeria.

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DR N.C. AKAEZE. **Date**

(Project Supervisor)

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DR N.C. AKAEZE. **Date**

(Head of Department)

DEDICATION

I dedicate this work to my loving mother, Mrs. Rose Onime Azeigbe, and her husband, Mr. Azeigbe, whose love and guidance have been my greatest strength.

Special appreciation goes to Mr. Osaige, my wonderful friends Redeem and Peace, and my dear siblings for standing by me with unwavering encouragement and affection.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Livestock production is an essential aspect of agriculture in sub-Saharan Africa, contributing significantly to food security, income, and employment, particularly in rural areas (FAO, 2021). The success of livestock farming is largely dependent on the availability and quality of feed resources, which can constitute up to 70% of the total production cost (Smith *et al*., 2021). Among the tropical forages, Guinea grass (*Megathyrsus maximus*) stands out due to its high yield potential, palatability, and adaptability to different ecological zones (Oduguwa and Adebayo, 2019). It is widely cultivated and used as a primary feed for ruminants in Nigeria, particularly in the southern regions including Edo State.

Beyond its foundational nutritional properties, such as crude protein and fibre content, Guinea grass also contains a diverse array of phytochemicals. These are naturally occurring plant compounds, often referred to as secondary metabolites, that exert significant biological effects on animals. These include tannins, flavonoids, saponins, alkaloids, and phenols (Ajayi, Aderinboye and Okunade, 2020). Phytochemicals, while not classified as essential nutrients, have been extensively reported to influence feed intake, nutrient digestion, rumen microbial activity, and even systemic immunity in livestock (Patra and Saxena, 2011; Onyeka and Chukwuma, 2021). Many act as natural antioxidants and antimicrobials, potentially reducing the need for synthetic additives and antibiotics in animal diets, which aligns with the growing consumer demand for natural animal products (Franz *et al*., 2021).

The concentration and diversity of these bioactive phytochemicals in forages like Guinea grass are not static; they are profoundly influenced by a complex interaction of environmental factors. These include soil type and fertility, water availability, climatic conditions, exposure to pollution, and agricultural management practices such as cutting interval and fertilizer application (Makkar, 2003; Onyeka and Chukwuma, 2021). For instance, studies have shown that plants under environmental stress can alter their phytochemical profiles as a defence mechanism (Akula and Ravishankar, 2011). As a result, the phytochemical composition of Guinea grass may vary significantly between geographical locations and even between farms in the same region.

Despite the growing global interest in natural feed additives and bioactive forages, there is a conspicuous insufficiency of data on the phytochemical variation of Guinea grass across different agro-ecological locations in Nigeria. Most current feeding practices are based on general assumptions about forage quality, primarily focused on macro-nutrients, without considering the significant regional differences in bioactive chemical composition (Akinfemi, 2015). This gap poses a substantial limitation for nutritionists and farmers aiming to precisely optimize animal diets for both productivity and health. Moreover, the use of Guinea grass with inconsistent or unknown phytochemical profiles could lead to unpredictable animal performance, especially when fed as a primary component of the ration. For example, high but variable tannin levels can either improve protein utilization or suppress digestibility, depending on their concentration and type (Mueller-Harvey, 2006).

Phytochemicals such as flavonoids and condensed tannins are particularly noted for their antioxidant, antimicrobial, and antiparasitic properties, which can directly improve animal health and reduce reliance on synthetic drugs (Ajayi *et al*., 2020; Hoste *et al*., 2015). Therefore, systematically documenting their presence and spatial variation is a critical step towards the broader goal of enhancing sustainable livestock productivity through natural means. Furthermore, the outcome of this study may provide a scientific basis for policy and extension recommendations, guiding farmers towards identified forage harvesting zones with superior and consistent bioactive properties.

Given the increasing importance of integrating health-promoting compounds into sustainable livestock feeding systems, there is a pressing need to analyze and compare the phytochemical contents of Guinea grass collected from various localities within Benin City. Such data is indispensable for ensuring feed quality, consistency in animal nutrition, and ultimately, the resilience and profitability of the livestock sector in Edo State.

1.2 Objectives of the Study

The general objective of this study is to determine the phytochemical composition of Guinea grass (*Megathyrsus maximus*) leaf meal collected from three different locations in Benin City.

The specific objectives are to:

1. identify the phytochemicals present in Guinea grass leaf meal from each location,

2. quantify the concentrations of selected phytochemicals in the samples,

3. compare the phytochemical profiles among the three locations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Phytochemicals

Phytochemicals refer to an extensive array of naturally synthesized organic compounds that plants produce through their metabolic activities. Unlike primary metabolites such as carbohydrates, proteins, and lipids that are fundamental to the plant's growth, reproduction, and basic physiological functions, phytochemicals are categorized as secondary metabolites. This distinction arises because phytochemicals are not directly essential for the plant's immediate survival or primary metabolism (Ajayi *et al.,* 2020; Oduguwa and Adebayo, 2019).

Nonetheless, these compounds serve pivotal ecological roles. They enable plants to adapt and thrive in their environments by acting as chemical defenses against herbivores and pathogens, shielding plant tissues from ultraviolet radiation, and helping mitigate various abiotic stresses such as drought or poor soil conditions (Onyeka and Chukwuma, 2021; Skoczylas *et al.,* 2022). For instance, the bitter taste or toxic effect of certain phytochemicals discourages herbivory, while others act as signaling molecules in plant-plant or plant-microbe interactions, enhancing resilience under stress conditions (Wink, 2018).

In the realm of animal nutrition, phytochemicals have attracted growing research and practical interest due to their wide-ranging biological effects that extend beyond mere nutrient supply. These bioactive compounds can exert significant influences on feed palatability and intake, modulate rumen microbial ecosystems, and affect the digestion and absorption of nutrients. Importantly, many phytochemicals have been shown to possess antioxidant, antimicrobial, anti-inflammatory, anthelmintic, and immunomodulatory properties, making them valuable candidates for improving animal health and productivity in ways that complement or reduce reliance on synthetic feed additives (Onyeka and Chukwuma, 2021; Patra and Saxena, 2017).

When present at suitable concentrations in forage plants, phytochemicals can enhance livestock performance by supporting immune functions, reducing internal parasite burdens, stabilizing rumen fermentation, and even mitigating methane emissions, thereby contributing to more sustainable animal production systems (Benchaar *et al.,* 2015; Ajayi *et al.,* 2020). However, excessive levels of some phytochemicals may have antinutritional or toxic effects, highlighting the need for balanced assessment (Makkar, 2018).

Thus, characterizing and understanding the phytochemical composition of forages like Guinea grass (*Megathyrsus maximus*) is crucial. This knowledge not only informs evaluations of their nutritional quality but also provides insights into their potential functional impacts on livestock health and productivity. It ensures that these naturally occurring compounds can be optimally harnessed to support sustainable feeding strategies while avoiding negative consequences associated with overconsumption (Mueller-Harvey *et al.,* 2019).

2.2 Classification of Phytochemicals

Phytochemicals encompass an exceptionally broad range of naturally synthesized plant compounds. They are typically classified based on shared chemical structures, biosynthetic origins, or functional characteristics. In forage science and animal nutrition, these groupings are essential because they help predict how such compounds will interact with animal digestive systems and metabolic processes, thereby influencing their potential benefits or risks when used in livestock feeding (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021).

Phytochemicals relevant to Guinea grass and other tropical forages can be grouped into the following major categories:

2.2.1 Phenolic Compounds (Including Tannins and Flavonoids)

Phenolic compounds form one of the largest and most studied groups of plant secondary metabolites. They are structurally identified by the presence of at least one hydroxyl group directly bonded to an aromatic hydrocarbon ring (Skoczylas *et al.,* 2022). These compounds serve various protective roles in plants and significantly influence ruminant nutrition.

Tannins:

Tannins are high-molecular-weight polyphenols recognized for their strong ability to bind proteins and other macromolecules. They are broadly categorized into hydrolyzable tannins, which can be broken down into simpler phenolics under acidic conditions, and condensed tannins, which are more stable. In ruminant systems, moderate levels of tannins can be advantageous by forming complexes with dietary proteins. This reduces excessive breakdown in the rumen, thereby allowing more amino acids to reach the small intestine for absorption, improving nitrogen utilization. However, excessive tannin intake may negatively impact feed intake and digestion by binding to digestive enzymes and essential nutrients, lowering overall digestibility (Oduguwa and Adebayo, 2019; Mueller-Harvey *et al.,* 2019).

Flavonoids:

Flavonoids are a diverse subgroup of low-molecular-weight phenolic compounds that include flavonols, flavones, flavanones, anthocyanins, and isoflavones. They are especially noted for their antioxidant properties, neutralizing harmful free radicals and mitigating oxidative stress, which supports animal health and reduces the risk of disease. Additionally, flavonoids exhibit anti-inflammatory and antimicrobial activities that help maintain gut integrity and support immune function in livestock (Ajayi *et al.,* 2020; Vasta *et al.,* 2019).

2.2.2 Saponins

Saponins are naturally occurring glycosides composed of a sugar chain linked to a hydrophobic aglycone known as sapogenin. They are easily identified by their ability to produce stable foams when mixed with water a property that historically led to their name (from “sapo,” Latin for soap) (Patra and Saxena, 2017).

In ruminant nutrition, saponins play a beneficial role by suppressing rumen protozoal populations, which indirectly promotes bacterial growth and improves microbial protein synthesis. This microbial shift also contributes to lower methane emissions, enhancing feed energy efficiency. However, if saponins are present in excessive quantities, they may irritate or damage the intestinal lining or cause red blood cell lysis (hemolysis) in sensitive species, underscoring the need to monitor their concentrations in forage resources (Onyeka and Chukwuma, 2021; Makkar, 2018).

2.2.3 Alkaloids

Alkaloids are nitrogen-containing compounds predominantly derived from plant amino acid metabolism. They often possess strong physiological and pharmacological activities (Wink, 2018).

In plants, alkaloids act primarily as defensive compounds, deterring herbivores through their bitter taste or toxic effects. In controlled amounts, certain alkaloids may offer medicinal properties, such as antimicrobial or anti-inflammatory actions, which could be beneficial in animal systems. However, excessive intake of alkaloids can pose serious risks, including neurological disturbances, reproductive challenges, or even toxicity, making it crucial to characterize their levels in any feed source (Ajayi *et al.,* 2020; Adejoro *et al.,* 2019).

2.2.4 Other Important Classes: Terpenoids and Glycosides

Terpenoids (Isoprenoids):

These compounds are structurally based on repeating five-carbon isoprene units and are responsible for many plant odors, pigments (like carotenoids), and flavors. Beyond their ecological roles, some terpenoids exhibit antimicrobial, antioxidant, or anti-inflammatory properties, which may indirectly benefit animal health when consumed as part of forage diets (Wink, 2018).

Glycosides:

Glycosides are molecules that consist of a sugar component bonded to a non-sugar moiety(aglycone). While many glycosides are inert in their intact form, they can be hydrolyzed in the animal digestive tract to release bioactive aglycones, some of which may exert therapeutic or toxic effects depending on their type and concentration. Careful evaluation of glycoside content is therefore important to balance benefits and mitigate risks (Adejoro *et al.,* 2019).

2.3 Nutritional and Bioactive Role of Phytochemicals in Livestock Feeding

Phytochemicals, recognized as secondary metabolites, are abundantly synthesized by forage plants such as Guinea grass (*Megathyrsus maximus*), where they serve as intricate biochemical tools enabling the plant to thrive. Within their native ecosystem, these compounds play a protective role, acting as natural deterrents against herbivores, inhibiting the growth of pathogenic microorganisms, and helping plants adapt to environmental stressors like drought and intense sunlight (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021). Remarkably, when these phytochemicals are consumed by livestock through their diet, they extend their utility by imparting a wide spectrum of nutritional and bioactive benefits. These include improving protein efficiency, modulating gut microbial populations, and enhancing immune resilience. Such multifunctional properties are now increasingly valued in modern livestock systems that seek to elevate productivity and animal health through more natural, eco-friendly feeding approaches, thereby reducing dependency on synthetic growth promoters and chemotherapeutics (Oduguwa & Adebayo, 2019; Patra, 2023).

2.3.1 Nutritional Contributions of Phytochemicals

From a nutritional perspective, various phytochemicals exert indirect yet significant influences on how ruminants utilize the nutrients provided in their diets. A prime example is tannins, which have long been characterized as antinutritional factors because of their propensity to bind dietary proteins and essential minerals, thereby potentially limiting their digestibility. However, contemporary research has revealed that when present at controlled, moderate concentrations, tannins can actually offer substantial nutritional advantages. Specifically, tannins form stable complexes with dietary proteins that are resistant to the extensive microbial degradation typically occurring in the rumen. This protective effect allows a greater proportion of intact protein to bypass the rumen and proceed to the abomasum and small intestine, where enzymatic digestion releases amino acids for absorption. This not only enhances the overall efficiency of protein utilization but also improves nitrogen retention in the animal's body, consequently minimizing nitrogen losses to the environment through excretion (Onyeka and Chukwuma, 2021; Mueller-Harvey *et al.,* 2019; Makkar, 2018).

In a similar vein, saponins contribute positively to ruminant nutrition by modulating the microbial ecosystem within the rumen. They exert a selective inhibitory action on protozoal populations microorganisms that, although part of the normal rumen community, compete with bacteria and often engulf them, reducing microbial protein supply. By suppressing protozoa, saponins facilitate a relative increase in beneficial rumen bacteria responsible for synthesizing high-quality microbial protein. This microbial protein flows into the small intestine, serving as a primary and readily digestible source of amino acids for the host animal (Oduguwa and Adebayo, 2019; Patra and Saxena, 2017). Additionally, the shift in rumen fermentation dynamics induced by saponins lead to a reduction in methane production. Since methane synthesis represents a diversion and loss of dietary energy, lowering its formation not only improves the feed energy efficiency but also contributes to more environmentally sustainable livestock production (Ajayi *et al.,* 2020; Benchaar *et al.,* 2015).

2.3.2 Bioactive (Functional) Roles in Animal Health and Production

Beyond their indirect contributions to nutrient metabolism, phytochemicals inherently possess a diverse array of biological activities that profoundly influence animal health, welfare, and overall productivity. These biofunctional properties are especially significant in modern livestock systems that prioritize natural strategies for disease prevention, performance enhancement, and environmental stewardship (Patra, 2023).

1. Antioxidant Properties

Phenolic compounds, notably flavonoids and tannins, serve as powerful natural antioxidants. They neutralize reactive oxygen species (free radicals) and mitigate oxidative stress a physiological imbalance often linked to cellular damage, impaired immune function, and higher disease susceptibility. This antioxidant mechanism is particularly valuable in intensive animal production settings, where stressors such as crowding, heat, and high metabolic demands elevate oxidative challenges. By curbing oxidative stress, these phytochemicals support stronger immune responses and reduce the incidence of disorders associated with tissue degeneration (Ajayi *et al.,* 2020; Vasta *et al.,* 2019).

2. Antimicrobial and Antiparasitic Activities

Several phytochemicals demonstrate notable antimicrobial properties, enabling them to selectively modulate the gut microbial ecosystem. Compounds like saponins and specific alkaloids can suppress populations of pathogenic bacteria and protozoa, thereby favoring the growth of beneficial microbes that contribute to optimal gut function. Additionally, their antiparasitic effects help control internal parasites, which are a common constraint in grazing systems. By naturally suppressing pathogens and parasites, these phytochemicals reduce the need for synthetic antibiotics and commercial anthelmintics, aligning livestock production with growing consumer demands for residue-free animal products (Onyeka and Chukwuma, 2021; Hoste *et al.,* 2015).

3. Immunomodulatory Effects

Flavonoids and related polyphenols are increasingly recognized for their ability to modulate immune functions. They influence cytokine expression and enhance the structural integrity of gut-associated lymphoid tissues (GALT) critical sites where immune surveillance against ingested pathogens occurs. This immunomodulatory action translates into a more robust defense system, improving the animal's resilience to infectious challenges and contributing to overall health and performance (Oduguwa and Adebayo, 2019; Vasta *et al.,* 2019).

4. Role in Methane Mitigation

Methane production from enteric fermentation is not only a major source of greenhouse gases but also represents an energy loss for the ruminant, with up to 12% of gross energy intake potentially lost as methane. Phytochemicals such as condensed tannins and saponins have been extensively documented to reduce methane emissions by altering rumen microbial communities and redirecting fermentation pathways. This dual impact improving feed energy efficiency while reducing the environmental footprint of livestock operations positions phytochemicals as important tools in the pursuit of more sustainable animal agriculture (Ajayi *et al.,* 2020; Patra and Saxena, 2017; Benchaar *et al.,* 2015).

2.3.3 Dual Nature: Beneficial Versus Antinutritional Effects

It is essential to recognize that the benefits of phytochemicals are often concentration-dependent. At low to moderate inclusion levels, these compounds confer the aforementioned advantages. However, when consumed in excessive quantities, they may exert antinutritional effects. For instance, high concentrations of tannins can lead to the formation of insoluble complexes with proteins and minerals, reducing their digestibility and potentially depressing voluntary feed intake. Likewise, elevated levels of saponins may irritate or damage the gut mucosa and, under certain conditions, cause red blood cell rupture (haemolysis) (Onyeka and Chukwuma, 2021; Makkar, 2018). This highlights the necessity of accurately quantifying phytochemical concentrations in forages like Guinea grass to ensure their inclusion enhances rather than impairs animal performance.

2.3.4 Implications for Feeding Strategies

A thorough understanding of the nutritional and bioactive roles of phytochemicals empowers livestock producers and nutritionists to optimize natural feed resources. Incorporating phytochemical-rich forages such as Guinea grass into balanced rations can:

A. Increase the efficiency of protein utilization by safeguarding dietary proteins from extensive rumen degradation (Mueller-Harvey *et al.,* 2019).

B. Strengthen immune function and elevate the animal's capacity to resist diseases (Vasta *et al.,* 2019).

C. Reduce reliance on synthetic antimicrobials and anthelmintics, aligning production with public health objectives and market preferences for residue-free products (Hoste *et al.,* 2015).

D. Enhance environmental sustainability by lowering methane emissions, thus improving overall feed energy conversion (Patra and Saxena, 2017; Benchaar *et al.,* 2015).

Evaluating the phytochemical composition of Guinea grass leaf meal harvested from different environmental locations, as undertaken in this study, is therefore critical. It ensures that these bioactive compounds are present at optimal levels that maximize their functional advantages without surpassing thresholds that could compromise animal health or performance (Makkar, 2018).

2.4 Overview of Guinea Grass (*Megathyrsus maximus*) as a Tropical Forage

Guinea grass (*Megathyrsus maximus*), which was previously taxonomically identified as Panicum maximum, stands out as a premier perennial tropical forage grass extensively cultivated and naturally dispersed across the humid and sub-humid regions of Africa, Latin America, Asia, and increasingly, other tropical zones worldwide (Riveros *et al.,* 2021; Muinga *et al.,* 2020). Its remarkable ecological plasticity and exceptional capacity for producing substantial biomass under a variety of environmental conditions have cemented its role as a fundamental component in tropical livestock feeding systems. It serves efficiently in both cut-and-carry operations where herbage is harvested and transported to animals and in pasture-based systems, supporting direct grazing by ruminants (Muinga *et al.,* 2020).

2.4.1 Botanical and Agronomic Features

Belonging to the family *Poaceae*, *Megathyrsus maximus* is distinguished by its pronounced tillering ability, which enables it to produce multiple stems from a single plant base, thereby enhancing ground cover and resilience under defoliation. Its leaves are typically long and narrow, contributing to a favorable leaf-to-stem ratio that supports higher digestibility, especially in younger regrowth stages (Riveros *et al.,* 2021). Structurally, the grass adopts an upright growth habit, attaining heights of 1.5 to 3 meters, which facilitates both manual and mechanical harvesting.

Ecologically, *M. maximus* exhibits a broad adaptability to different climatic and edaphic conditions. It is capable of thriving across diverse soil types, from well-drained loams to moderately acidic soils, and demonstrates a moderate tolerance to periods of water deficit due to its relatively deep and fibrous root system (Riveros *et al.,* 2021). This makes it particularly suited to rain-fed systems common in tropical agriculture.

One of its key agronomic advantages is its rapid establishment and persistence. The species can be propagated both by seeds, which germinate reliably under favorable moisture and temperature regimes, and by vegetative means, such as root splits or stem cuttings. Following establishment, it exhibits vigorous regrowth after cutting or grazing, a trait that supports frequent harvesting intervals and makes it highly compatible with rotational grazing systems, thereby optimizing forage utilization and maintaining pasture productivity (Muinga *et al.,* 2020).

2.4.2 Adaptability and Ecological Role

Guinea grass is widely celebrated for its remarkable ecological adaptability, thriving across diverse climatic regimes that typify tropical and subtropical zones. While it exhibits its most vigorous growth under annual rainfall ranging from 900 to 1500 mm, this resilient grass is also capable of sustaining productive stands under comparatively drier conditions. Its adaptability to moisture stress is largely due to its deep-penetrating root system, which enables it to exploit subsurface water reserves inaccessible to many shallow-rooted forage species (Riveros *et al.,* 2021).

Furthermore, Guinea grass demonstrates a unique capacity to perform well under partial canopy shade, a trait that has facilitated its successful integration into silvopastoral systems innovative land-use models that combine trees with forage production. Within these systems, Guinea grass flourishes beneath tree cover, thus maximizing land use efficiency while contributing to enhanced biodiversity, improved microclimate conditions, and carbon sequestration (Muinga *et al.,* 2020).

In addition to its climatic adaptability, Guinea grass plays a critical role in maintaining soil health and landscape stability. Its dense growth provides excellent ground cover, effectively shielding the soil from the erosive forces of wind and rainfall. Through its continuous cycle of biomass accumulation and decomposition, it contributes substantial organic matter back into the soil, enriching soil fertility, promoting beneficial microbial activity, and reinforcing overall agroecological resilience (Muinga *et al.,* 2020).

2.4.3 Nutritional Profile and Forage Value

From a nutritional perspective, Guinea grass stands out among tropical forages for its commendable crude protein content, which typically ranges between 8% and 14% of dry matter, contingent upon growth stage, soil fertility, and management intensity (Muinga *et al.,* 2020; Ajayi *et al.,* 2020). Younger regrowth stages are particularly valued, as they generally present higher leaf-to-stem ratios and more digestible structural carbohydrates, translating to superior nutritive quality.

Beyond its protein contribution, Guinea grass offers moderate levels of metabolizable energy, making it well-suited to support the maintenance and productive needs—such as growth, lactation, and reproduction of a broad spectrum of ruminant livestock, including cattle, sheep, and goats. Its consistent biomass yield and satisfactory nutritional balance underpin its widespread adoption as a basal forage in many tropical grazing and cut-and-carry systems (Riveros *et al.,* 2021; Muinga *et al.,* 2020).

Significantly, Guinea grass is also a natural repository of diverse phytochemicals, including tannins, saponins, and flavonoids (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021). These secondary metabolites impart additional functional value to the forage. Tannins, for instance, can form protective complexes with dietary proteins, reducing excessive ruminal degradation and thereby enhancing post-ruminal amino acid absorption. Saponins have been shown to selectively modulate rumen microbial populations, often resulting in reduced protozoal counts, improved nitrogen utilization, and decreased methane emissions a key consideration in climate-smart livestock production. Flavonoids, with their potent antioxidant properties, help mitigate oxidative stress and support immune competence. Together, these bioactive constituents elevate Guinea grass from merely a source of macronutrients to a strategic functional feed, integral to modern approaches that aim to promote animal health, reduce environmental impacts, and lessen dependency on synthetic feed additives (Ajayi *et al.,* 2020; Patra, 2023).

2.4.4 Role of Guinea Grass in Livestock Systems

Guinea grass stands out as a foundational component of tropical livestock systems, primarily due to its outstanding forage biomass productivity, which can frequently exceed 20 tons of dry matter per hectare annually under well-managed regimes that include timely fertilization and strategic cutting (Riveros *et al.,* 2021; Muinga *et al.,* 2020). Such high herbage yields translate directly into the ability to sustain substantial stocking rates, thereby supporting intensive meat and milk production enterprises across diverse tropical and subtropical landscapes.

One of the most compelling advantages of Guinea grass lies in its remarkable flexibility across different forage utilization systems. It can be harvested as fresh cut herbage and fed immediately in cut-and-carry operations, reducing overgrazing pressures on natural pastures. Alternatively, during periods of surplus growth, the grass can be conserved as hay or ensiled, providing a reliable feed reserve to bridge the nutritional deficits typical of dry seasons. Its palatability and relatively stable nutrient profile post-harvest make it an effective forage base for year-round feeding programs (Muinga *et al.,* 2020).

An additional key feature of Guinea grass systems is their compatibility with legume intercropping, often involving nitrogen-fixing species such as *Stylosanthes spp*. or *Centrosema spp.*

Moreover, the integration of Guinea grass into diversified pasture systems help buffer against the volatility associated with monocultures, offering resilience against pests, diseases, and variable climatic conditions. Its structural robustness and deep rooting also aid in maintaining pasture productivity during periods of intermittent drought, ensuring a more consistent feed supply (Riveros *et al.,* 2021).

In practical terms, these combined attributes mean that Guinea grass serves not merely as a bulk feed but as a strategic forage species, essential for underpinning the productivity and profitability of ruminant enterprises in the tropics. It supports the physiological demands of cattle, sheep, and goats across various production goals from weight gain in meat systems to sustaining lactation in dairy operations thereby reinforcing food security and rural livelihoods in many developing regions (Muinga *et al.,* 2020; Ajayi *et al.,* 2020).

2.5 Environmental Factors Affecting Phytochemical Variation

The composition and concentration of phytochemicals in forage species like Guinea grass (*Megathyrsus maximus*) are far from static characteristics; rather, they represent highly dynamic traits that fluctuate in response to a multitude of interacting influences. Chief among these are environmental conditions, the plant's inherent genetic makeup, and its physiological stage of growth or maturity (Makkar *et al.,* 2021; Skoczylas *et al.,* 2022).

Grasping the nuances of how these factors govern phytochemical expression is essential, given that such variations have profound implications for both the nutritional quality and the functional (bioactive) properties of the forage. These shifts can affect the efficiency with which livestock utilize nutrients, influence rumen fermentation dynamics, and either enhance or compromise animal health, depending on the type and concentration of specific compounds. Additionally, understanding these determinants is critical for ensuring that phytochemical levels remain within biologically safe thresholds, thereby safeguarding the palatability, digestibility, and overall suitability of the forage for inclusion in animal diets (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021).

2.6 Overview of Guinea Grass (*Megathyrsus maximus*) as a Tropical Forage

Guinea grass (*Megathyrsus maximus*), which was previously taxonomically identified as *Panicum maximum*, stands out as a premier perennial tropical forage grass extensively cultivated and naturally dispersed across the humid and sub-humid regions of Africa, Latin America, Asia, and increasingly, other tropical zones worldwide (Skerman Riveros, 1990; Facheux *et al.,* 2018). Its remarkable ecological plasticity and exceptional capacity for producing substantial biomass under a variety of environmental conditions have cemented its role as a fundamental component in tropical livestock feeding systems. It serves efficiently in both cut-and-carry operations where herbage is harvested and transported to animals and in pasture-based systems, supporting direct grazing by ruminants (Muinga *et al.,* 2010).

2.6.1 Botanical and Agronomic Features

Belonging to the family *Poaceae*, *Megathyrsus maximus* is distinguished by its pronounced tillering ability, which enables it to produce multiple stems from a single plant base, thereby enhancing ground cover and resilience under defoliation. Its leaves are typically long and narrow, contributing to a favorable leaf-to-stem ratio that supports higher digestibility, especially in younger regrowth stages (Facheux *et al.,* 2018). Structurally, the grass adopts an upright growth habit, attaining heights of 1.5 to 3 meters, which facilitates both manual and mechanical harvesting.

Ecologically, *M. maximus* exhibits a broad adaptability to different climatic and edaphic conditions. It is capable of thriving across diverse soil types, from well-drained loams to moderately acidic soils, and demonstrates a moderate tolerance to periods of water deficit due to its relatively deep and fibrous root system (Skerman and Riveros, 1990; Muinga *et al.,* 2010). This makes it particularly suited to rain-fed systems common in tropical agriculture.

One of its key agronomic advantages is its rapid establishment and persistence. The species can be propagated both by seeds, which germinate reliably under favorable moisture and temperature regimes, and by vegetative means, such as root splits or stem cuttings. Following establishment, it exhibits vigorous regrowth after cutting or grazing, a trait that supports frequent harvesting intervals and makes it highly compatible with rotational grazing systems, thereby optimizing forage utilization and maintaining pasture productivity (Facheux *et al.,* 2018; Muinga *et al.,* 2010).

2.6.2 Adaptability and Ecological Role

Guinea grass is widely celebrated for its remarkable ecological adaptability, thriving across diverse climatic regimes that typify tropical and subtropical zones. While it exhibits its most vigorous growth under annual rainfall ranging from 900 to 1500 mm, this resilient grass is also capable of sustaining productive stands under comparatively drier conditions. Its adaptability to moisture stress is largely due to its deep-penetrating root system, which enables it to exploit subsurface water reserves inaccessible to many shallow-rooted forage species (Skerman and Riveros, 1990).

Furthermore, Guinea grass demonstrates a unique capacity to perform well under partial canopy shade, a trait that has facilitated its successful integration into silvopastoral systems innovative land-use models that combine trees with forage production. Within these systems, Guinea grass flourishes beneath tree cover, thus maximizing land use efficiency while contributing to enhanced biodiversity, improved microclimate conditions, and carbon sequestration (Lascano *et al.,* 2005; Facheux *et al.,* 2018).

In addition to its climatic adaptability, Guinea grass plays a critical role in maintaining soil health and landscape stability. Its dense growth provides excellent ground cover, effectively shielding the soil from the erosive forces of wind and rainfall. Through its continuous cycle of biomass accumulation and decomposition, it contributes substantial organic matter back into the soil, enriching soil fertility, promoting beneficial microbial activity, and reinforcing overall agroecological resilience (Muinga *et al.,* 2010; Lascano *et al.,* 2005).

2.6.3 Nutritional Profile and Forage Value

From a nutritional perspective, Guinea grass stands out among tropical forages for its commendable crude protein content, which typically ranges between 8% and 14% of dry matter, contingent upon growth stage, soil fertility, and management intensity (Muinga *et al.,* 2010; Ajayi *et al.,* 2020). Younger regrowth stages are particularly valued, as they generally present higher leaf-to-stem ratios and more digestible structural carbohydrates, translating to superior nutritive quality.

Beyond its protein contribution, Guinea grass offers moderate levels of metabolizable energy, making it well-suited to support the maintenance and productive needs such as growth, lactation, and reproduction of a broad spectrum of ruminant livestock, including cattle, sheep, and goats. Its consistent biomass yield and satisfactory nutritional balance underpin its widespread adoption as a basal forage in many tropical grazing and cut-and-carry systems (Skerman and Riveros, 1990; Muinga *et al.,* 2010).

Significantly, Guinea grass is also a natural repository of diverse phytochemicals, including tannins, saponins, and flavonoids (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021). These secondary metabolites impart additional functional value to the forage. Tannins, for instance, can form protective complexes with dietary proteins, reducing excessive ruminal degradation and thereby enhancing post-ruminal amino acid absorption. Saponins have been shown to selectively modulate rumen microbial populations, often resulting in reduced protozoal counts, improved nitrogen utilization, and decreased methane emissions a key consideration in climate-smart livestock production. Flavonoids, with their potent antioxidant properties, help mitigate oxidative stress and support immune competence. Together, these bioactive constituents elevate Guinea grass from merely a source of macronutrients to a strategic functional feed, integral to modern approaches that aim to promote animal health, reduce environmental impacts, and lessen dependency on synthetic feed additives (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021; Patra and Saxena, 2011).

2.6.4 Role of Guinea Grass in Livestock Systems

Guinea grass stands out as a foundational component of tropical livestock systems, primarily due to its outstanding forage biomass productivity, which can frequently exceed 20 tons of dry matter per hectare annually under well-managed regimes that include timely fertilization and strategic cutting (Skerman and Riveros, 1990; Muinga *et al.,* 2010). Such high herbage yields translate directly into the ability to sustain substantial stocking rates, thereby supporting intensive meat and milk production enterprises across diverse tropical and subtropical landscapes.

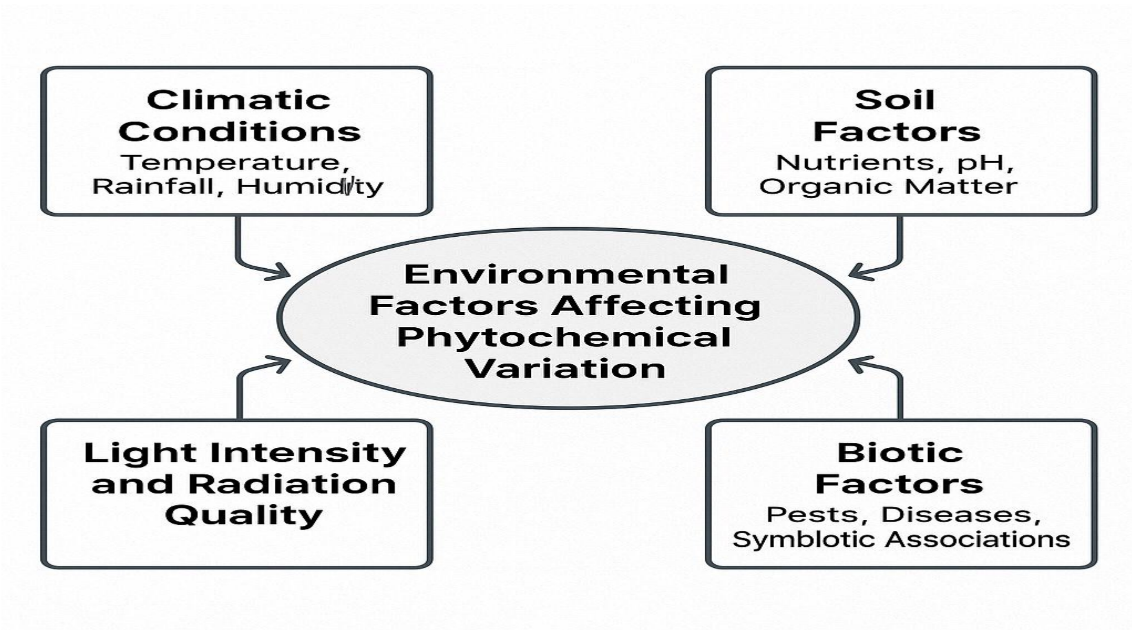
One of the most compelling advantages of Guinea grass lies in its remarkable flexibility across different forage utilization systems. It can be harvested as fresh cut herbage and fed immediately in cut-and-carry operations, reducing overgrazing pressures on natural pastures. Alternatively, during periods of surplus growth, the grass can be conserved as hay or ensiled, providing a reliable feed reserve to bridge the nutritional deficits typical of dry seasons. Its palatability and relatively stable nutrient profile post-harvest make it an effective forage base for year-round feeding programs (Muinga *et al.,* 2010; Ajayi *et al.,* 2020).

Beyond its primary function as a direct source of nutrients, Guinea grass plays an integral role in supporting ecosystem services within livestock production systems. Its vigorous ground coverage protects the soil from erosion, maintains soil structure, and promotes water infiltration. Through regular defoliation and subsequent regrowth, it contributes significant amounts of organic residues back to the soil, enhancing soil organic carbon levels and stimulating beneficial microbial activity (Lascano *et al.,* 2005; Facheux *et al.,* 2018).

An additional key feature of Guinea grass systems is their compatibility with legume intercropping, often involving nitrogen-fixing species such as Stylosanthes spp. or Centrosema spp. When cultivated together, these legumes synergistically enrich the pasture, not only by raising the crude protein content of the total forage on offer, but also by contributing to soil nitrogen balance through biological nitrogen fixation. This reduces the reliance on synthetic nitrogen fertilizers, lowers input costs, and minimizes environmental nitrogen runoff all critical considerations in advancing sustainable, climate-smart livestock systems (Muinga *et al.,* 2010; Skerman and Riveros, 1990).

Moreover, the integration of Guinea grass into diversified pasture systems helps buffer against the volatility associated with monocultures, offering resilience against pests, diseases, and variable climatic conditions. Its structural robustness and deep rooting also aid in maintaining pasture productivity during periods of intermittent drought, ensuring a more consistent feed supply (Facheux *et al.,* 2018; Lascano *et al.,* 2005).

In practical terms, these combined attributes mean that Guinea grass serves not merely as a bulk feed but as a strategic forage species, essential for underpinning the productivity and profitability of ruminant enterprises in the tropics. It supports the physiological demands of cattle, sheep, and goats across various production goals — from weight gain in meat systems to sustaining lactation in dairy operations — thereby reinforcing food security and rural livelihoods in many developing regions (Muinga *et al.,* 2010; Ajayi *et al.,* 2020).



2.7 Effects of Location on Forage Quality and Composition

The geographical location in which a forage species such as Guinea grass (*Megathyrsus maximus*) is cultivated holds a fundamental influence over its nutritional attributes and phytochemical constitution. This influence is not governed by a single factor but is instead the integrated outcome of diverse and interdependent elements, including regional climate patterns (temperature, rainfall distribution, humidity), edaphic factors (soil fertility, pH, organic matter content), topographical variations, and site-specific biotic interactions such as pest pressures and symbiotic microbial communities (Ajayi *et al.,* 2020; Muir *et al.,* 2022).

Collectively, these environmental determinants drive key physiological and metabolic pathways within the plant, thereby shaping not only the accumulation of primary metabolites like proteins and carbohydrates but also the synthesis and relative proportions of secondary metabolites such as tannins, flavonoids, and saponins (Onyeka and Chukwuma, 2021). Understanding this location-driven variability is essential, as it directly impacts the forage's suitability for livestock feeding, its functional role in modulating rumen fermentation, and its capacity to contribute to animal health and productivity (Riveros *et al.,* 2021).

2.7.1 Climatic Variability and Its Nutritional Consequences

The nutritional quality and secondary metabolite profile of forage species such as Guinea grass are profoundly shaped by the climatic regimes under which they are grown. Locations inherently differ in thermal environments, precipitation patterns, atmospheric humidity, and solar radiation, all of which exert pivotal influences on plant metabolic activities.

For instance, in agroecological zones characterized by moderate water deficits or elevated light intensities, Guinea grass frequently demonstrates an increased accumulation of secondary metabolites, notably tannins and flavonoids, as part of an adaptive response to abiotic stressors (Ajayi *et al.,* 2020). These phytochemicals serve to mitigate oxidative stress and protect cellular structures, thereby enhancing the forage's resilience. However, when present at elevated concentrations, these same compounds can exhibit antinutritional properties by reducing palatability and impairing protein digestibility in ruminants (Mueller-Harvey *et al.,* 2019).

Conversely, environments marked by ample and evenly distributed rainfall coupled with moderate temperatures typically encourage vigorous vegetative growth. Under such conditions, the rapid expansion of biomass can lead to a dilution of nutrient and phytochemical concentrations, often resulting in forage with higher moisture content and occasionally lower crude protein levels due to the partitioning of nitrogen across a larger volume of plant tissue (Onyeka and Chukwuma, 2021). This underscores the intricate balance between climatic factors and the qualitative attributes of forage, with direct implications for its nutritive and functional value in livestock systems.

2.7.2 Soil Variability and Its Role in Forage Composition

Beyond climatic influences, edaphic factors play a decisive role in determining forage quality and phytochemical characteristics. Soils across different locations exhibit considerable heterogeneity in terms of pH, texture, organic carbon content, cation exchange capacity, and the availability of macro and micronutrients, each of which modulates plant metabolic processes in distinct ways (Muir *et al.,* 2022).

For example, Guinea grass cultivated on soils with adequate nitrogen availability and balanced levels of phosphorus, potassium, and trace elements generally manifests higher concentrations of crude protein, thereby enhancing its feed value for ruminants. However, it has also been observed that excessive nitrogen fertilization may inversely affect the synthesis of certain secondary metabolites, such as tannins, which are integral to protecting dietary proteins from ruminal degradation (Oduguwa and Adebayo, 2019).

In contrast, in soils that are deficient in phosphorus or potassium, plants often reallocate metabolic resources toward the increased production of phenolic compounds as a physiological strategy to cope with nutrient stress. This can lead to forage that is more astringent due to elevated tannin content, potentially influencing voluntary intake and modifying rumen fermentation dynamics (Ajayi *et al.,* 2020). Furthermore, soil pH and organic matter levels influence microbial activity and nutrient solubility, indirectly shaping both primary nutrient accumulation and secondary metabolite profiles.

2.7.3 Impacts on Fiber Content, Structural Development, and Digestibility

Topographic and soil moisture variations across locations also critically affect plant growth rates, morphological characteristics, and the balance between structural and non-structural constituents. In fertile, low-lying terrains with superior moisture retention, Guinea grass often achieves greater plant height and biomass yields, which is typically associated with increased deposition of cell wall carbohydrates reflected in higher neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin contents (Onyeka and Chukwuma, 2021). While this augments total forage mass available for harvest, it can simultaneously reduce voluntary intake and overall digestibility, limiting the efficiency of nutrient extraction by ruminants.

Conversely, Guinea grass established on upland sites or soils with marginal fertility frequently exhibits more compact growth forms, characterized by higher leaf-to-stem ratios. Such morphological traits generally favor improved digestibility and energy availability, alongside relatively elevated crude protein content due to slower dilution of nitrogen across plant biomass (Ajayi *et al.,* 2020). These site-specific morphological and compositional differences highlight why identical forage species may present markedly different feeding values when sourced from varied agroecological settings.

2.7.4 Integrated Perspective and Practical Implications

Taken together, these multifactorial influences underscore the complex interplay between climatic conditions, edaphic properties, and topographical nuances, all of which shape the balance of primary nutrients and secondary metabolites in Guinea grass across different locations. This environmental modulation is particularly critical in the context of your study, which seeks to assess the phytochemical profiles of Guinea grass leaf meal harvested from three distinct sites within Benin City. Understanding these location-driven variations is essential not only for optimizing ration formulations to enhance animal performance but also for predicting and managing the functional impacts of bioactive compounds on rumen fermentation, methane mitigation, and animal health (Onyeka and Chukwuma, 2021; Patra, 2023).

2.8 Variability in Phytochemical Composition Due to Location

Phytochemicals such as tannins, saponins, and flavonoids which represent some of the most functionally significant secondary metabolites in Guinea grass (*Megathyrsus maximus*) are notably plastic traits, whose synthesis and accumulation are intricately regulated by environmental stimuli. This inherent plasticity means that the same genotype grown under divergent environmental conditions can exhibit markedly different phytochemical profiles, with substantial implications for its nutritive and functional utility in ruminant nutrition (Ajayi *et al.,* 2020; Onyeka and Chukwuma, 2021).

2.8.1 Influence of Stress and Solar Inputs

In locations characterized by intense solar radiation and moderate water deficits, plants often undergo elevated levels of oxidative stress, prompting the upregulation of pathways responsible for the biosynthesis of polyphenolic compounds such as tannins and flavonoids. These phytochemicals act as potent antioxidants, safeguarding cellular structures from reactive oxygen species and contributing to photoprotection. In the context of animal feeding, such elevated levels of tannins can be beneficial by forming stable complexes with dietary proteins, thereby shielding them from excessive microbial degradation in the rumen and allowing greater amino acid absorption in the intestines (Mueller-Harvey *et al.,* 2019). Similarly, flavonoids exert a range of bioactive functions, including antimicrobial and immunomodulatory effects, which can enhance gut health and overall animal resilience (Vasta *et al.,* 2019).

However, it is critical to recognize the threshold-dependent nature of these benefits. When tannin or saponin concentrations exceed optimal levels, they may begin to reduce palatability, depress voluntary feed intake, and impair nutrient digestibility by forming insoluble complexes not only with proteins but also with essential minerals such as iron and zinc (Onyeka and Chukwuma, 2021). This dualistic role underscores the necessity of carefully characterizing phytochemical concentrations in Guinea grass sourced from different agroecological zones.

2.8.2 Effect of Shadier or Humid Microclimates

Conversely, when Guinea grass is cultivated under conditions of greater canopy cover, frequent cloudiness, or in environments with high relative humidity and ample rainfall, plants often prioritize rapid growth and biomass accumulation. The accompanying reduced light intensity and generally lower oxidative stress diminish the need for protective secondary metabolites, resulting in lower concentrations of flavonoids, tannins, and related compounds. While this leads to forage that is typically more palatable and more readily consumed by livestock, it may concurrently lack sufficient bioactive levels to effectively modulate rumen fermentation, reduce methane emissions, or suppress internal parasites, thereby necessitating alternative strategies to achieve these functional outcomes (Muir *et al.,* 2022).

2.8.3 Modulating Role of Local Biotic Factors

Equally influential in shaping the phytochemical landscape of Guinea grass are the local biotic interactions that vary across geographic locations. Each site harbors distinct assemblages of soil microbiota, fungal endophytes, pathogenic organisms, and herbivorous pests, all of which can exert selective pressures on plant secondary metabolism.

2.8.4 Microbial and Mycorrhizal Interactions

For example, associations with arbuscular mycorrhizal fungi (AMF) can alter the plant's nutrient uptake dynamics, particularly phosphorus acquisition, which in turn may affect the carbon-to-nutrient balance and drive the allocation of resources toward or away from secondary metabolite production. Enhanced nutrient acquisition under strong mycorrhizal colonization may sometimes dampen the synthesis of defensive phytochemicals, while plants experiencing nutrient limitations—even if mediated by reduced AMF effectiveness—may invest more in phenolic and tannin pathways as part of stress adaptation (Thirkell *et al.,* 2017).

2.8.5 Pests, Pathogens, and Induced Defense

Local variations in pest and pathogen pressures also play a crucial role. Plants attacked by herbivores or exposed to pathogenic fungi and bacteria often activate induced systemic resistance pathways, which can elevate the biosynthesis of compounds such as flavonoids, alkaloids, and tannins (Onyeka & Chukwuma, 2021). While this can incidentally enhance the forage's functional capacity to modulate gut microbiota or inhibit protozoa in the rumen, excessive accumulation may again risk reducing palatability and nutrient availability if not properly balanced.

Broader Implications for Livestock Nutrition and the Relevance to This Study

This location-dependent variability in phytochemical and nutritional composition underscores a central challenge in tropical forage-based systems: even genetically identical Guinea grass planted across different microenvironments within a single region—such as the varied agroecological niches found within Benin City—can produce forages that differ significantly in crude protein, fiber fractions (NDF, ADF, lignin), mineral composition, and concentrations of key functional phytochemicals (Ajayi *et al.,* 2020; Muir *et al.,* 2022).

Practical Feeding Strategy Adjustments

For livestock producers, this variability necessitates nuanced feeding strategies:

- Forages from areas with elevated tannin or saponin levels may be incorporated at lower inclusion rates, potentially complemented with tannin-binding agents like polyethylene glycol (PEG) or by blending with low-tannin forages to prevent declines in voluntary intake.
- Conversely, Guinea grass from more humid or shaded locations may require strategic supplementation with protein meals or functional feed additives to compensate for reduced phytochemical-mediated protein sparing and methane mitigation effects.

2.9 Implications of Phytochemical Rich Forage in Animal Health and Production

The growing emphasis on integrating phytochemical-rich forages such as Guinea grass (*Megathyrsus maximus*) into livestock feeding systems stems from their remarkable capacity to deliver benefits that extend well beyond the provision of basic nutrients.

Unlike conventional forage considerations that focus primarily on crude protein, fiber, and energy content, these tropical grasses are distinguished by their diverse suite of secondary metabolites, notably tannins, saponins, flavonoids, alkaloids, and various phenolic constituents.

Collectively, these phytochemicals endow the forage with a multifaceted bioactive profile, imparting a range of physiological and metabolic effects that can have profound implications for ruminant health, growth performance, feed efficiency, and even the quality attributes of resulting animal products (Ajayi *et al.,* 2020; Patra, 2023).

2.9.1 Enhanced Efficiency of Protein Utilization and Improved Nitrogen Dynamics

Protective Binding of Dietary Proteins

A primary nutritional advantage conferred by phytochemical-laden forages stems from the action of condensed tannins, which have a remarkable propensity to form pH-sensitive complexes with dietary proteins. Within the near-neutral pH environment of the rumen, tannins bind to soluble and structural proteins, thereby shielding them from rapid proteolytic degradation by ruminal microbes (Onyeka and Chukwuma, 2021).

This protective mechanism markedly reduces the transformation of protein into ammonia and its subsequent incorporation into microbial protein. Instead, more undegraded dietary protein (UDP) escapes the rumen, proceeding to the small intestine where it is digested by endogenous proteases and absorbed as amino acids an outcome that bolsters nitrogen retention and supports muscle accretion or milk synthesis.

Nitrogen Retention and Environmental Benefits

This strategic shift in nitrogen partitioning also carries significant environmental ramifications. By diminishing ammonia accumulation in the rumen, there is less nitrogen lost via urine, reducing volatilization into ammonia gas and nitrate leaching, which are key contributors to environmental contamination from livestock operations (Benchaar *et al.,* 2015).

Nevertheless, these benefits are inherently concentration-dependent. Optimal improvements are typically observed when condensed tannins are present at moderate levels (2–4% of dietary dry matter). When concentrations surpass this safe range, tannins may bind irreversibly with proteins, rendering them indigestible throughout the gastrointestinal tract, ultimately compromising feed conversion efficiency and reducing the bioavailability of essential amino acids (Mueller-Harvey *et al.,* 2019).

2.9.2 Modulation of Rumen Fermentation Profiles and Mitigation of Methane Emissions

Selective Alterations in Microbial Populations

Beyond protein metabolism, phytochemicals exert profound influences on the ruminal microbial ecosystem and fermentation dynamics. Saponins, characterized by their amphiphilic glycoside structures, exhibit targeted antiprotozoal activities. By selectively suppressing populations of ruminal protozoa which are primary hosts for methanogenic archaea saponins indirectly limit the population of methanogens, thereby reducing enteric methane production and conserving more dietary energy for productive purposes (Patra and Saxena, 2017).

Shifts in Volatile Fatty Acid Production

Similarly, tannins and flavonoids can modulate rumen fermentation pathways, promoting a shift in volatile fatty acid (VFA) profiles toward higher propionate and reduced acetate formation. This metabolic alteration is particularly advantageous, as propionate serves as a key substrate for hepatic gluconeogenesis, directly supporting glucose homeostasis critical for lactating and growing animals. Simultaneously, reduced acetate-to-propionate ratios lower hydrogen availability for ethanogenesis, synergistically curbing methane emissions while improving energy capture from the feed.

Climate and Economic Synergies

Such phytochemically mediated shifts not only enhance nutrient utilization efficiency but also align ruminant feeding systems with climate-smart agriculture initiatives, offering a natural strategy to reduce greenhouse gas outputs without the need for costly feed additives or chemical inhibitors (Patra, 2023).

2.9.3 Reinforcement of Animal Health through Antioxidant and Immunomodulatory Pathways

Neutralization of Reactive Oxygen Species

Phytochemical-rich forages contribute importantly to the antioxidant status of the animal, chiefly through their abundant content of flavonoids and phenolic acids. These compounds act by directly scavenging reactive oxygen species (ROS) and by upregulating intrinsic antioxidant defense systems such as superoxide dismutase and catalase, thereby protecting cellular lipids, proteins, and nucleic acids from oxidative injury (Vasta *et al.,* 2019).

Given that oxidative stress underlies a vast array of production diseases from mastitis and reproductive inefficiencies to metabolic syndromes bolstering antioxidant defenses plays a crucial role in maintaining overall herd health and sustaining high performance.

Modulation of Immune and Gut Integrity

In addition to their antioxidant activities, several phytochemicals exert immunomodulatory effects, influencing the expression of cytokines and enhancing the architecture of gut-associated lymphoid tissue. This dual action strengthens mucosal immunity and systemic defenses, reducing susceptibility to enteric pathogens and systemic infections outcomes that directly translate to lower veterinary costs and improved productivity metrics (Adejoro *et al.,* 2019).

2.9.4 Natural Control of Internal Parasites and Gut Pathogens

Among the most strategically important roles of phytochemicals in forages like Guinea grass is their capacity to function as natural antiparasitic and antimicrobial agents. This becomes critically relevant in tropical and sub-tropical livestock systems, where gastrointestinal nematodes (GINs) and pathogenic bacteria are persistent threats, undermining animal health, feed efficiency, and overall farm profitability.

Tannins, particularly condensed tannins, have demonstrated strong anthelmintic activities. These compounds can interfere with the life cycle of parasitic nematodes by disrupting egg hatchability, impairing larval development, or reducing the establishment and fecundity of adult worms in the gastrointestinal tract. This translates into lower faecal egg counts and reduced parasite burdens, diminishing the need for frequent administration of synthetic anthelmintics (Hoste *et al.,* 2015). Given the escalating problem of drug-resistant parasite strains, such bioactivity has immense practical significance.

Similarly, saponins have shown the ability to alter gut wall integrity and exert direct toxic effects on parasites. They also possess membrane-active properties that can disrupt the structural stability of protozoa and some bacteria.

Beyond parasites, these phytochemicals can help regulate gut microbial populations. Moderate levels may inhibit pathogenic organisms such as E. coli or Salmonella, while favoring populations of beneficial bacteria that support fiber digestion and overall gut health (Patra and Saxena, 2017).

Together, these effects not only enhance animal resilience to infections but also align with the growing consumer demand and regulatory pressures to reduce the use of antibiotics and chemical dewormers in livestock production systems.

2.9.5 The Double-Edged Sword: Antinutritional and Toxic Risks

While the benefits of phytochemicals are compelling, it is equally important to recognize that these compounds are not universally benign. Their impacts on ruminant nutrition and health are often dose-dependent, and surpassing optimal thresholds can pivot their effects from beneficial to detrimental. This dual nature necessitates careful attention:

- Feed Intake and Palatability: Elevated tannin concentrations increase astringency by binding salivary proteins, creating a puckering mouthfeel that reduces voluntary feed intake. Likewise, high levels of saponins and many alkaloids impart bitterness that animals may instinctively avoid. If intake drops, animals fail to meet their energy and protein requirements, leading to suboptimal growth or milk yields.

- Nutrient Digestibility: Excessive tannins can form stable, insoluble complexes not only with proteins but also with carbohydrates and minerals, effectively locking away nutrients from microbial fermentation in the rumen and subsequent absorption in the intestines (Onyeka and Chukwuma, 2021). This can result in reduced fiber digestibility and poorer feed conversion efficiency.
- Physiological Toxicities: At high intake levels, saponins exhibit hemolytic properties, meaning they can disrupt the lipid membranes of red blood cells, leading to hemolysis and potential anemia. Some alkaloids may exert neurotoxic or reproductive effects depending on their type and concentration (Adejoro *et al.,* 2019).

These potential drawbacks reinforce the necessity of systematically quantifying phytochemical contents especially when sourcing Guinea grass from diverse agroecological locations where environmental conditions may drive wide variability in these compounds.

2.9.6 Influence on Animal Product Quality and Human Health Benefits

The impact of phytochemicals does not stop at the animal level; it extends into the quality of animal-derived foods, with both technological and nutritional consequences for end consumers.

- Improved Oxidative Stability: Certain flavonoids, phenolic acids, and even residual tannins absorbed or stored in tissues can help slow oxidative spoilage in meat and milk by neutralizing free radicals. This leads to longer shelf life and improved sensory characteristics, such as color stability in meat and reduced rancidity in dairy products (Vasta *et al.,* 2019).

- Functional or "Healthier" Animal Products: There is emerging interest in the concept of functional foods, where meat or milk from animals fed phytochemical-rich forages might deliver enhanced antioxidant properties or natural anti-inflammatory compounds to human diets. This has market potential in premium segments seeking foods perceived as more natural or health-promoting.

- Possible Off-Flavors: On the flip side, very high concentrations of certain phytochemicals can impart atypical flavors to meat or milk, which may be undesirable in some consumer markets. This highlights the importance of managing forage phytochemical profiles not only for animal performance but also to safeguard sensory quality of products.

2.10 Summary of Reviewed Literature

A comprehensive examination of existing literature highlights the increasingly recognized importance of tropical forage species such as Guinea grass (*Megathyrsus maximus*), which serve not only as fundamental sources of energy and protein for ruminant animals but also as carriers of a wide array of bioactive phytochemicals. Numerous studies have established that Guinea grass harbors substantial quantities of secondary metabolites, notably tannins, saponins, flavonoids, alkaloids, and assorted phenolic compounds (Ajayi *et al.,* 2020; Skoczylas *et al.,* 2022). These compounds imbue the forage with a dual functionality: providing essential nutrients while simultaneously exerting physiological and metabolic effects that can profoundly influence animal health and production outcomes.

Several investigations have shown that moderate levels of condensed tannins present in Guinea grass play a pivotal role in enhancing protein metabolism in ruminants (Mueller-Harvey *et al.,* 2019). By forming reversible complexes with dietary proteins within the rumen environment, tannins effectively safeguard these proteins from premature microbial degradation. This mechanism facilitates greater passage of undegraded dietary protein (UDP) into the small intestine, where enzymatic hydrolysis ensures superior amino acid absorption. The resultant improvement in nitrogen utilization efficiency translates directly into better growth rates, superior muscle deposition, and concurrently lowers nitrogen excretion in urine, thereby mitigating the environmental burden associated with livestock waste (Patra, 2023).

Similarly, the presence of saponins and flavonoids in Guinea grass has garnered attention for their ability to influence rumen microbial ecosystems. Saponins have demonstrated efficacy in selectively suppressing protozoal populations, which indirectly diminishes the habitats of methanogenic archaea (Patra and Saxena, 2017). This interaction is crucial for reducing enteric methane emissions, leading to more efficient energy capture from feed. Additionally, flavonoids and tannins can modulate ruminal volatile fatty acid (VFA) profiles, favoring increased propionate production at the expense of acetate, which inherently supports reduced hydrogen availability for methane synthesis (Benchaar *et al.,* 2015). Such shifts not only optimize feed energy utilization but also advance climate-smart livestock production objectives.

Beyond these nutritional efficiencies, the literature extensively documents the antioxidant, antimicrobial, antiparasitic, and immunomodulatory roles of phytochemicals prevalent in Guinea grass. Flavonoids and other phenolic compounds function as potent antioxidants that quench reactive oxygen species, thereby reducing oxidative stress, preserving cellular integrity, and lowering susceptibility to metabolic and infectious diseases (Vasta *et al.,* 2019). Moreover, by modulating cytokine responses and enhancing gut-associated lymphoid tissue function, these phytochemicals contribute to a more resilient immune system. Their antimicrobial and anthelmintic actions also help curtail gastrointestinal parasite loads and pathogenic bacterial populations, reducing dependence on synthetic pharmaceuticals and aligning with modern consumer demands for residue-free animal products (Hoste *et al.,* 2015).

Nevertheless, the literature consistently underscores the dose-sensitive nature of these phytochemicals. While their presence at controlled levels yields pronounced benefits, excessive concentrations may provoke antinutritional repercussions (Makkar, 2018). High tannin levels, for instance, can lead to undesirable astringency, diminish voluntary feed intake, and form insoluble complexes not only with proteins but also with carbohydrates and minerals, thereby impairing overall nutrient digestibility. Likewise, elevated saponin intake has been associated with cytotoxic and hemolytic effects, underscoring the delicate balance required to harness these compounds safely.

Additionally, a recurring theme across the reviewed studies is the substantial variability in phytochemical profiles of Guinea grass, driven by diverse environmental determinants such as temperature, rainfall, solar radiation, soil fertility parameters (notably nitrogen, phosphorus, and organic matter), plant maturity at harvest, and interactions with local biotic communities (Muir *et al.,* 2022). These factors can cause pronounced differences in phytochemical accumulation, even among genetically similar stands cultivated across different geographical or agroecological settings.

Despite the depth of global research on phytochemical impacts in tropical forages, there exists a notable scarcity of localized data focusing on Guinea grass grown under the specific environmental conditions prevalent in Benin City, Nigeria. This gap creates uncertainty in predicting how such forage may perform nutritionally and functionally within local livestock systems. Consequently, the present study is both timely and justified, aiming to systematically investigate and compare the phytochemical composition of Guinea grass leaf meals collected from three ecologically distinct sites within Benin City. Such an endeavor is pivotal for developing site-specific feeding strategies, ensuring the optimal use of this versatile forage to bolster animal productivity, enhance health, and maintain product quality, all while carefully managing the risks associated with excessive intake of bioactive compounds.

2.11 Identified Research Gap

Despite the extensive body of literature that underscores the agronomic importance and general nutritive value of Guinea grass (*Megathyrsus maximus*) as a leading tropical forage, critical gaps persist in our understanding of its phytochemical dynamics, particularly when examined through the lens of localized agroecological variability. These knowledge deficiencies are especially evident in the Nigerian context and even more so within the microclimatic mosaic of Benin City. The principal gaps identified from the literature can be elaborated as follows:

2.11.1 Insufficient Location-Specific Phytochemical Characterization

While numerous investigations have explored Guinea grass with respect to fundamental nutritional indices—such as crude protein content, neutral detergent fiber (NDF), acid detergent fiber (ADF), and digestible energy there remains a conspicuous scarcity of studies that systematically evaluate its secondary metabolite composition, namely tannins, saponins, flavonoids, and alkaloids, across different geographical or ecological gradients (Ajayi *et al.,* 2020). Current literature is largely dominated by broad regional or country-level assessments, often neglecting micro-scale heterogeneities that exist within localities such as Benin City.

Given that phytochemical biosynthesis in plants is highly responsive to edaphic conditions (soil pH, organic matter, nutrient availability), climatic variables (rainfall patterns, temperature fluctuations, humidity), and biotic pressures (pathogen and pest incidence, symbiotic associations), the absence of finely resolved, location-specific data leaves a significant void (Muir *et al.,* 2022). This means that potential intra-regional differences in the phytochemical fingerprints of Guinea grass are largely undocumented, limiting our ability to make informed decisions about its optimal utilization under diverse local production systems.

2.11.2 Lack of Integrated Studies Linking Phytochemical Profiles to Functional and Antinutritional Implications

Additionally, although isolated studies have reported the occurrence of individual phytochemicals such as tannins or saponins in tropical grasses, there is a notable deficiency of holistic research frameworks that simultaneously quantify these compounds in Guinea grass and correlate their concentrations with potential nutritional, bioactive, and antinutritional outcomes under specific environmental contexts.

This lack of integrated data is particularly problematic in the formulation of evidence-based feeding strategies. Without localized phytochemical benchmarks, nutritionists, extension agents, and livestock producers are compelled to rely on generalized or extrapolated information, which may not accurately reflect the bioactive properties or safety thresholds of Guinea grass cultivated within the distinct agroecological landscape of Benin City (Makkar, 2018). Consequently, opportunities to strategically harness the benefits of phytochemicals such as enhanced rumen protein bypass, methane reduction, improved antioxidant status, and natural parasite control while avoiding risks like depressed feed intake or impaired nutrient digestibility, remain underutilized.

2.11.3 Lack of Comparative Evaluations Across Benin City's Distinct Agroecologies

Even within the relatively compact geographical confines of Benin City and its peri-urban surroundings, there exists considerable environmental heterogeneity including variations in soil texture and fertility (ranging from ferralsols to hydromorphic soils), subtle shifts in micro-topography that influence drainage and water availability, as well as localized differences in microclimatic factors such as humidity, solar intensity, and wind exposure. Each of these environmental parameters plays a critical role in regulating secondary metabolic pathways in plants, thereby shaping the synthesis and accumulation of phytochemicals like tannins, saponins, flavonoids, and alkaloids (Skoczylas *et al.,* 2022).

Despite these well-recognized ecological gradients, there remains an almost complete absence of systematic, multi-site comparative studies focused on Guinea grass cultivated across these different localized environments. Without such studies, there is no empirical basis to quantify how location-specific factors in Benin City distinctly influence the phytochemical composition of Guinea grass leaf meal. This represents a critical oversight because it implicitly assumes uniformity in phytochemical profiles across all harvesting sites — an assumption that is unlikely to hold true given the known responsiveness of phytochemical biosynthesis to environmental stresses and soil nutrient dynamics.

Practically, this means farmers, feed processors, and livestock nutritionists in the region currently lack evidence-based information to discriminate between Guinea grass sourced from different localities. Consequently, they may inadvertently incorporate forage with suboptimal or excessively high levels of certain phytochemicals, potentially affecting animal intake, nutrient utilization, or even health outcomes. This local data void directly limits the capacity to implement site-adapted harvest strategies or to selectively utilize forages best aligned with specific nutritional or bioactive feeding objectives.

2.11.4 Insufficient Baseline for Developing Location-Tailored Feeding Strategies in Sustainable Livestock Systems

Modern livestock production — especially under the paradigm of climate-smart and residue-free agriculture — increasingly seeks to exploit the multifunctional properties of phytochemical-rich forages. For instance, moderate tannin concentrations are harnessed to protect dietary proteins in the rumen, thereby improving amino acid absorption and reducing nitrogen excretion into the environment (Mueller-Harvey *et al.,* 2019). Similarly, saponins are valued for their roles in modulating rumen protozoal populations, with implications for methane mitigation and improved microbial protein synthesis (Patra & Saxena, 2017). Flavonoids and phenolics contribute antioxidant and immune-supportive effects that may lower disease incidence without resorting to synthetic antimicrobials (Vasta *et al.,* 2019).

However, the strategic incorporation of such phytochemical benefits into feeding programs is only feasible when there is robust, location-specific data detailing the typical ranges of these bioactive compounds under local agronomic and environmental conditions. In Benin City, the near-total lack of such baseline phytochemical profiling means that nutritionists and farmers cannot confidently formulate site-sensitive rations that balance nutritional adequacy with biofunctional impacts. This forces continued reliance on broad generalizations or imported reference values from other agroecological zones, which may not accurately reflect local realities.

As a consequence, opportunities to optimize Guinea grass inclusion rates — ensuring that its phytochemicals act as functional additives (for protein sparing, methane reduction, parasite control) rather than as antinutritional liabilities remain largely untapped (Makkar, 2018). Without this foundation, sustainable feeding interventions that could simultaneously boost productivity, reduce input costs, and minimize environmental footprints are severely constrained.

2.12 Overall Justification for This Study

Despite the extensive utilization of Guinea grass (*Megathyrsus maximus*) as a principal forage resource in tropical livestock systems, there remains a substantial deficit in localized scientific data concerning its phytochemical composition, particularly under the varying agroecological conditions that characterize Benin City and its environs. This gap is especially noteworthy given the increasing recognition of the multifaceted roles that phytochemicals—such as tannins, saponins, flavonoids, and other phenolic compounds—play in modern sustainable animal production (Patra, 2023).

Existing literature has predominantly centered on the general nutritive attributes of Guinea grass, emphasizing crude protein, fiber fractions, and digestible energy, while largely overlooking comprehensive phytochemical profiling across different local microenvironments. Moreover, there is an almost complete absence of comparative studies evaluating how soil variability, microclimatic differences, and local biotic pressures influence the accumulation of these bioactive compounds within Benin City (Muir *et al.,* 2022). This represents a critical oversight, as such environmental factors are well documented to significantly affect secondary metabolite synthesis in forage species.

Without robust, location-specific data on phytochemical profiles, livestock nutritionists and farmers are compelled to rely on generalized feeding standards or assumptions that may not reflect the actual functional properties of locally sourced Guinea grass. This not only limits the ability to strategically exploit beneficial phytochemical effects such as enhanced protein utilization, methane mitigation, and natural parasite suppression but also raises the risk of inadvertently exceeding safe inclusion thresholds that could impair feed intake, nutrient digestibility, or even animal health (Makkar, 2018).

Furthermore, in the context of climate-smart and residue-free livestock production, there is a growing imperative to leverage naturally occurring phytochemicals as alternatives to synthetic feed additives, antibiotics, and anthelmintics (Hoste *et al.,* 2015). However, such strategies are inherently dependent on accurate characterization of these compounds within locally available forages. The lack of integrated studies that concurrently quantify phytochemical concentrations and assess their potential nutritional and biofunctional implications underpins the necessity of this research.

By systematically evaluating and comparing the phytochemical composition of Guinea grass leaf meal collected from three distinct locations within Benin City, this study seeks to fill these critical knowledge gaps. The outcomes are anticipated to provide an empirical basis for formulating location-tailored feeding strategies that maximize the nutritional and functional potentials of Guinea grass while minimizing associated risks. This, in turn, supports more precise, sustainable, and cost-effective livestock production practices that align with both producer goals and consumer expectations for environmentally responsible and health-conscious animal agriculture.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Benin City, the capital of Edo State, located in the southern part of Nigeria. The city lies approximately on latitude 6.34°N and longitude 5.62°E. Benin City experiences a tropical rainforest climate with two distinct seasons: the rainy season (April to October) and the dry season (November to March). The annual rainfall ranges between 1,500 mm and 2,000 mm, and average temperatures range from 25°C to 32°C, (National Meteorological Department, 2023, p12). The three sampling locations were chosen within Benin City to capture potential variations in soil composition, microclimate, and environmental conditions that may influence phytochemical profiles. The locations selected were:

Location	**LGA**	**CODE**
Uniben	Egor	A
Ekosodin	Ovia North East	B
Ekuahen	Oredo	C

3.2 Collection of Plant Material

Fresh Guinea grass (*Megathyrsus maximus*) leaves were harvested from each of the three selected locations within Benin City, Edo State, Nigeria. Approximately 2 kg of fresh leaf material was collected per location from multiple plants to obtain a representative sample. Care was taken to ensure uniform maturity (pre-flowering stage) and time of harvest (between 7:00 and 9:00 AM) to minimize variability due to plant developmental stage or diurnal fluctuations in phytochemical content (Muir *et al.,* 2022). The samples were collected during the same week in July to control for seasonal variation.

3.3 Sample Preparation

Each sample was processed separately to avoid cross-contamination.

Washing and Air-Drying:

The harvested Guinea grass leaves were washed thoroughly with clean tap water to remove dust, epiphytic microorganisms, and other debris. The clean leaves were then air-dried at room temperature (25–28°C) for 7–10 days in a well-ventilated laboratory setting, away from direct sunlight to prevent the degradation of heat-sensitive and photosensitive phytochemicals such as flavonoids and certain alkaloids (Ajayi *et al.,* 2020).

Grinding and Sieving:

Once fully dried, the leaves were ground into a fine powder using a laboratory-grade mechanical grinder (Thomas-Wiley Mill Model 4, USA). The powdered samples were sieved using a 1 mm mesh sieve to ensure uniform particle size, which is critical for consistent solvent extraction and analytical precision. The homogenized samples were stored in airtight, labeled amber glass containers and kept in a cool, dark, and dry place until analysis to preserve phytochemical integrity (Skoczylas *et al.,* 2022).

3.4 Phytochemical Screening

The phytochemical composition of the Guinea grass leaf meal was determined using a combination of qualitative and quantitative methods, following standard procedures widely cited in contemporary phytochemical and forage science literature.

3.4.1 Qualitative Phytochemical Screening

The presence of major phytochemical groups was tested for each sample using standard chemical tests as adapted from established protocols (Onyeka and Chukwuma, 2021; Sofowora, 1993).

Phytochemical Test Method

Alkaloids Wagner's and Mayer's reagent test

Tannins Ferric chloride test

Saponins Froth test

Flavonoids Lead acetate test

Terpenoids Salkowski's test

Glycosides Keller-Killiani test

Phenols Ferric chloride test

Steroids Liebermann-Burchard test

The intensity of color formation or the volume of precipitate was used to semi-quantitatively estimate relative abundance (e.g., + = present, ++ = moderate, +++ = abundant).

3.4.2 Quantitative Phytochemical Analysis

The actual concentrations of selected phytochemicals were determined using spectrophotometric and gravimetric methods. All analyses were carried out in triplicate to ensure precision and reliability. The phytochemicals quantified included:

- Total Phenolic Content: Determined using the Folin-Ciocalteu method as described by Skoczylas *et al*. (2022). Gallic acid was used as the standard, and absorbance was measured at 765 nm. Results were expressed as mg Gallic Acid Equivalents (GAE) per g of dry weight.

- Total Flavonoid Content: Quantified using the aluminum chloride colorimetric assay according to Onyeka and Chukwuma (2021). Quercetin was used as the standard, and absorbance was measured at 510 nm. Results were expressed as mg Quercetin Equivalents (QE) per g of dry weight.

- Condensed Tannins: Estimated by the butanol-HCl method as outlined by Mueller-Harvey *et al.* (2019). Purified quebracho tannin was used as the standard, and absorbance was measured at 550 nm.

- Total Alkaloids: Determined using a gravimetric method following extraction with acidic ethanol (10% acetic acid in ethanol) and precipitation with concentrated ammonium hydroxide (Adejoro *et al.,* 2019).

- Total Saponins: Quantified using a gravimetric method based on Makkar (2018), which involves extraction with aqueous methanol and purification with diethyl ether.

- Phytates: Determined using a spectrophotometric method based on the reaction between phytate, ferric ions, and sulfosalicylic acid, measuring the decrease in absorbance of the iron-phytate complex at 500 nm (Oduguwa and Adebayo, 2019).

The absorbance for all spectrophotometric analyses was read using a UV-Visible spectrophotometer (Shimadzu, UV-1800, Japan), and concentrations were calculated using calibration curves prepared with relevant analytical-grade standards.

3.5 Data Analysis

Data from the quantitative phytochemical analyses were expressed as mean ± standard deviation (SD) of triplicate determinations. Differences in phytochemical composition among the three locations were analyzed using One-Way Analysis of Variance (ANOVA) at a significance level of $p < 0.05$. Where significant differences occurred, Tukey's Honest Significant Difference (HSD) post-hoc test was used to separate the means. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Version 25.0, IBM Corp., 2017).

3.6 Limitations of the Methodology

- While locations were selected for their perceived environmental differences, specific edaphic factors such as soil macro-nutrient composition, micronutrient content, and potential pollution levels were not directly analyzed. These unmeasured factors may be significant contributors to the observed variation in phytochemical profiles (Muir *et al.,* 2022).

- The study focused on a selected range of phytochemicals (phenolics, tannins, flavonoids, saponins, alkaloids, phytates). Other potentially beneficial bioactive compounds, such as specific terpenoids or cyanogenic glycosides, were not included in the analysis.

- Although air-drying at ambient temperature helps preserve some thermolabile compounds, it may not completely prevent the slow oxidative degradation of certain volatile or highly sensitive phytochemicals compared to freeze-drying (lyophilization).

CHAPTER FOUR

PHYTOCHEMICAL COMPOSITION OF GUINEA GRASS (*Megathyrsus maximus*) LEAVES FROM DIFFERENT LOCATIONS IN BENIN CITY

4.1 Phytochemical Composition of Guinea Grass Leaves from Different Locations

The phytochemical analysis of Guinea grass (*Megathyrsus maximus*) leaves collected from Ekehuan, University of Benin, and Ekosodin within Benin City is presented in Table 4.1. The compounds examined include alkaloids, saponins, tannins, and phenols. The results revealed significant ($p < 0.05$) differences in the concentrations of these bioactive constituents among the various sampling locations, suggesting that environmental and ecological conditions had a substantial impact on the synthesis and accumulation of secondary metabolites in the grass.

As shown in the table, Ekosodin samples exhibited the highest concentrations of all the measured phytochemicals, followed by those from the University of Benin, while Ekehuan had the lowest levels. Specifically, alkaloid content ranged from 0.403 mg/100 g in Ekehuan to 12.500 mg/100 g in Ekosodin. Saponin levels varied between 0.560–9.800 mg/100 g, while tannin and phenol contents ranged from 1.847–5.457 mg/100 g and 2.100–4.200 mg/100 g, respectively.

These variations may be attributed to differences in soil fertility, moisture availability, light intensity, and other microclimatic factors across the locations, which influence the biosynthesis of phytochemicals in plants. The findings align with previous studies showing that environmental variability significantly affects secondary metabolite concentration in tropical forage species (Oduguwa and Adebayo, 2019; Ajayi *et al.,* 2020).

Table 4.1: Phytochemical composition (mg/100 g DM) of Guinea grass leaves from different locations in Benin City

Parameter	Ekehuan	UNIBEN	Ekosodin	SEM
Alkaloid	0.403^{a}	0.640^{a}	12.500^{b}	0.205
Saponin	0.560^{a}	1.250^{b}	9.800^{c}	0.1065
Tannin	1.847^{a}	4.200^{b}	5.457^{c}	0.0557
Phenol	2.100^{a}	3.800^{b}	4.200^{c}	0.0289

Means with different superscripts (a, b, c) differ significantly ($p < 0.05$).

4.2 Alkaloid Content

Alkaloid concentration in M. maximus varied significantly ($p < 0.05$) across the three locations, ranging from 0.403 mg/100 g in Ekehuan to 12.500 mg/100 g in Ekosodin. The UNIBEN sample (0.640 mg/100 g) was statistically similar to Ekehuan but significantly lower than Ekosodin.

The higher alkaloid level in Ekosodin suggests that this site provides conditions favorable for alkaloid biosynthesis, likely due to factors such as soil nutrient profiles, moisture stress, or biotic interactions that stimulate defensive metabolic pathways (Wink, 2018; Onyeka and Chukwuma, 2021). Alkaloids protect plants against pathogens and herbivores, and their synthesis is often upregulated under environmental stress.

From a livestock perspective, while certain alkaloids possess antimicrobial properties, excessive quantities can cause antinutritional or toxic effects by interfering with neuro-muscular function and reducing feed intake (Adejoro *et al.,* 2019). Therefore, the high alkaloid content in Ekosodin grass necessitates caution in feed formulation to avoid potential toxicity.

4.3 Saponin Content

Saponin levels also differed significantly ($p < 0.05$), with concentrations ranging from 0.560 mg/100 g in Ekehuan to 9.800 mg/100 g in Ekosodin, and 1.250 mg/100 g at UNIBEN. The elevated saponin content in Ekosodin may reflect stress-induced synthesis in response to local environmental pressures (Muir *et al.,* 2022).

Saponins possess notable bioactive properties. At moderate levels, they can reduce rumen protozoal populations, enhance microbial protein synthesis, and lower enteric methane emissions, thereby promoting sustainable ruminant nutrition (Patra and Saxena, 2017; Benchaar *et al.,* 2015). However, excess saponins can depress feed intake or cause hemolytic effects (Makkar, 2018).

Hence, the high saponin level in Ekosodin grass may offer biofunctional benefits for methane mitigation but requires balanced inclusion in livestock diets to prevent adverse effects.

4.4 Tannin Content

Tannin concentrations ranged from 1.847 mg/100 g in Ekehuan to 5.457 mg/100 g in Ekosodin, with UNIBEN samples showing intermediate values (4.200 mg/100 g). The progressive increase from Ekehuan to Ekosodin suggests that environmental stress enhances tannin synthesis through the phenylpropanoid pathway (Skoczylas *et al.,* 2022).

Moderate tannin levels are beneficial in ruminant diets, forming reversible complexes with dietary proteins to reduce rumen degradation and improve post-ruminal amino acid absorption, while also offering antioxidant and anthelmintic effects (Mueller-Harvey *et al.,* 2019; Hoste *et al.,* 2015). Excessive levels, however, depress voluntary intake and nutrient digestibility by forming insoluble complexes.

The UNIBEN grass exhibited a concentration that may represent a favorable balance, whereas Ekosodin samples, though rich in tannins, may require processing (such as ensiling or the addition of binding agents like polyethylene glycol) before feeding to optimize their benefits.

4.5 Phenol Content

Phenol concentrations also differed significantly ($p < 0.05$), being highest in Ekosodin (4.200 mg/100 g), followed by UNIBEN (3.800 mg/100 g) and Ekehuan (2.100 mg/100 g). This trend is consistent with the general stress-response pattern observed for other phytochemicals, where conditions such as higher light intensity promote phenolic accumulation as part of a photoprotective mechanism (Vasta *et al.,* 2019).

Phenolic compounds are potent antioxidants that help mitigate oxidative stress in both the plant and the consuming animal. Their presence in forage can enhance animal health by supporting immune function and improving the oxidative stability of meat and milk (Vasta *et al.,* 2019). However, high concentrations can sometimes reduce protein digestibility.

Thus, Ekosodin grasses demonstrate strong antioxidant potential, while UNIBEN samples may offer an optimal balance between nutritive value and functional benefits.

4.6 Summary of Phytochemical Variations and Discussion Overview

Phytochemical profiles of *M. maximus* from the three locations in Benin City revealed significant ($p < 0.05$) locational differences. Ekosodin consistently recorded the highest levels of alkaloids, saponins, tannins, and phenols, followed by UNIBEN and Ekehuan. These variations strongly reflect the influence of environmental and edaphic factors, such as soil composition, microclimate, and potential biotic stress, on the plant's secondary metabolism (Muir *et al.,* 2022; Onyeka and Chukwuma, 2021).

From a livestock nutrition perspective, this gradation indicates that forages from different locations possess distinct functional properties. Moderate levels of these phytochemicals are advantageous: saponins can suppress rumen protozoa and reduce methane; tannins enhance protein utilization; and phenols improve antioxidant status (Patra, 2023; Mueller-Harvey *et al.,* 2019). However, the high concentrations found in Ekosodin samples could impair feed digestibility or pose toxicity risks if not managed carefully.

Therefore, location-specific utilization strategies are essential. Forages from Ekosodin may be strategically used for their bioactive properties but likely require processing or dilution before feeding. In contrast, grasses from Ekehuan and UNIBEN might be more suitable for direct use in high-nutrient diets where high palatability and intake are priorities.

This study provides a critical baseline understanding of the spatial variation in Guinea grass phytochemistry, supporting improved forage selection, precision feeding, and the development of sustainable livestock management practices in tropical systems.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary of Findings

This research investigated the phytochemical composition of Guinea grass (*Megathyrsus maximus*) leaves collected from three ecologically distinct locations within Benin City, Edo State namely Ekehuan (Eke), University of Benin (Uni), and Ekosodin (Eko) to evaluate how local environmental and edaphic variations affect the biosynthesis and accumulation of key secondary metabolites. The phytochemical constituents examined included alkaloids, saponins, tannins, and phenolic compounds, all of which play crucial roles in determining the nutritional, functional, and medicinal qualities of tropical forages.

The results revealed significant ($p < 0.05$) differences in the concentrations of these phytochemicals across the three sampling sites, highlighting the influence of microclimatic and agroecological factors on metabolite production in Guinea grass. Specifically, alkaloid concentrations ranged from 0.403 mg/100 g in Ekehuan to 12.500 mg/100 g in Ekosodin, showing more than a 30-fold difference between the lowest and highest sites. Saponin levels exhibited a similar pattern, increasing from 0.560 mg/100 g at Ekehuan to 9.800 mg/100 g at Ekosodin, while tannin and phenol contents varied from 1.847–5.457 mg/100 g and 2.100–4.200 mg/100 g, respectively.

Overall, the Ekosodin samples consistently recorded the highest concentrations of all phytochemical parameters, followed by the University of Benin, whereas Ekehuan presented the lowest levels. This pattern suggests that the unique ecological conditions of Ekosodin possibly including soil composition, microbial activity, and specific biotic or abiotic stressors favored the activation of metabolic pathways responsible for secondary metabolite synthesis. Environmental factors such as soil fertility, light intensity, and temperature fluctuations are known to regulate the expression of enzymes involved in phytochemical biosynthesis (Muir *et al.,* 2022; Skoczylas *et al.,* 2022). Thus, variations in these conditions across Benin City likely contributed to the distinct phytochemical signatures observed.

From a nutritional and functional standpoint, the findings have several important implications. Moderate concentrations of phytochemicals particularly tannins, saponins, and phenolic compounds can exert beneficial physiological effects in ruminant feeding systems. For example, tannins at optimal levels protect dietary proteins from excessive rumen degradation, thereby improving nitrogen utilization efficiency and amino acid absorption (Mueller-Harvey *et al.,* 2019). Saponins help reduce rumen protozoal populations, enhancing microbial protein synthesis and lowering enteric methane emissions (Patra and Saxena, 2017; Benchaar *et al.,* 2015), while phenolic compounds exhibit strong antioxidant properties that can enhance animal health and product stability (Vasta *et al.,* 2019). Additionally, low to moderate alkaloid levels may contribute to plant defence mechanisms, though their role in direct animal health benefits is more complex and requires careful management (Adejoro *et al.,* 2019).

However, the study also reaffirms that excessive accumulation of these metabolites may have antinutritional consequences, including reduced voluntary feed intake, lower digestibility, and in some cases, cytotoxic effects, especially when alkaloid or saponin levels surpass safe thresholds (Makkar, 2018). This underlines the importance of quantitative assessment and controlled dietary inclusion of Guinea grass leaf meal to balance the nutritional advantages against potential risks.

In essence, this research establishes that Guinea grass grown in different ecological zones within Benin City exhibits distinct phytochemical fingerprints that are reflective of localized environmental pressures and adaptive metabolic responses. The findings contribute valuable baseline data for the region and provide a foundation for developing location-specific feeding strategies that optimize the use of Guinea grass in sustainable livestock production systems. Such insights are essential for improving animal productivity, health, and product quality, while minimizing reliance on synthetic growth promoters and ensuring eco-friendly feed resource management (Patra, 2023).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study examined the phytochemical composition of Guinea grass (*Megathyrsus maximus*) leaves collected from three different locations within Benin City, Ekehuan, University of Benin, and Ekosodin to evaluate the extent to which local environmental variations influence the biosynthesis and accumulation of secondary metabolites. The findings revealed significant ($p < 0.05$) differences in the concentrations of major phytochemicals such as alkaloids, saponins, tannins, and phenolic compounds among the sampling sites.

Among the three locations, Ekosodin exhibited the highest levels of all analyzed phytochemicals, followed by the University of Benin, while Ekehuan recorded the lowest concentrations. This distinct variation suggests that microclimatic factors (such as temperature, humidity, and light intensity) and edaphic conditions (including soil fertility, organic matter content, and moisture availability) play a decisive role in regulating the metabolic pathways responsible for phytochemical synthesis in Guinea grass.

The results provide meaningful insights into the impact of environmental heterogeneity on forage bioactivity and quality. Moderate concentrations of phytochemicals particularly tannins and saponins are beneficial to livestock nutrition, as they can enhance protein utilization, improve rumen fermentation efficiency, and offer antioxidant and antimicrobial effects that promote animal health. Conversely, excessive accumulation of these compounds may lead to antinutritional consequences, such as reduced feed intake, lower digestibility, and potential interference with nutrient absorption.

From a practical perspective, the study establishes that Guinea grass from different ecological zones within Benin City displays unique phytochemical profiles. These biochemical distinctions should be carefully considered in livestock feed formulation and when exploring the grass as a natural source of bioactive compounds for use in animal nutrition or phytopharmaceutical applications.

In conclusion, the research provides a strong scientific foundation for location-based forage assessment, underscoring the importance of integrating environmental and management factors into forage utilization strategies to achieve sustainable livestock production and enhanced feed efficiency in tropical systems.

6.2 Recommendations

Based on the outcomes of this research, the following recommendations are proposed:

1. Adopt Site-Specific Forage Utilization Practices

Livestock farmers and feed formulators should take into account the geographical source of Guinea grass when determining its inclusion levels in feed rations. Forages harvested from nutrient-rich or stress-prone areas such as Ekosodin, which exhibit higher phytochemical concentrations, should be used in controlled proportions to prevent possible antinutritional or toxic effects on animals.

2. Incorporate Phytochemical Profiling into Forage Evaluation Programs

Routine phytochemical screening should be included as part of forage quality assessment and monitoring systems. This will help ensure that livestock receive feeds that are both nutritionally balanced and biologically safe, preventing negative impacts associated with excessive secondary metabolites.

3. Conduct Further Studies on Seasonal Variation

Future research should investigate how seasonal changes particularly between the wet and dry seasons affect phytochemical accumulation in Guinea grass. Such studies will help determine the optimal harvesting period that maximizes both nutritive value and bioactive potential of the forage.

4. Integrate Phytochemical, Proximate and Mineral Analyses

For a more comprehensive understanding of forage quality, future studies should combine phytochemical evaluation with proximate composition (crude protein, fiber, lipid, ash) and mineral profiling. This integrative approach will provide a holistic assessment of Guinea grass's nutritional and functional properties for livestock feeding.

5. Promote Utilization in Sustainable Livestock Systems

Given its moderate phytochemical content, Guinea grass has the potential to serve as a natural alternative to synthetic feed additives. Farmers and feed producers are encouraged to explore its use for enhancing animal productivity, reducing methane emissions, and promoting eco-friendly and sustainable livestock production systems.

6. Establish a Regional Forage Database

Collaboration between government agencies, research institutions, and universities should be encouraged to develop a regional database documenting the phytochemical and nutrient composition of commonly used tropical forages. Such a database would guide feed formulation, policy development, and forage management strategies across Nigeria and similar agroecological regions.

7. Enhance Soil and Environmental Management Practices

Since soil fertility, moisture, and organic matter strongly influence phytochemical synthesis, farmers should adopt improved soil amendment, organic enrichment, and irrigation practices to optimize both the yield and quality of Guinea grass. This will ensure consistent forage productivity and improved livestock performance.

This study has conclusively demonstrated that environmental location and microecological variability exert a significant influence on the phytochemical composition of Guinea grass (*Megathyrsus maximus*) cultivated within Benin City. The variations observed in the concentrations of alkaloids, saponins, tannins, and phenolic compounds across the three study sites clearly highlight the dynamic relationship between plant metabolism and environmental factors such as soil fertility, moisture availability, and microclimatic conditions. By providing empirical evidence on how site-specific conditions shape the accumulation of bioactive compounds, this research contributes meaningfully to the scientific understanding of forage chemistry and its implications for livestock nutrition. The findings not only establish a baseline for local phytochemical profiling but also emphasize the importance of integrating environmental management practices with forage production and feeding strategies.

Furthermore, this study supports the global drive toward sustainable and climate-smart livestock systems, where natural feed resources like Guinea grass can serve as functional alternatives to synthetic feed additives. By recognizing and managing the biochemical diversity of forages, animal nutritionists and farmers can achieve improved productivity, animal health, and environmental sustainability. In essence, this research bridges the gap between plant biochemistry, environmental ecology, and animal nutrition, offering valuable insights for future studies, forage agronomists, and policymakers interested in advancing the frontiers of sustainable forage utilization and precision livestock feeding.

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