BIOMASS YIELD, NUTRITIONAL VALUE, AND *IN VITRO* RUMINAL FERMENTATION KINETICS OF GUATEMALA GRASS (*Tripsacum laxum*) AT DIFFERENT HARVESTING STAGES AND AGRO-ECOLOGIES IN SOUTHERN ETHIOPIA

BY:

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JIMMA ETHIOPIA

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MSc. Thesis

Submitted to Department of Animal Sciences, School of Graduates Students, College of Agriculture and Veterinary Medicine, Jimma University, In Partial Fulfillment of the Requirements for the Degree of Master of Science in Animal Production

Major Advisor: Metekia Tamiru (PhD candidate, MSc, Lecturer)

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I have completed my thesis work as per the approved proposal and it has been evaluated and accepted by my advisors. Hence, I hereby kindly request that the department allow me to present the findings of my work and submit my thesis.

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We, the thesis advisors, have evaluated the content of this thesis and found it to be satisfactory, executed according to the approved proposal, written according to the standard and format of the university, and ready to be submitted. Hence, we recommend the thesis to be submitted.

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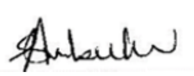
Co- Advisor: -Dr. Jane Wamatu (PhD)  01/12/2023

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DEDICATION

I dedicate this thesis to my beloved parents, my father Abarijal Aba jebel and my mother Karida Abamecha, who devoted their time and resources to support and strengthen me, guiding me towards success in my life.

STATEMENT OF THE AUTHOR

I hereby declare that this Thesis is the result of my genuine work, and I have duly acknowledged all sources used writing it. This Thesis has been submitted in partial Fulfillment of MSc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to users in accordance with the library's regulations. I solemnly declare that this Thesis is not submitted to any other institution for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis may be used without special permission, if proper acknowledgement is given to the source. Permission to quote extensively from or reproduce any part of this thesis, either partially or in its entirety can be granted by the head of the major department or the dean of the graduate school, provided they deem the intended use of the material to be for scholarly purposes. For all other purposes, authorization must be obtained directly from the author.

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BIOGRAPHICAL SKETCH

The author was born to his mother, Karida Abamecha, and father, Abarijal Aba jebel, in Kersa district, Jimma zone of Oromia Regional State, Ethiopia, on September 3, 1989. He attended his elementary and junior school education in Serbo and completed his high school studies at Serbo High School and Jimma Preparatory School, respectively. After successfully passing the Ethiopian Higher Education Entrance Examination, he joined Madawalabu University in 2009 and graduated with a B.Sc. degree in Animal and Range Sciences in July 2011. In October 2011, he was employed at the Kersa District of Agriculture office and served as an animal nutrition expert and team leader. Later, in September 2021, he enrolled at Jimma University, College of Agriculture and Veterinary Medicine, Department of Animal Science for the MSc study program in animal production.

LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
CP	Crude Protein
CSA	Central Statistical Agency
DM	Dry Matter
DMY	Dry Matter Yield
FAO	Food and Agricultural Organization
GLM	Generalized Linear Model
ICARDA	International Center for Agricultural Research in the Dry Areas
ILRI	International Livestock Research Institute
INL	Inter-Node Length
IVOMD	*In-vitro* Organic Matter Digestibility
ME	Metabolizable Energy
NDF	Neutral Detergent Fiber
NLPP	Number of Leaf per Plant
NNPP	Number of Node per Plant
NTPP	Number of Tiller per Plant
PH	Plant Height
RCBD	Randomized Complete Block Design

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Biomass yield, nutritional value, and *in vitro* ruminal fermentation kinetics of Guatemala grass (*Tripsacum laxum*) at different harvesting stages and agro-ecologies in southern Ethiopia

ABSTRACT

The study was conducted to evaluate the effect of agro-ecologies and harvesting date on morphological traits, biomass yield, chemical composition, in vitro dry matter digestibility and in vitro ruminal fermentation kinetics of the Guatemala grass in southern Ethiopia. The experiment was laid out in a factorial arrangement of two agro-ecologies and three harvesting dates in a randomized complete block design with three replications. Data collection included morphological parameters, dry matter yield, chemical composition, and in vitro gas production. Data was analyzed using GLM procedure of SAS (SAS, 2014, Ver. 9.3). The results indicated that harvesting stage significantly ($p<0.05$) influenced morphological parameters, while plant height, Number of nodes per plant and dry matter yield were tended to be affected by variations in agro-ecologies. Guatemala grass harvested from mid agro-ecologies and early harvesting stage were significantly higher ($P<0.05$) in CP, IVOMD and ME. The proximate composition, nutritive value, IVOMD and gas production potential of forage grass showed great variability across different agro-ecologies and harvesting stages. During the incubation period, from 3 hours to 24 hours, gas production was notably higher ($p<0.05$) at grass harvested from lower agro-ecologies. There was a strong and negative correlation between the CP content and NDF ($r= -0.415$), ADF ($r= -0.170$), and ADL ($r= -0.559$), while a strong positive correlation was observed between CP content and both IVOMD($r=0.802$) and ME($r=0.710$). Methane exhibited a strong positive correlation ($r =517$) with total gas production during the in vitro microbial fermentation process. Based on these findings, Guatemala grass has potential as an alternative ruminant feed in mid and lowland agro-ecologies in southern Ethiopia. However, this study was limited to one sampling period; therefore, further research is needed to explore the changes in chemical composition and nutritional values across different seasons.

Keywords: Guatemala grass, *in vitro* gas production, methane, nutritional value.

1. INTRODUCTION

1.1 Background and Justification

This diversity showcases a significant potential in livestock genetic resources. The array of livestock products and by-products, including meat, milk, honey, eggs, cheese, and butter, play a crucial role in enhancing the nutritional well-being of the population by providing essential animal proteins Ethiopia is home to Africa's largest animal population, with 70.29 million cattle and 42.9 million sheep, 52.5 million goats, 57 million chickens, 8.1 million camels, 6.9 million bee colonies and 12.9 million equines and has high potential in livestock genetic resources (CSA, 2022). The livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply provide the needed animal protein that contributes to the improvement of the nutritional status of the people.

Livestock plays an important role in providing export commodities, such as live animals, hides, and skins thereby earning foreign exchange for the country. This is due to the surplus output and preference of the breeds by the Middle East Countries (CSA, 2022). However, the productivity of livestock in Ethiopia lags behind the African average, primarily due to inadequate supply of feed and suboptimal feeding practices (Asresie *et al*., 2015; Mekonnen *et al.*, 2022). The productivity of this sector is constrained by several factors, including poor genetics, low reproductive performance, poor quality and varying seasonal availability of feed, frequent disease incidence and parasite challenge, along with limited access to services and inputs (Adnew *et al*., 2019).

The primary sources of livestock feed in Ethiopia are mainly green fodder, crop residues with smaller contributions from improved pasture, forage crops, and agro-industrial by-products (CSA, 2022). However, these major feed resources are inherently low in their nutritional value which cannot even meet basic maintenance requirements of livestock (Gunders and Bloom, 2017). Natural grazing plays a significant role as a feed source for livestock in Ethiopia. Presently, grazing lands are overgrazed and degraded, leading to reduced biomass, a lack of variety in forage species, and low nutritional quality. These feed resources are deficient in crude protein (CP), vitamins, and metabolizable energy (ME). The grazing land is constantly being converted to cropping land in response to the food demand of the human population. Therefore, it is crucial to improve the existing grazing land by introducing improved grasses and studying their agronomic performance and chemical composition. Planting improved grass can enhance marginal lands; improve carbon sequestration to mitigate climate change and increase the value of natural assets and system reliance (Kane and Solutions, 2015). Additionally, tackling the problem of feed scarcity can be addressed through identification, evaluation, and promotion of forage species with better forage yield and nutritive value, that are also adapted to drought and low soil fertility (Mengistu *et al.,* 2017). According to Getnet (2012), the integration of improved forage crops in agricultural systems has many advantages, including soil conservation, reduction of weeds, pests, and diseases, in addition to their primary use as high-quality animal feeds.

One of the candidate forages that can help alleviate ruminant feed shortages and thereby enable the country to exploit livestock resource potential is *Tripsacum laxum*, also known as Guatemala grass. It is a perennial fodder grass that is commonly grown in large parts of Africa as a source of livestock feed (Munyasi, 2015). Guatemala grass originated from Mexico and South America and has been introduced for fodder in many tropical countries. The main attributes of Guatemala grass include its high productivity under favorable conditions. The ready availability of its planting material makes it a good candidate for forage cultivation. The average DM yield is about 18- 22 t/ha/year (Gong *et al*., 2022; Zahui *et al.,* 2021).

Guatemala grass also provides several environmental benefits, particularly preventing soil erosion (Tahir *et al*., 2018). The species is relatively good in nutritional value, with a protein content of about 10% and low fiber content (average NDF < 70%). However, it is low in DM (average 22%), which tends to increase over time, inversely affecting its nutritional value. A notable aspect of Guatemala grass is its capacity to stay leafy even at advanced stages of growth. The performance of forage species varies across locations due to differences in soil types, temperature and amount and distribution of rainfall. Testing the adaptability and yield potential of different forage crops across various agro-ecological zones is very important to identify the best-bet accessions for utilization (Kebede *et al*., 2017).

In southern Ethiopia, Guatemala grass is one of the early introduced high yielding forage species to the agro forestry systems and it has high biomass production, making it a valuable source of feed for livestock (Andualem & Hundessa, 2022). However, little is known about its adaptability related to growth features, forage productivity and forage quality. Knowing information on morphological parameters such as plant height, number of leaves, leaf length, and number of tillers, days to maturity, growth habit, and production potential of the grass are important to integrate this grass in animal feed system of the country. Therefore, with the above backgrounds in mind, the proposed study is conceived with the following general and specific objectives.

1.2 Objective

1.2.1 General objective

- The general objective of the study was to evaluate the morphological traits, biomass yield, nutritional value, *in vitro* dry matter digestibility and in vitro ruminal fermentation kinetics of Guatemala grass under different agro-ecologies and harvesting stage to integrate this grass in animal feed system of the southern Ethiopia.

1.2.2 Specific objectives

The specific objectives of this study were:

- To determine the effect of agro-ecologies on morphological traits, biomass yield, chemical composition, *in vitro* organic matter digestibility and *in vitro* ruminal fermentation kinetics of the Guatemala grass.

- To evaluate the effect of harvesting stage on morphological traits, biomass yield, chemical composition, *in vitro* organic matter digestibility and *in vitro* ruminal fermentation kinetics of the Guatemala grass.

- To evaluate the interaction effect of agro-ecologies and harvesting stage on morphological traits, biomass yield, chemical composition, *in vitro* organic matter digestibility and *in vitro* ruminal fermentation kinetics of the Guatemala grass.

2. LITERATURE REVIEW

2.1 Overview of Feed Resources in Ethiopia

One of the key factors affecting livestock productivity is the availability of sufficient and high-quality feed resources (Duguma and Janssens, 2021). Wondatir, (2010) claims that one of the main causes of Ethiopia's low productivity is a lack of both sufficient and high-quality livestock feeds, particularly during the dry season. In Ethiopia, natural grazing land is the main source of feed for animals. Green fodder (grazing) is the primary form of animal nutrition in rural areas of the nation, followed by agricultural residue, hay, and by-products (CSA, 2022). However, due to decreasing grazing field size and the use of native hay's constrained coverage, the function of natural pasture is occasionally waning (Lemlem and Tesfay, 2010). There is not much land.

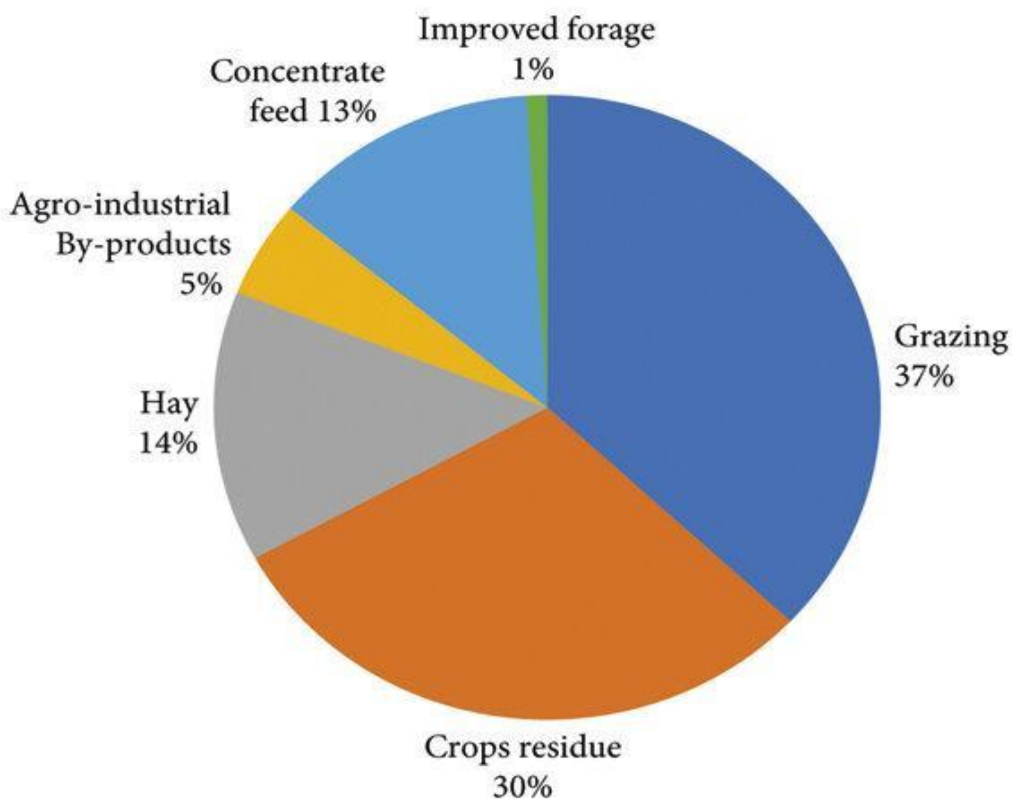

Figure 1. Proportion of animal feed resources in Ethiopia (Source: CSA 2021)

2.1.1 Natural pasture

Most of the animal feed is derived from natural pasture, which is overgrazed and made up of local forage species. In developing nations, natural pasture is a significant source of ruminant cattle feed (Fuglie *et al.*, 2021). On permanent areas, fallow land, and after-harvest land, grazing takes place. Following crop harvest, both fallow ground and crop stubble offer subpar grazing for a very brief time. The amount and standard of natural pasture vary according to altitude, precipitation, soil type, and crop intensity. The pastoral zones with increased rainfall are distinguished by low carrying capacity and extensive stands of thorny vegetation. Continuous grazing and rotational grazing are the two fundamental kinds of grazing systems (Lemlem and Tesfay, 2010).

In Ethiopia recently, over 54.23 percent of most of the animal feed is derived from natural pasture, which is overgrazed and made up of local forage species. In developing nations, natural pasture is a significant source of ruminant cattle feed (Fuglie *et al.,* 2021). On permanent areas, fallow land, and after-harvest land, grazing takes place. Following crop harvest, both fallow ground and crop stubble offer subpar grazing for a very brief time. The amount and standard of natural pasture vary according to altitude, precipitation, soil type, and crop intensity. The pastoral zones with increased rainfall are distinguished by low carrying capacity and extensive stands of thorny vegetation. Continuous grazing and rotational grazing are the two fundamental kinds of grazing systems (Lemlem and Tesfay, 2010).

2.1.2 Crop residues

The potential and abundance of crop wastes that might, in most situations, be used to feed cattle in Ethiopia. About half of the total feed source for ruminant livestock is provided by cereal crop wastes in the highland mixed crop-livestock farming systems in the Ethiopian highlands. During the dry seasons of the year, agricultural leftovers can contribute up to 80% of the total (Tolera and Abebe, 2007). On subsistence farm holdings, a wide range of arable crops are grown, and many of these crops have residues that, once grain is harvested, can be a significant source of livestock feed. grain yield equivalent to 13.7 million tons using a multiplier (13.6 million tons in rural areas and 136 thousand tons in urban areas) from cereals having CP values ranging from 3.1 - 6.7% with a digestibility level of about 40.7-54.1%. They are suited for all classes of livestock in the country according to their nutritional characteristics. Stover is the leaves and stalks of corn (maize), sorghum, or soybean plants that are left in a field after harvest. It can be directly grazed by cattle or dried for use as fodder. Stover has attracted some attention as a potential fuel source and as biomass for fermentation or as a feedstock for cellulosic ethanol production (Lemlem and Tesfay, 2010).

Cereal straw from teff, barley, and wheat is the largest component of livestock diet in the intermediate and highland areas that are not obtained in situ. Straw is stacked after threshing and fed to animals during the dry season, as are pulse-crop residues (e.g., horse bean, chickpeas, haricot beans, field peas, and lentils). At lower altitudes in the highland areas' maize, sorghum, and millet stovers occur to a greater extent than at higher altitudes. Teff is grown at intermediate altitudes and barley replaces wheat at higher altitudes, where pulses are also primarily also grown. The nutritive values of the different residues vary. Whereas teff straw is equivalent to medium-quality hay, the residue of other cereal crops is only of poor to fair quality. On the other hand, pulse haulms are high-quality roughage with 5-8% protein (Mengistu *et al*., 2017). Crop residues are generally characterized by low crude protein content but high cell wall and cell wall constituents (Bediye *et al.,* 2007).

Crop residues are characterized by high fiber content and low digestibility and feed intake (Wondatir, 2010). The CP content of crop residues ranges from 2.4- 7% and the value of IVDMD for straw is between 34 and 52%. However, the nutritional values of crop residues vary according to the type of crop used. Residues from leguminous crops are of better quality than the residues from cereals. Legume straws contain less fiber, and higher digestible protein than cereal straws (Bogale, 2004). Crop residue is used for different purposes in Ethiopia for instance (Tsigie *et al.*, 2011). Bediye *et al*., (2007) reported that crop residues are used for feed, fuel, construction, and bedding purposes. The combinations of these crop residues tradeoffs will in general reduce the contributions of crop residues to livestock feed resources (Tsigie *et al.,* 2011). Hence, there is a need to look for other alternative feed resources as complementary feeds to crop residues.

2.1.3 Agro-industrial by-products

Topical feed resources in the country are crop residues and natural pasture, with agro-industrial by-products and manufactured feed contributing much less (Gebremedhin, 2009). Agro-industrial by-products that were once classified as waste are now considered valuable livestock feeds, he current trends of increasing urban population have a significant effect on the establishment of agro-industries due to the corresponding increasing demand for edible main products (Lemlem and Tesfay, 2010). Agro-industrial by-products have special value in feeding livestock, mainly in urban and peri-urban livestock production situations where the productive potential of the animal is relatively high and requires a high nutrient supply. Agro-industrial by-products are mainly used for dairy, fattening, and commercial poultry production, and the scope for their wider use by smallholder producers is low due to availability and price (Tegegne *et al.*, 2013). Natural pasture and crop residues are naturally of low quality and do not fulfill the nutrient requirements of animals. Hence, high-producing animals such as dairy cattle and fattening animals should be supplemented with high-energy and/or protein concentrates (Kobayashi *et al.,* 2021). In Ethiopia, the major agro-industrial by-products commonly used for animal feeds are obtained from flour milling industries (wheat bran, wheat short, wheat middling, and rice bran), edible oil extracting plants (Noug cake, cottonseed cake, peanut cake, linseed cake, sesame cake, sunflower cake, etc.), breweries and sugar factories (Molasses).

2.1.4 Improved forages

Mengistu *et al*. (2017) noted that improved multipurpose forage crops and trees, such as Sesbania spp., *Leucaena leucocephala*, Calliandra spp., and *Chamaecytisus palmensis*, were introduced, made more well-known, and used in Ethiopia's mixed crop-livestock system starting in the 1970s to supplement the nation's abundant roughage feed resources. Even though the demand for feed may increase in these conditions, the adoption of forage is hampered by competition with food crops, particularly as farmers are often reluctant to forsake food. It complements crop production by maintaining soil fertility through nitrogen fixation. While grazing depletes the fertility of the land, forage growing improves soil health. Improved fodder species generally have higher herbage yield potential than natural pasture about 13, 8, and 10.5 t/ha DM yield for grasses, herbaceous legumes, and browse trees, respectively, while the average herbage DM yield obtained from seasonally rested pasture and continuously grazed pasture was 4 and 1 t/ha, respectively (Mengistu *et al.,* 2016).

According to Feyissa et al., (2015), the overall average productivity of the improved fodder crops per unit area has been found to exceed the productivities of seasonally rested and continuously grazed natural pastures by about 3-fold and 10-fold, respectively. The use of improved forages would reduce pressure on natural pasture, improve soil fertility and erosion of marginal lands, and improve carbon sequestration to mitigate climate change, support the system substantially, and enhance natural assets and system reliance (Tekalign, 2014). They also have a long growing season and help extend the green feed period to provide useful nutrients, mainly in rural areas where the availability and accessibility of agro-industrial by-products are limited. Moreover, improved fodder crops, especially those that implement crop production, maintain soil fertility via fixation and accumulation of nitrogen, helping to prevent soil erosion and replenishing degraded land when integrated into natural resource management schemes (Mengistu *et al.*, 2016). In most parts of the country, introduced fodder trees (Leucaena, Sesbania sp. tree Lucerne) and grasses like Napier grass, Oat, Rhodes grass, desho grass, etc., legumes like Desmodium, Vetch, and sweet lupine are on the way of production, These forages had been used mostly within the soil-erosion control program, irrigation canals, around farmers' homesteads and in small amount in the crop cultivated lands (Bediye *et al.*, 2007)

2.1.5. Non-conventional feeds

Vegetable rejects, sugar cane leaves, enset leaves, fish offal, and other non-conventional feed types are examples of conventional feed. Yeshitila Ademasu (2008) also noted non-traditional meals, such as the leftovers of regional beverages including coffee, areke, tela, chat called geraba, and fruits and vegetables that were rejected. In Ethiopia, non-conventional feed contributes 1.85 percent of the total feed for livestock, according to CSA (2022). Animals in the southwest Shewa zone were fed unconventional foods such as abish (*Trigonela foenum gracium*), tobacco, and mineral soils (ESAP, 2009). Due to incorrect collection, the contribution of non-traditional feeds to livestock is quite low.

2.2. Role of Grasses in Livestock Production

Grass is a common word that generally describes a monocotyledonous green plant in the family Poaceae (Kellogg, 2015). It occupies a greater area of the world’s surface than any other plant family, occurs in almost every terrestrial environment and provides a vital source of food for humans and animals (Tillich, 2007). They provide energy and nutrients for animal growth and maintenance. Their leaves are more palatable than stems and re-growths more nutritious than old tissues. Forage grasses can be either annual or perennials with a wide spectrum of adaptation and diverse growth habits and thus they are distributed in all continents and climatic zones. Herrera (2004) argued that pasture turns out to be an appropriate source of food for ruminants, mainly in countries with tropical climates. This is due to the high number of species that can be used, the possibility of cultivating them throughout the year, capacity of ruminant using fibrous foods, does not compete as food for humans and tends to be a cheap economical source. Grasses are more easily accessible, better in taste and quicker in digestion than shrubs and trees. During the rains, with proper stocking rate, the pasture will be more than cattle require; excess pasture can be conserved in the form of hay and grass silage.

The productivity of the different grass species could be distinctly different and is also influenced by area of origin, including temperature, light intensity, total rainfall, soil type, fertilization level, and by stage of maturity (Wassie *et al.,* 2018). Nutritive quality of forage has been defined as the product of the voluntary intake, digestibility and efficiency of nutrients that are used by the animal. Tropical grasses drastically reduce in their nutritive value with an advance in harvesting. Cutting at proper growth stage is a crucial management practice to determine herbage dry matter yield (DMY), crude protein (CP), *in vitro* dry matter digestibility (IVDMD) and other constituents (Van Soest, 1994).

2.2.1 General description of Guatemala grass (*Tripsacum laxum*)

Guatemala grass (*Tripsacum laxum* Scrib and Merr) is a robust, strongly rhizomatous, tufted, and leafy perennial grass that can form large bunches. The stems can grow up to 3.5-4.5 m high and 1-5 cm (about 1.97 in) in diameter. The plant remains leafy for a long time and stems and stems develop at a very late stage. The roots are shallow, and the plant does not grow well during a long dry season. As the grass matures, the roots become stronger and store nutrients that are necessary for re-growth after cuttings (Cook *et al.*, 2005). The leaves are tall (0.4-1.2 m long x 9 cm broad), glabrous or sparsely hairy and the inflorescences are sub digitate with 3 to 8 slender, elongated racemes (up to 20 cm long) containing male and female spikelets (3-5 mm long). *Tripsacum laxum* originated from Mexico and South America and has been introduced as fodder species in many tropical areas (FAO, 2004).

It grows from sea level up to 180 m above sea level at temperatures ranging from 18°C to 30°C. It does better under good soil moisture but can withstand neither short droughts while it can neither bear waterlogging nor flooding (Cook *et al*., 2005). However, it can grow on a wide range of soil (including podsols, Ultisols, Oxisols, peats, acid sulfate soils, and very acid coastal marine sands) and withstand low pH provided the soils are well-drained. *Tripsacum laxum* is usually propagated by stem cuttings or rooted splits at the beginning of the rainy season (Cook *et al.*, 2005). It can be planted with fast-growing twinning or shrub legumes. The average DM yield is about 18-22 t/ha/year (Cook *et al*., 2005; Nivyobizi *et al*., 2010). The species is relatively good in nutritional value, with a protein content of about 10% and low fiber content (average NDF < 70%). It is also low in DM (average 22%), which increases over time while the nutritive value decreases. An important feature of *Tripsacum laxum* is its ability to remain leafy at a very late stage of development (Vargas-Rodríguez and Rutt, 2009).

2.2.2 Benefits of Guatemalan grass

Guatemalan grass is a perennial fodder grass that belongs to the genus Tripsacum in Gramineae (http://www.iplant.cn/info/Tripsacum). This species is commonly grown in large parts of Africa as a source of livestock feed (Luo *et al*., 2021). Guatemala grass with a good adaptation can be tolerant to drought and acid, which is widely introduced as forage in other tropical regions of the world (Paul *et al*., 2016). Guatemala grass is cultivated primarily for fodder in cut-and-carry systems. It can also be used to make silage. Guatemala grass provides several environmental benefits, notably against soil erosion and the development of pests and diseases in neighboring crops (Cook *et al*., 2005). Guatemala grass helps to control weeds, which, in turn, results in a reduction in nematode infestation. For instance, it is used in tea plantations for rejuvenating soils during fallow and preventing Meloidogyne nematode infestations. In Burundi, Guatemala grass has been used to reduce bacterial wilt in potato plantations (Cook *et al.,* 2005). Guatemala grass can be used as mulch to improve soil (Cook *et al*., 2005). In tea plantations, Guatemala grass mulch increased 3-year-old tea yields by 10%. Though contour stripping with Guatemala grass has many advantages, it is reported to harbor more rodents than other grasses, causing a potential health hazard (plague) (Kamugisha, 2007).

2.2.3 Distribution of Guatemala grass

Guatemala grass originated in Mexico and South America and has been introduced for fodder in many tropical countries. It is a warm season grass that grows from sea level up to an altitude of 1800 m, at temperatures ranging from 18 to 30°C. It does better under good soil moisture but can withstand short droughts. It is intolerant of waterlogging and flooding. It can grow on a wide range of soils (including podsols, Ultisols, Oxisols, peats, acid sulfate soils, and very acidic coastal marine sands) and withstand low pH and the presence of Al, provided the soils are well-drained (FAO, 2012; Cook *et al*., 2005).

2.2.4 Forage management

Guatemala grass is usually propagated from stem cuttings or rooted culms at the beginning of the rainy season. The first cut can be done 4 to 6 months after planting. Guatemala grass can be planted with fast growing twinning or shrub legumes such as *Desmodium intortum*, *Desmodium uncinatum, Calliandra calothyrsus, Leucaena leucocephala, Leucocephala diversiflora, and Sesbania sesban* (Cook *et al*., 2005). *The association* of Guatemala grass with *Leucaena diversifolia* or *Calliandra calothyrsus* produces as much DM as Guatemala grass alone and improves the production of digestible protein (Akyeampong *et al*., 1996). The average DM yield is about 18-22 t/ha/year (Cook *et al.,* 2005). In the eastern highlands of Africa, yields ranging from 9 to 50 t DM were recorded (Nivyobizi et al., 2010). Since most of the biomass is produced during the wet season, it is generally recommended to use Guatemala grass in cut-and-carry systems (Wandera, 1997). It can also be stored as silage for dry season supply (Sarwatt *et al*., 1992).

Smallholder dairy producers in the highland areas of East Africa (Kenya and Tanzania) have been encouraged since the 1970s to grow Guatemala grass as a high-yielding fodder (Myoya *et al.,* 1988; Boonman, 1993). However, such initiatives have met with a mixed reception: in Kenya, when compared to other forage species evaluated for herbage dry matter yields and farmer acceptance, Guatemala grass was ranked lowest, mainly because of foliar diseases and poor regeneration after defoliation (Muyekho *et al*., 2003).

2.2.4.1 Cut-and-carry system

When harvested for direct feeding in cut-and-carry systems, Guatemala grass should be cut when it reaches 100-120 cm high and not closer than 10-25 cm from the ground. Guatemala grass does not withstand heavy grazing or frequent cutting. Cutting frequency should be about every 30 days during the wet season and every 42-45 days during the dry season. It has higher persistence than elephant grass (*Pennisetum purpureum*) but lower yield and nutritive value (Cook *et al.*, 2005). It is sometimes recommended to let Guatemala grass completely established by not harvesting in the first year after planting. This encourages the perennial nature of Guatemala grass and results in healthier regrowth (Vargas-Rodriguez, 2009).

2.2.4.2 Silage

When Guatemala grass is intended for silage, it is recommended that the regrowth (after first cut for direct feeding) reach 0.8-1 m high but is still in the vegetative state (8-week). Ensiling Guatemala grass results in 12% DM losses during the process (FAO, 2012). Guatemala grass has relatively high moisture content (69%) and a relatively low content in water soluble carbohydrates (3–9% DM) (Sarwatt *et al*., 1992), which can result, as in most tropical grasses, in ineffective pH reduction during ensiling and low storage stability (Webster *et al*., 1980). It is thus recommended to wilt the Guatemala grass before ensiling in order to increase dry matter and the content of water-soluble carbohydrates and to reduce nutrient losses through lixiviation (McDonald *et al*., 1991; Humphreys, 1991; Nussio, 2005). Wilting combined with chopping may increase the availability of water-soluble carbohydrates to the fermenting micro-organisms, increase crude protein content and slightly decrease NDF and ADF contents (Lavezzo *et al*., 1989).

2.3 Effect of Harvesting Stage and Altitude on Morphological Characteristics

2.3.1 Plant height

Plant height is an important parameter contributing to biomass yield in forage crops. Plant height of forage maize at harvest was affected by different spatial arrangements of plants. Plants attained maximum height before the end of the vegetative phase of growth. Mean plant height was low in the early stage of growth, but for harvesting after 120 days, enhanced growth was observed in Desho grass (Asmare *et al*., 2017). According to Adnew *et al*., (2019) study there is a significant effect on mean plant heights for the Brachiaria grasses generally increased and were significantly different throughout the growth period. According to Kanak *et al.,* (2013), there is no significant effect on plant height was observed among Para, germen, and dhal grasses at different harvestings.

2.3.2 Number of tillers per plant

According to Asmare *et al.,* (2017) both harvesting age and plant spacing had significant effects on tiller numbers. The mean tiller number per plant increased from 36.4 at 75 days of growth to 106.4 at 135 days, while corresponding numbers for different plant spacings were 75.3tillers/plant at 10 cm and 83.9tillers/plant at 50 cm in Desho grass. The number of tillers per plant in the studied grasses was significantly increased in all altitudes about the advance in harvesting dates of plants because of the development of new shoots bearing on each plant, which resulted in a greater number of tillers as the plant matured (Adnew *et al.,* 2019). According to Tilahun *et al.,* (2017), the number of tillers per plant increased as plant spacing increased. An individual plant tends to produce more tillers to fill the available space when plant stands are thinner or uneven. Thus, lower densities of Gama grass (*Tripsacum* dactyloides) per unit area had a lower number of tillers compared to higher plant density. Taye Bayable (2004) indicated that plants harvested at 120 days produced higher numbers of tillers per plant as compared to the numbers of tillers per plant harvested at 60 days.

2.3.3 Number of leaves per plant

The number of leaves (NLPP) increased with an increase in harvesting day, and fewer total leaves per plant were produced from short harvesting intervals of grass due to cutting at the younger stage of growth, while leaf length per plant was highest at reduced harvesting intervals (Butt *et al.,* 1993). Leaf length (LLPP) in grasses plays an essential role in shaping the physical structure of the grass canopy and consequently in competition for light within the sword (Adnew et al., 2019). According to Tilahun *et al.* (2017), leaf number per plant, which, in part, determines the photosynthetic capacity of the plants, was significantly affected by harvesting age, while plant spacing had no effect. The leaf length of the Brachiaria cultivars was significantly affected by harvesting stages (Adnew *et al.*, 2019). Ghiwot (2019), the length of leaf per plant (43.96 cm) observed at 60 days of harvesting was significantly lower as compared with 120 days in Buffel Grass (Cenchrus ciliaris Linn). According to Adnew *et al.*, (2019), a study on Brachiaria hybrid Mulato II found that the maximum number of leaves (8.33) and the maximum length of leaves (34.30cm) were absorbed in the late (120 days) harvesting stages. Mamila *et al*. (2020) reported that Para grass (*Brachiaria mutica*) grass harvested at 45 and 30cm plant spacing from late harvesting (120 days) recorded the longest LLPP (26.7 cm and 26.5 cm) and the highest NLPP recorded from wider and intermediate plant spacing at late harvesting age (1280.7 and 1142.1 leaves, respectively).

2.3.4 Number and length of internodes

The effects of genotype, temperature, and photoperiod on canopy size and availability of moisture influence the number of leaves initiated on the main stem. Changes in leaf number are themselves associated with changes in the number of internodes and, thus, the length of stems. The internode length of elephant grass increased significantly with increased days of harvesting.

The highest number of internodes obtained at late days of harvesting may be due to undisturbed vegetative growth for longer periods and the lowest may be due to the removal of photosynthetic materials during the harvesting time (Butt *et al*., 1993; Tessema Zewdu, 2000). As harvesting days increase, the number and length of internodes increase. According to Wanania (2019), absorption on buffel grass (Cenchrus ciliaris linn), the recorded number of internodes per plant is 74.45±4.6 at 60, 94.9±4.4 at 90 and 115±5.5 at 120 stages of harvesting and the length of internodes per plant is 11.34±0.6 at 60, 11.64±0.4 at 90 and 11.71±0.2 at 120 stages of harvesting.

2.3.5 Dry matter yield

Dry matter yield (DMY) is the percentage of the forage that is not water. Grasses that yield the highest DM should be the most sought since they can supply the highest amount of forage to livestock. According to Tilahun *et al.* (2017), there is no significant effect of plant spacing on DMY in desho grass. The highest DMY at the narrow spacing could be attributed to a greater number of plants per unit area, where the closer the distance or the higher the plant population, the greater the amount of total DMY compared to wider spacing (Pholsen and Sornsungnoen, 2004). Yasin *et al.* (2003) recorded similar trends in narrow spacing. Njoka *et al.* (2004) also indicated a higher yield was attributed to high plant populations that allowed the fodder crop to thrive well in terms of nutrient uptake from soil and solar interception in the early period of plant growth and development. According to Adnew *et al*., (2019), the harvesting stage can affect the forage DM yield and nutritive values of Brachiaria grasses. Mamila *et al*., (2020) reported that the total DMY of Para grass (*Brachiaria mutica*) at the late harvesting stage (120 days) and at narrow plant spacing (15 cm) was the highest (20.19 t/ha), while the lowest DMY (8.59 t/ha) was recorded at the early harvesting stage (60) at narrow plant spacing (15 cm).

2.4 Effect of Harvesting Stage and Altitude on Yield and Chemical Composition of Forage

2.4.1 Dry matter content

The studies reported that the DM content of grasses increased with an increase in the growth and development of plants and a longer harvest time. According to Asmare *et al.* (2017), 2017) highest total DM yield observed at the last harvest stage (150 d) indicated that the time of harvesting had a high influence on dry matter yield. DM content increases as the harvesting stage increases. The DM content of Desho grass (*Pennisetum pedicellatum) increased* with an increase in the growth and development status of the plants and with advancements in harvesting dates (Asmare *et al*., 2018; Mamila, 2020) reported that the content of DM of Para grass (Brachiaria mutica) at the late harvesting stage (120 days) and at narrow plant spacing (15 cm) was the highest (20.19 t/ha), while the lowest DMY (8.59 t/ha) was recorded at the early harvesting stage (60) at narrow plant spacing (15 cm). According to Yiberkew et al., (2020), a study on *Brachiaria* hybrid Mulato II has high dry matter content (90.21% and 90.14%) obtained at intermediate (30 cm) and narrow (15 cm) plants. While the lowest dry matter content (89.87%) was observed at wider plant spacing, according to Tilahun *et al. (2017)* observations, on desho grass, the highest DM (89.00%) at late (135) harvesting day and the lowest DM (88.20%) at early (75) harvesting day were recorded.

2.4.2 Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within forage. The quantity of ash in any feed is a positive indicator of the inorganic (mineral) content. Generally, most forage has an ash content ranging from 3% to 12% (Linn and Martin, 1999). The ash content of Para (*Brachiaria mutica*) grass was reduced with an increase in the stage of maturity (Mamila, 2020). The majority of the six studied Brachiaria grasses in different locations had high minerals at early harvesting (Adnew *et al.*, 2019). The available minerals are absorbed into the plant tissue with minimal competition on the wider spacing and their accumulation in the plant tissue is indicative of the observed higher total ash content. The higher number of tillers contained more green leaves on the wider spacing, which had a higher content of minerals in the leaf fractions compared to the narrow spacing.

2.4.3 Crude protein content

Crude protein (CP) and digestible dry matter (DDM) are the most important components of feed. Protein is commonly measured as crude protein (CP), which is 6.25 times the nitrogen content of forage. The CP content varies widely among forage plants, but in all species, it declines with the increasing age of forages. The crude protein content of Napier grass showed an increasing trend with a reduced day of harvesting. This could be attributed mainly to the dilution of the CP contents of the forage crops by the rapid accumulation of cell wall carbohydrates at the later stages of growth (Van Soest, 1994). According to Asmare, (2017), finding, the CP of Desho grass (*Pennisetum pedicellatum*) decreased with an increase in harvest days. Tilahun *et al*., (2017) report that crude protein (CP) concentration was significantly affected by harvesting age (declining from 10.9% at 75 days to 9.3% at 135 days). Munyasi (2015) reported that the CP content of the stem and leaf of Mott elephant grass was higher at the narrowest spacing. Guinea grass (*Panicum maximum*) established on 30 x 30 cm, 40 x 40 cm, and 50 x 50 cm spacing resulted in increased CP content on relatively wider plant spacing (50 x 50 cm). (Yiberkew *et al.*, 2020) and Tilahun *et al.*, (2017) report, plant spacing was not a significant difference of crude protein concentration on Brachiaria hybrid Mulato II and Desho grass respectively.

2.4.4 Neural detergent fiber content

Neutral detergent fiber (NDF) is the insoluble portion of the forage that contains cellulose, hemicelluloses, lignin, and silica (Van Soest *et al.*, 1991). It is a good indicator of fiber contents in forages; they do not measure how digestible that fiber is, but NDF is a good indicator of "bulk" and thus feed intake. The intake potential of feeds is negatively related to NDF contents. Tropical grasses are characterized by low nutritive value due to the higher lignin content and less degradable materials in their cell walls due to the rapid rate of achieving maturity. The increase in cell wall constituents (NDF) is a very important limiting factor in the nutritive value of feeds (Marten *et al.*, 2015). An increasing trend of NDF content was observed with extended days of harvesting. Neutral detergent fiber content varied from 58% to 63% in Napier grass harvested from 68 days to 114 days (Tessema Zewdu *et al.*, 2002). The NDF content of grass was highest (77.68%) from late harvesting (150 days after planting), while it was comparatively lower for earlier harvesting periods (72.78% at 90 days and 73.96% at 120 days) (Asmare, 2017). NDF concentration in desho grass increased significantly as harvesting age and plant spacing increased (Tilahun *et al*., 2017).

2.4.5 Acid detergent fiber content

Acid detergent fiber (ADF) is the portion of the forage that remains after treatment with a detergent under acidic conditions. It includes cellulose, lignin, and silica. Acid detergent fiber is a good indicator of digestibility and thus energy intake (Van Soest *et al.*, 1991). ADF is important because it is negatively correlated with how digestible forage may be when fed. As ADF increases, the forage becomes less digestible. ADF is a good indicator of digestibility and, thus, energy intake. ADF is important because it is negatively correlated with how digestible forage may be when fed. ADF concentration increased significantly with an increase in harvesting age but was unaffected by plant spacing in desho grass (Tilahun et al., 2017). According to Taye and Lemma (2009), a recorded increase in ADF when Napier grass was harvested at 60, 90, and 120 days should be expected with increasing grass maturity. The ADF content of grass was highest (42.60%) from late harvesting (135 days of harvesting), while it was comparatively lower for earlier harvesting periods (33.10% at 75 days of harvesting and 37.60% at 105 days of harvesting) in desho grass (Tilahun *et al.*, 2017). Yiberkew *et al.*, (2020) in Brachiaria hybrid 'Mulatto II' and Tilahun *et al.*, (2017) in Desho grass reported that ADF was unaffected by plant spacing.

2.4.6 Acid detergent lignin content

Acid detergent fiber (ADL) is the portion of the forage that remains after treatment with detergent under acidic conditions. It includes cellulose, lignin, and silica. Lignin is the non-carbohydrate component of the forage cell wall and is identified as limiting fiber digestibility. ADL is a good indicator of digestibility and, thus, energy intake. The ADL content of desho grass increased from 17.3 to 20.7% as the days of harvesting increased from 75 to 135 (Tilahun *et al*., 2017). Ghiwot (2019) reported that plants harvested at early (60) harvesting days had the lowest (7.63±0.5) ADL content, whereas the higher (10.17±0.6) ADL content was recorded at late (120) harvesting days in buffel grass (*Cenchrus ciliaris* Linn).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The research was carried out into two agro-ecologies (low and middle altitudes) under rain-fed conditions. The mid-altitude area was represented by the Mante Dubo sub-research site of Areka Agricultural Research Center in Boloso Sore Woreda. Mante Dubo is located at a distance of about 300km south of the capital, Addis Ababa, and at an altitude of 1711 m.a.s.l and situated at N 07' 06.4312` and E037' 41.688`. The rain fall is bimodal and ranges between 1201 and 1600mm with highest from July to September. The mean annual temperature ranges between 22 and 24°C (Getachew et al, 2016). The lowland region was represented by Humbo district in Wolita Zone. Humbo district geographically, it is located at 6°43'44"N latitude and 37°45'51"E longitude, at elevations varying from 1500–2500 meters above sea level. The mean annual temperature is 22°C. The rainfall is erratic with an annual average ranging from 843 to 1403 mm (Humbo Woreda Agricultural Office, 2012).

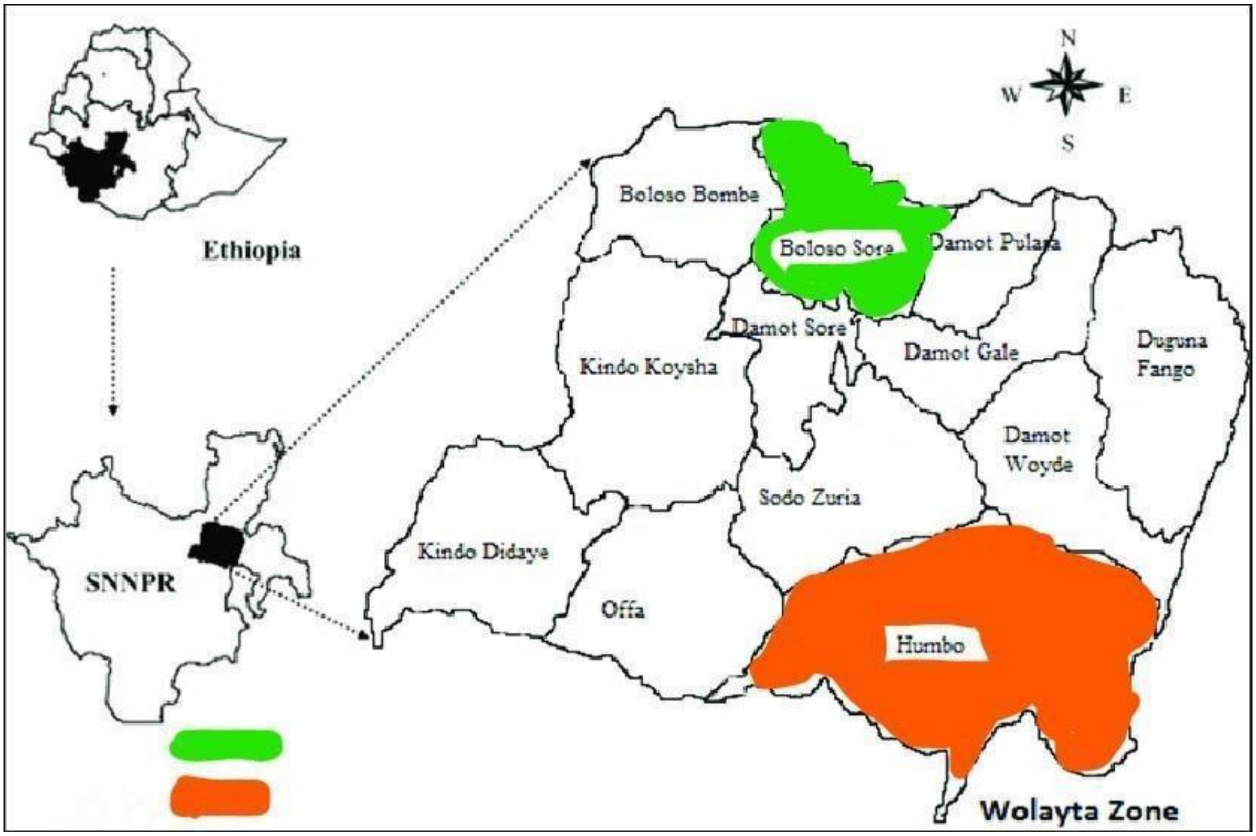

Figure 2. Administrative map of the study area (Source: Werissaw Haileselassie, 2020)

3.2. Experimental Layout, Design, and Treatments

A total area of 361 m2 (19 m*19 m) was selected from the locations. The land was ploughed and harrowed with oxen in July 2022. The experiment was laid out in a factorial arrangement of two agro-ecologies (lowland and midland) and three harvesting dates (90, 120, and 150 days) in a randomized complete block design (RCBD) with three replications. Replicates are arranged in three blocks, and each block contains three plots in a row, for a total of nine plots. The plot size of each treatment was 25 m^2 (5 m x 5 m). Rhizomes, made from root cuttings, were collected from the forage nursery site of the Areka Agricultural Research Center (ARC) for planting. A total of 70 cutting rhizomes of approximately 5cm in length were planted on established plots with spacing between rows and plants of 75cm and 50cm, respectively. There was a 1-meter width between plots and blocks. Urea fertilizer was applied at a rate of 100kg/ha during planting and 100kg/ha during the growth period, respectively (Cameron *et al.*, 2008).

Guatemala (*Tripsacum laxum*) grass was established during the onset of the main rainy season, and all agronomic management (fertilizer, weeding, and draining excess water from each plot) was done according to protocols recommended by Areka ARC.

Table 1. The treatments used in the experiment.

Midland

B1	90 HD	150 HD	120 HD
B2	120 HD	90 HD	150 HD
B3	150 HD	120 HD	90 HD

Lowland

B1	90 HD	150 HD	120 HD
B2	120 HD	90 HD	150 HD
B3	150 HD	120 HD	90 HD

HD=Harvesting days

3.3 Data Collection, Sampling, and Measurements

The study involved collecting all the morphological parameters of Guatemala grass.

3.3.1 Plant height

Plant height was consistently monitored by measuring the primary shoot from the soil surface to the base of the top leaf with a meter rule, following the method outlined by Rayburn *et al.* (2007). For accuracy, two rows were chosen at random from the seven in each plot, avoiding the two outermost rows. From these, five tillers were selected randomly, and their average height was recorded.

3.3.2 Number of tillers per plant

The number of tillers was counted and recorded on the same tagged plants. The number of tillers per plant was counted from five randomly selected plants in the middle row of each plot at each harvesting stage from different locations, and the mean was obtained.

3.3.3 Number of leaves per plant and internode length

In an experimental plot area, the number of leaves per tiller was counted in 10 randomly selected tillers at each harvesting stage from different agro-ecologies. By multiplying the number of leaves per tiller by the number of tillers per plant, the total number of leaves per plant was obtained. Three randomly selected plants per plot in each row were measured for internode length.

3.3.4 Number of nodes per plant

The number of nodes per plant was counted and recorded for 10 randomly selected plants from each plot.

3.3.5 Dry matter yield

Following the completion of each harvest phase for every plot, the dry matter yield (DMY) was determined. Manual harvesting was performed with a sickle, ensuring a remaining stubble height of 8 cm from the ground level (Adnew *et al.*, 2019). For assessing the fresh biomass, a digital scale was used, and then from the net harvested biomass, sub-samples of approximately 500 grams of fresh plants were collected. These sub-samples were then oven-dried to measure dry weights. Consequently, the total dry matter yields for each plot were computed by applying the dry matter percentage and fresh biomass yield from the respective plot's sample area, and these were subsequently converted into tons per hectare. The dry matter yield was ascertained after drying the samples in a forced-air drying oven at 65°C for 72 hours. The dry matter yield was determined as follows:

DMY (t/ha) = (10 x TFW x SSDW) / (HA x SSFW) (James et al., 2008).

Where: 10 is the constant for the conversion of yields from kg/m^2 to t/ha.

TFW: total fresh weight (kg)
SSDW: sub-sample dry weight (g)
HA: harvest area (m^2)
SSFW: sub-sample fresh weight (g)

3.4 Chemical Analysis

The chemical analysis and in vitro organic matter digestibility were determined at the International Livestock Research Institute (ILRI) Animal Nutrition Laboratory, Addis Ababa, Ethiopia. Samples were dried at 65°C for 72 hours and ground to pass through a 1mm sieve. About 500g of oven-dried samples were used for laboratory analysis. Dry matter was determined by oven drying at 65ºC overnight (method 934.01). Ash was determined according to (method 942.05). Nitrogen content was determined by the Kjeldhal method using Kjeldhal (protein/nitrogen) Model 1026 (Foss Technology Corp.) (Method 954.01). A conversion factor of 6.25 was used to convert nitrogen to crude protein. Neutral detergent fiber, acid detergent fiber (ADF) and lignin were determined as described by Van Soest and Robertson (1985).

Neutral detergent fiber did not involve the use of heat stable amylase and the result was expressed exclusive of residual ash. Acid detergent fiber was expressed without residual ash. Lignin was determined by solubilization of cellulose with sulfuric acid. *In vitro* organic matter digestibility was measured in rumen microbial inoculum using in vitro gas production techniques. The buffer solution was prepared according to the method described by Menke and Steingass (1988). Rumen fluid was collected prior to morning feeding using a vacuum pump from three ruminally cannulated cows fed a total mixed ration of grass hay (790 g/kg), wheat bran (203 g/kg), salt (3.2 g/kg) and a mineral and vitamin mixture (4.6 g/kg) on a DM basis. The use of cows was assessed and approved by the Environmental and Occupational Health and Safety Unit of ILRI. The rumen fluid from the cows was composited (1:1, v/v), filtered through four layers of cheese cloth, and added to the buffer solution (1:2, v/v), which was maintained in a water bath at 39 ^{o}C under continuous flushing with CO_2. The buffered rumen fluid (30 ml) was pipetted into 100 ml syringes containing 0.2 g of sample and immediately placed into a water bath at 39°C. Gas production was recorded after 24 hours of incubation and used to calculate IVOMD according to Menke et al.'s (1979) equations suitable for legume hays as follows: IVOMD (g / kg) = 14.88 + 0.889GP +0.45CP+ 0.0651XA

Where GP: 24 h net gas production (ml/200 mg); CP: crude protein (g/kg DM); XA: Ash content (g/kg DM). All chemical analyses were carried out in triplicate.

3.5 *In vitro* Gas Production and Methane Emission Potential of Guatemala Grass

Rumen fluid was obtained from the rumen of fistulated sheep from Dilla University farms that were housed in individual cages and fed on sample hay daily with free access to water and mineral licks. Rumen liquid samples were collected before the morning meal in pre-warmed thermos flasks and transported immediately to the adjoining laboratory, where they were strained through three layers of cheesecloth and kept at 39 ^{o}C under a CO_2 atmosphere. Filtered rumen liquor was pooled together to achieve a homogenous inocula. 30 ml of inoculum was introduced into 200 mg of samples in syringes containing cheesecloth-strained rumen liquor and buffer (NaHCO3 + Na2HPO4 + KCl + NaCl + MgS04. 7H2O + CaCl2. $2H_2O$) (1:2 v/v) under continuous flushing with CO_2. Vaseline was applied to the pistons to ease movement and prevent the escape of gas. The syringes were pre-warmed at 39°C before the addition of 30 ml of buffer mixture and rumen liquor to each syringe. The syringes were agitated 30 minutes after the start of incubation and every hour for the first 10 hours of incubation. The gas production was measured at 3, 6, 12, 24, 48, and 72 hours of incubation, and after 72 hours, 4 ml of NaOH (10M) was introduced to estimate the methane production as reported by Fievez *et al.* (2005). Each run encompassed three blanks and a standard sample of known gas production. The average volume of gas produced from the blanks was deducted from the volume of gas generated per sample, following the standards for known gas production. The volume of gas produced at intervals was plotted against the incubation time, and from the graph, the gas production characteristics were estimated using the equation.

$Y = a + b\ (1\text{-exp}^{-ct})$ described by Ørskov and McDonald (1979).

Where: Y is the gas production (ml/200mg DM) at time *t*, a is the intercept of the gas production curve (gas produced from soluble fraction), *b* is the extent of gas production (potentially degradable fraction), $a + b$ is the potential gas production (ml/200 mg DM), and *c* is the rate constant of gas production of b. t is the incubation time.

Methane content was measured by the absorption of CO_2 with 40% NaOH (Fievez et al., 2005). Using this method, it was assumed that in vitro gases primarily contained methane and CO_2, while other gases produced during fermentation were relatively insignificant. At the end of incubations (72 h) and after recording the final gas volume as V_1, the lower end of the syringe was connected to the lower end of another syringe containing 4 mL of NaOH (10 mL). The NaOH was then introduced into the incubated contents, preventing gas escape. Mixing the contents with NaOH allowed the absorption of CO_2, with the remaining gas (V_2) in the syringe considered to be CH_4 (Fievez *et al*., 2005). The *in vitro* gas production and methane emission of grass samples were analyzed at the Dilla University Animal Nutrition Laboratory.

3.6 Statistical Analyses

The collected data was managed and organized with MS Excel 365. Data were subjected to the General Linear Model (GLM) procedure of SAS version 9.3 (2011) for analysis of variance. Mean comparisons were done using Duncan's Multiple Range Test (DMRT). The ANOVA test was applied to compare the mean nutritional value for the three stages of maturity and agro-ecologies, as well as the interaction effect of agro-ecologies and stages of maturity. Pearson's correlation coefficient was used to measure the relationship between nutrient contents. Differences between means were declared significant at $P<0.05$.

The following statistical model was used:

$Yijk = \mu + Ai + Bj + A^* B (ij) + e (ijk)$,

Where: Y = parameter studied (DM yield, morphological characteristics, chemical composition, ME, IVOMD, GP, and CH_4)

μ = Overall mean

Ai =the effect of location (i= 1, 2)

Bj = the effect of stage of maturity (j = 1, 2, 3)

*A*B (ij)* = the interaction effect of location and stages of maturity and

e (ijk) = The error term

4. RESULTS AND DISCUSSION

4.1 Effect of Agro-ecologies and Harvesting Stage on Morphological Characteristics and Dry Matter Yield of Guatemala Grass

Agro-ecologies, harvesting stage and their interaction effect on the morphological traits and dry matter yield of Guatemala grass are shown in Table 2. Plant height (PH), number of nodes per plant (NNPP) and dry matter yield (DMY) were significantly ($p<0.05$) affected by agro-ecologies. Harvesting stage had a significant ($p<0.05$) effect on all examined morphological parameters and dry matter yield of Guatemala grass. Their interaction effect had a non-significant ($p>0.05$) effect on all examined morphological and dry matter yield of Guatemala grass.

4.1.1 Plant height

Harvesting stage, agro-ecologies and their interaction had a significant ($p<0.05$) effect on plant height (PH) of Guatemala grass as shown in Table 2. Significantly ($p<0.05$) highest PH was recorded from low agro-ecologies with the mean value of 106.57cm compared to mid agro-ecologies (95.88cm). The PH difference between agro-ecologies could be due to the differences in moisture content and soil fertility of the testing environments. Moreover, the variation in PH in different agro-ecologies could be due to the difference between the physiological changes of plants observed during the growing periods. In accordance with this result, Adnew *et al.*, (2019) reported that the highest significant ($p<0.05$) difference of PH at low agro-ecologies of all cultivars of brachiaria grass. In line with this finding, Asmare *et al.*, (2018) reported that PH is significantly affected by agro-ecologies and harvesting date.

As indicated in Table 2, plant height was significantly affected by different harvesting stage, where highest PH (128.86cm) was recorded during the late harvesting stage (150 days) followed by intermediate cutting stage (120 days) with the mean value of 103.60cm. This shift in the plant's growth could be the result of its extensive root system and effective nutrient uptake, which allows the plant to keep growing and getting taller. Increased PH at the point of late harvest may be the result of extensive root growth and effective nutrient uptake, which enable the plant to continue growing taller (Andualem and Hundessa, 2022).Similarly, Asmare et al., (2017) reported that PH was lower during early cutting stage, but increased growth was observed after 120 days of harvesting for desho grass. Contrary to the current result, Andualem and Hundessa, (2022) reported that the highest PH was recorded at high agro-ecologies followed by low agro-ecologies.

Plant height is an important parameter contributing to yield in forage crops (Atumo *et al*., 2021). The overall mean PH of Guatemala grass observed in the current study (101.23cm) was lower than the value (214cm) reported by Andualem and Hundessa, (2022) for the same grass species from southern Ethiopia and this difference comes from soil fertility, harvesting stage, management, and climatic condition. Plant height increment was consistent with plant maturity. Alarming increments in plant height are one of the major acclimatization responses to light competition in plants, i.e., leaf length during the vegetative period in grasses (Adnew and Asmare, 2023).

4.1.2 Number of tillers per plant

Number of tillers per plant (NTPP) of Guatemala grass was significantly ($p<0.05$) affected by the harvesting stage as shown in Table 2. Agro-ecologies, as well as the combination of harvesting stage and agro-ecologies, were observed to have an insignificant effect ($p>0.05$) on the NTPP. Notably, the highest NTPP (10.64) was recorded during the late harvesting stage (150 days), surpassing the count observed at the intermediate cutting stage (120 days), with this difference being statistically significant ($p<0.05$).

The minimum NTPP (5.67) was recorded during the early cutting stage (90 days). This indicated that the total NTPP increases linearly with increase in harvesting stage. The increase in the tiller number might be due to longer days of maturity and the associated continuous increment in the photosynthetic rate of the grass. When the plants approached maturity, numerous fine branches appeared, growing out from the leaf axils of the main stems (Bantihun et al., 2022). The increment in NTPP was in accordance with that of Mupenzi *et al.,* (2017) who reported that NTPP was significantly ($p<0.05$) affected by harvesting date. According to Andualem and Hundessa, (2022), the NTPP increased with the advance in harvesting stage of plants as the result of the development of new shoots bearing on each plant resulting in a more significant NTPP matures.

The mean NTPP (7.84) of Guatemala grass recorded in the current study was lower than the value (11.9) reported by Andualem and Hundessa, (2022) for the same grass species. This difference might be due to agro-ecologies, maturity stage, weather condition, soil type, and management system and agro ecology. Tillers density is an important attribute of grasses as it increases the chances of survival and the amount of available forage (Bantihun *et al.,* 2022). Moreover, it is an indicator of resource use efficiency by the different grass species and that the weight of a plant's tillers determines its productivity (Adnew, 2022). The large numbers of tillers produced by some grass species allow them to attain maximum growth at an earlier age and recover faster after defoliation. Tillering is also important in forage plants because it influences leaf-area production and dry matter yield (Njarui *et al*., 2016; Escobar Charry *et al.*, 2020; Mijena *et al*., 2022). Tillering performance is an important morphological characteristic to be considered during selection of appropriate forage crop species for better improvement of production and productivity (Bantihun *et al*., 2022).

4.1.3 Number of leaves per plant

Harvesting stage had a significant ($p<0.05$) effect on number of leafs per plant, (NLPP), whereas agro-ecologies had no significant ($p>0.05$) effect on the NLPP (Table 2). A significantly higher NLPP was recorded during the late cutting stage (150 days) with the mean value of 93.05. On the other hand, during the early cutting stage (90 days) significantly lower NLPP (50.80) was recorded form Guatemala grass. From this study result, we observed that with the increase in the stage of maturity, a greater number of leaves were produced which are important for the photosynthesis and transpiration surface for the newly emerging tillers. In concur with this result, Tilahun *et al.*, (2017) reported that NLPP, which in part determines the photosynthetic capacity of the plants, was significantly ($p<0.05$) affected by harvesting age.

The mean NLPP of the current study (70.64) was much higher than the value reported by Andualem and Hundessa, (2022) who reported 11.9 for Guatemala grass (*tripsacum andersonii*) harvested at three stages of maturity in Gedeo agroforestry systems, southern Ethiopia. The variation might be due to plant species, genetic variation, soil fertility and season. The increasing tendency in the NLPP with the advanced stages of harvesting indicated that the time of harvesting had a significant influence on the number of leaves. This might be due to the extended growth; there was increment in plant height, the number of tillers, and the number of nodes that produce a comparable number of leaves. On the other hand, Zemene *et al.* (2020) reported that the mean NLPP increased from 232.2 leaves at 60 days to 1211.1 leaves at 120 days in *Brachiaria mutica* grass, but this result was greater than our finding. Hence, it could be concluded that the production of leaves from new tillers generally increased with an increase in the days of harvesting because the longer the vegetative phase and the taller the plant, the greater the number of leaves produced (Moher *et al.,* 2022).

4.1.4 Number of nodes per plant

Harvesting stage, agro-ecologies and their interaction found to have significant ($p<0.05$) effect on number of nodes per plant (NNPP) of Guatemala grass. As shown in Table 2, significantly ($p<0.05$) higher NNPP was recorded from Guatemala grass harvested from low agro-ecologies. On the other hand, significantly ($p<0.05$) higher NNPP was observed in late cutting stage (150 days) followed by intermediate harvesting stage (120 days). The mean NNPP of tested grass species was 4.21. From this study result, we observed that with the increase in the stage of harvesting, NNPP was produced. This might be because when the age of harvesting increases, the tiller or stem remains vegetative; the apical meristem is indeterminate and theoretically can produce an infinite number of new nodes and leaves.

As with other agronomic traits, stem elongation is also influenced by variation in soil type, temperature, amount and distribution of rainfall, genotypes, and harvesting stage interaction effects. There was a maximum of 6.16 Guatemala grass nodes per plant at the late harvesting stage (150 days) and a maximum of 4.13 nodes per plant at the intermediate harvesting stage (120 days). This could be due to prolonged harvesting days, continued plant growth until maturity, and additional factors like possible increases in tiller length and internode count. This study, which is also in agreement with Yigzaw, (2019), reported that in buffel grass (Cenchrus ciliaris Linn), the number of internodes per plant significantly increased as harvest time approached. The reason for differences in the length of internodes would probably be the longer physiological and anatomical growth of the plants during late harvest.

4.1.5 Dry matter yield (t/ha)

Harvesting stage and agro-ecologies found to have significant ($p<0.05$) effect on dry matter yield (DMY) of tested grass as indicated in Table 2. Significantly ($p<0.05$) higher DMY were recorded from Guatemala grass harvested from low agro-ecologies (9.69t/ha) compared to mid agro-ecologies (7.95t/ha). On the other hand, the total dry matter of the late harvesting stage (150 days) was the highest (14.31t/ha), whereas the lowest DMY (4.76t/ha) was produced from the early harvesting stage (90 days). The current result indicated that the dry matter content increased with harvesting age increases. This might be due to maturity of grass, increment of number of tillers, leaves and structural carbohydrate in the plant tissues that increases the dry matter yield. This result agrees with other studies (Andualem and Hundessa, 2022; Asmare *et al*., 2017; Bantihun *et al.,* 2022) for other types of grasses.

The highest total DM yield observed in late harvesting stage (150 days) agreed with Mijena *et al.,* (2022) who reported that the time of harvesting had a high influence on dry matter yield. The increasing trend of dry matter yield in Guatemala grass could be attributed to the development of more tillers in the grass, leaf formation, leaf elongation, stem development and vegetative growth of the plant (Asmare *et al.,* 2017). At late harvesting, DMY increased due to the cumulative effect of plant growth and environmental factors. This condition influences the energy distribution and soil nutrients mobilizing, to sustain aboveground regrowth through photosynthesis.

The mean DMY (8.82t/ha) of the current study result was comparable with the finding of Mijena *et al*. (2022) and Adnew *et al.* (2019) were 8.22 t/ha and 10.38 t/ha from Bracharia grass in Ethiopia. On the other hand, the result obtained from late harvesting (150 days) 14.31 t/ha was in line with the value reported by Andualem and Hundessa, (2022) who reported 14.9t/ha for Guatemala grass (*tripsacum andersonii*) harvested at the mid-stage (150 days) in Gedeo agroforestry systems, southern Ethiopia.

Table 2. The effect of agro-ecologies and harvesting stage on morphological parameters and dry matter yield

Factors	Morphological Parameters					
	PH (cm)	**NTPP(Count)**	**NLPP(Count)**	**NNPP(Count)**	**INL (cm)**	**DMY (t ha^{-1})**
Agro-ecologies						
Midland	95.88^{b}	7.50^{a}	70.14^{a}	3.85^{b}	2.60^{a}	7.95^{b}
Lowland	106.57^{a}	8.19^{a}	71.13^{a}	4.56^{a}	2.72^{a}	9.69^{a}
Mean	101.23	7.84	70.64	4.21	2.66	8.82
Harvesting Stage						
90 days	71.23^{c}	5.63^{c}	50.80^{c}	2.33^{c}	1.95^{c}	4.76^{c}
120 days	103.60^{b}	7.26^{b}	68.06^{b}	4.13^{b}	2.70^{a}	7.39^{b}
150 days	128.86^{a}	10.64^{a}	93.05^{a}	6.16^{a}	3.33^{a}	14.31^{a}
Mean	101.23	7.84	70.64	4.21	2.66	8.82
Sources of Variation						
Agro-ecologies	<0.0001	0.0580	0.7827	0.0079	0.2811	0.0002
H.S	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AE x HS	0.0063	0.4744	0.4990	0.6102	0.4590	0.8800
SEM	5.980	0.533	4.489	0.4015	0.1462	1.009
CV	25.062	28.844	26.965	40.454	23.312	48.522

The means in each column with different superscripts are significantly different at $p<0.05$. NS=Non-Significant, NNPP= Number of Node Per Plant, INL=Inter Node Length, NLPP=Number of Leaf Per Plant, PH=Plant Height, NTPP=Number of Tiller Per Plant, DMY=dry matter yield cm=Centimeter, AE= agro-ecologies, HS=Harvesting Stage, ***=significantly different.

4.2 The Effect of Agro-ecologies and Harvesting Stage on Chemical Composition and *In Vitro* Organic Matter Digestibility of Guatemala Grass

Agro-ecologies, harvesting stage and their interaction effect on chemical composition and IVOMD of Guatemala grass is shown in Table 3. Dry matter percentage and ash were not significantly ($p<0.05$) affected by harvesting stage, agro-ecologies and their respective interaction. Similarly, agro-ecologies have found to have non-significance ($P>0.05$) difference on NDF, ADF, ADL and ME content of tested grass. On the other hand, harvesting stage had a significant ($P<0.05$) effect on all proximate composition and IVOMD except DM and ash content.

4.2.1 Dry matter content

Dry matter content (DM, %) of Guatemala grass was not significantly ($P>0.05$) affected by harvesting stage and agro-ecologies as indicated in Table 3. DM content was increased with the increasing of the harvesting stage. Increasing DM content with delayed harvesting time might be because of decreased moisture content in leaves as the plants aged and became lignified. In agreement with this result, Demlew *et al*. (2019) reported that forage species and harvesting time interaction had no significant effect ($P>0.05$) on DM content of Buffel grass at different harvesting time periods. In contrary with this finding, Andualem and Hundessa, (2022) reported that DM content of Guatemala grass was significantly affected by agro-ecologies and different harvesting stage. Additionally, Mijena *et al.* (2022) also reported that DM content of Bracharia grass was significantly ($P<0.001$) affected by the harvesting stage. It might be due to the type of grass or genetics of grasses, season, and management system in the area where the study was conducted.

The overall mean dry matter content of tested grass species was 91.68%. In line with this study, Bantihun *et al*., (2022) and Mijena *et al*., (2022) reported the average dry matter content of brachiaria grass were 91.0% and 92.89% respectively. However, Andualem and Hundessa, (2022) reported that (22.2%) the mean dry matter content of Guatemala grass after 180 days of harvesting stage which is quite lower than the current study result. This result reported a lower DM content than in the current result; this might be due to environment conditions, management system, and harvesting stage differences in the area where the current experiment was conducted.

4.2.2 Ash content

The ash content (%) of Guatemala grass showed a non-significant difference (*P* > 0.05) at both the harvesting stage and agro-ecologies. As shown in Table 3, numerically, higher ash content was observed on early harvesting days as compared to later and intermediate harvesting days. The ash content of 12.66% at 90 days, 12.63 at 120 days, and 12.16% at 150 days of harvesting was recorded. The decline of total ash with plant maturity might be due to the natural dilution and translocation of nutrients during the growth and development of plant tissue (Yigzaw, 2019). Unlike the current study result, Bantihun *et al.*, (2022) reported that harvesting stage had a very high significant (P<0.05) effect on ash content of different grass species. Similarly, Andualem and Hundessa, (2022) reported that ash content of Guatemala grass showed a significant difference (p<0.05) at different harvesting stages as well as among agro-ecologies. The same author indicated that the ash content was recorded at high agro-ecologies (14.9 % DM) followed by low altitude (13.4 % DM) whereas the least was from medium agro-ecologies (11.8 % DM). The overall mean ash content of Guatemala grass was 12.49%. In concur with this result, Andualem and Hundessa, (2022) reported that the mean ash content of Guatemala grass at mid agro-ecologies was 11.8%. Furthermore, Lyimo *et al*., (2016) reported 9.5% of the mean ash content of Guatemala grass which is lower than our current study result.

4.2.3 Crude protein content

Agro-ecologies, harvesting stage and their interaction showed a significant (*P*<0.05) effect on CP content (%) of Guatemala grass. Significantly (*P*<0.05) higher CP content was recorded from tested grass harvested from mid agro-ecologies (10.49%), whereas lower was from low agro-ecologies (8.68%). This could be due to the temperature, rainfall, soil fertility and organic matter content. This result was not in agreement with the finding of Andualem and Hundessa, (2022) in Guatemala grass (Tripsacum Anderson), who reported that the highest CP content was recorded from low agro-ecologies (9.80% DM) and the least was from medium agro-ecologies (8.69%). The highest CP content (11.46%) was recorded at early harvesting time (90 days) compared to the two late harvesting days (at 120 days 9.47% and 150 days 7.83% CP). This result indicated that the CP content significantly decreased with increase in harvesting stage and or in advance of plant maturity. The CP content of Guatemala grass was decreasing with increasing days of harvesting at both agro-ecologies. The increase in age in grasses is usually negatively associated with CP content. The decline in CP content with advancing stage of maturity is due to accretion of higher proportion of NDF corresponding to plant growth. This might be due to an increase in the structural carbohydrate and lignin content of forage materials and a reduced leaf-to-stem ratio (Yigzaw, 2019). In line with this result, Bantihun *et al*., (2022) reported that the highest CP content (10.3) was recorded during the early harvesting period (60 days), followed by the CP content of 10.12 during the intermediate harvesting stage (90 days) and CP content of 7.7 during the late harvesting stage (120 days). Similarly, Adnew and Asmare, (2023) reported that the highest CP concentration was obtained at the earliest stage of harvesting; with values declining as harvesting was delayed.

CP is one of the major criteria for determining the nutritional quality of a feed because as the level of CP increases, the DM intake by livestock and rumen microbial growth would also increase (Chanthakhoun *et al.*, 2012). The overall mean CP content (9.59%) of Guatemala grass evaluated in this experiment met the minimum requirements for ruminants (>7%), i.e., 6.9% for maintenance, but not met 10.0% for beef production, and 11.9% for milk production (Adnew et al., 2019). The current study result (9.59%) was comparable with the value (9.80%) reported by Andualem and Hundessa, (2022) for similar grass species.

4.2.4 Neutral detergent fiber content

Neutral detergent fiber (NDF, %) of Guatemala grass was significantly ($p<0.05$) affected by different harvesting stage, whereas agro-ecologies and their interaction showed non-significant ($p>0.05$) effect on NDF content of the plants. The highest mean value of NDF was recorded at 150 days of harvesting (64.57% DM), followed by 120 days of harvesting (62.999%% DM), whereas the least was from 90 days of harvesting (62.10 % DM). According to the result obtained from the current study, as harvesting stage extended, the NDF content also increased. This could be demonstrated by the reduction in crude protein content and a substantial increment in cell wall content. This might be related to such factors as temperature and moisture stress of the cultivation environment which affects the nature of cell content and leading to less carbohydrate content. The content of neutral detergent fiber increased with the plant maturity increased. This is due to an increment of insoluble proportions of the forage like cellulose; hemicelluloses, lignin and silica with plant matured increased (Jagadeesh *et al.*, 2017). In line with this finding, Andualem and Hundessa, (2022); Yigzaw, (2019); Asmare *et al*., (2017) reported that increasing trend of NDF content were observed with extended days of harvesting in Guatemala (Tripsacum Andersoni), Buffel, and desho grasses, respectively. Similarly, harvesting age had a significant effect on the NDF content of grass species as reported by Tilahun *et al.*, (2017) at 75 (45.26), 105 (46.26), and 135 (51.7) days of harvesting of Desho grass.

The NDF content of plants is reported to result in decreased voluntary feed intake, feed conversion efficiency, and longer rumination time dry matter intake will decrease (Haselmann *et al.*, 2019). The mean value (63.22%) of NDF content was comparable with the finding of Mijena *et al.,* (2022); Adnew *et al*., (2019); Andualem and Hundessa, (2022) who reported 64.66%, 61.98% and 61.3% for brachiaria and Guatemala grass respectively. Forage plants containing above 72% of NDF will cause a low intake of forage. In this study, NDF content of Guatemala grass was below 72% NDF. Therefore, it has high intake by animals and produces a high milk yield and weight gain of animals since milk yield and/or weight gain are closely related to feeding intake. Forage NDF is relevant to the improvement of the forage nutritional value and can be an important parameter to define the forage quality because the more fibrous pasture occupies more space for longer and limits the intake rate (Adnew and Asmare, 2023).

4.2.5 Acid detergent fiber content

The harvesting stage had a significant (P< 0.05) effect on the acid detergent fiber content (ADF, %) of *Guatemala* grass, whereas ADF value was not significantly (P>0.05) affected by agro-ecologies (Table 3). Significantly (P<0.05) higher ADF content was recorded during late harvesting stage (150 days) followed by intermediate harvesting stage (120 days). From the current study, ADF content of 36.95, 38.41 and 39.33% were recorded at 90, 120, and 150 days of harvesting respectively with the overall mean value of 38.23%. The increment of ADF content as age increases might be associated with the development of the structural component/cell wall constituents of the grass as age increases. This might be because as plants become mature there is a greater development of structural carbohydrates. The decreased leaf-to-stem ratio and more stem content increased lignification with delayed days of harvesting

(Asmare *et al.,* 2017; Kefyalew *et al*., 2020). In line with this study, Yigzaw, (2019) reported that plants harvested at 60 days had the lowest NDF, ADF, and ADL content; whereas the higher NDF, ADF, and ADL content was recorded in the plots harvested at 120 days. Similarly, Mijena *et al.*, (2022) showed that the ADF increased with the extended harvesting stage with the lowest ADF content from the harvesting stage at 45 days after total clearing.

The overall mean value of ADF obtained from the current study (38.23%) was lower than the finding of Andualem and Hundessa, (2022) who reported 42.3% of ADF for Guatemala grass, whereas (Adnew *et al.,* 2019) reported similar ADF (38.31) value for brachiaria grass in northwestern Ethiopia. This difference could come from planting systems, environment, agro-ecologies, soil type, soil fertility, and harvesting age in the area where the current experiment was conducted. The recommended minimum ADF content in the feed is 170 to 210 g kg-1 DM feed, but it depends on various factors such as particle size, feeding methods, supplements, rate and extent of fermentation of fiber source (Varga *et al.,* 1998). Forage with higher ADF has lower cellulose digestibility in the rumen, thereby reducing the energy available to the lactating cow for milk production. ADF is the value that refers to the cell wall portions of the forage that are made up of cellulose and lignin (Adnew *et al.,* 2019). The digestibility of the feed is related to fiber because the indigestible portion has a proportion of ADF, and the higher the value of ADF the lower the feed digestibility (Faji *et al*., 2022).

4.2.6 Acid detergent lignin (%)

The harvesting stage had a significant (P<0.05) effect on the acid detergent lignin content (ADL %) of *Guatemala* grass (Table 3). However, agro-ecologies and the interaction between harvesting stage and agro-ecologies had non-significant (P>0.05) effect on ADL% of tested grass. ADL content of 4.46% at 90 days, 5.80% at 120 days, and 5.99% at 150 days of harvesting time were recorded respectively. The ADL content of Guatemala grass was progressively increased with harvesting day. This could be because the plant produced more lignin and complex sugars in its cell wall. In accordance with the current study, previously reported finding concluded that ADL value of grass was increased with progressive stage of maturity (Bantihun *et al*., 2022; Mijena *et al*., 2022; Tilahun *et al.*, 2017). This might be due to the increase in structural carbohydrates (cellulose and hemicelluloses) and the increase in lignin concentration in plant tissues (Schmatz *et al*., 2020). Lignin is the non-carbohydrate component of the forage cell wall and is identified as limiting fiber digestibility. The late stage had the highest lignin content which would bind the cellulose and hemicellulose and prevent them from being digested and are utilized efficiently by the rumen microbes (Bantihun *et al.*, 2022).

The overall mean ADL content of Guatemala grass (5.42%) recorded from the current study lower than the value (7.23%) reported by Andualem and Hundessa, (2022) for the same grass species. This difference might be due to the variation in planting system, harvesting stage, agro-ecologies, soil type, and fertility in the area where the experiment was conducted.

4.2.7 Metabolizable energy (ME MJ/kg)

Metabolizable energy (ME) yield of Guatemala grass was significantly ($P<0.001$) influenced by harvesting stage, whereas agro-ecologies and the interaction between harvesting stage had non-significant ($P>0.05$) effect on Metabolizable energy. Significantly ($P<0.05$) higher ME of tested grass was recorded from early harvesting stage (90 days). The ME content of 8.48 MJ/kg at 90 days, 8.04 MJ/kg at 120 days, and 7.93 MJ/kg at 150 days of harvesting was recorded as shown in Table 3. The results indicated that the ME content of Guatemala grass decreased with the extended harvesting stage. A decreasing trend in ME yield with advancing stages of plant maturity in the current study is consistent with the report of (Adnew *et al.*, 2019; Onjai-uea *et al.,* 2022). The overall mean value (8.15MJ/Kg) of ME of Guatemala grass in the current study was similar with the value (8.64MJ/Kg) reported by Mijena et al., (2022). Furthermore, Asmare *et al*., (2017) reported the lower mean value of desho grass (6.23MJ/Kg) compared to the current study result. The difference could be due to the variation in plant species, agro-ecological zone, harvesting stage, and management practices.

The mean ME of the current study (8.15MJ/Kg) was above ME of 7 MJ/kg DM might be better to supply energy to ruminant livestock. It was explained by McDonald et al. (2002) that the later the cutting date, the larger the DMY was due to the higher indigestible plant part (cell wall components), which leads to a reduced digestibility of the pasture, which was also reflected in the lower ME value of grasses.

4.2.8 *In vitro* organic matter digestibility (%)

Agro-ecologies and harvesting stages had shown a significant ($P<0.05$) effect on the In vitro dry matter digestibility (IVOMD) of Guatemala grass ($P<0.05$) as shown in Table 3. Significantly ($P<0.05$) higher IVOMD were recorded in Guatemala grass harvested from mid agro-ecologies compared to low agro-ecologies. On the other hand, significantly ($P<0.05$) higher IVOMD was recorded from tested plant harvested during early stage (90 days) with the mean value of 59.50% followed by intermediate harvesting stage (120 days) with the mean value of 56.18%. Significantly, lower IVOMD was recorded from grass harvested during the later stage (150 days). The results of the study were in agreement with those of Andualem and Hundessa, (2022), who claimed that Guatemalan IVDMD grass was superior in the early stages of maturity in relation to both mid and late-harvesting stages. Similarly, Kitaba and Tamir, (2007) reported that advancing harvesting stage significantly ($P\leq0.05$) decreased IVOMD and increasing nutrient levels significantly ($P\leq0.05$) increased IVDMD. The same author reported that IVOMD declined markedly with increased days of harvesting at the same or different levels of nutrients.

The result indicated that, IVOMD of Guatemala grass was better at the early days of maturity related to both mid and late harvesting stages. The fall in digestibility with the increasing harvesting stage is related to the drops in CP content and an increase in fibers portion (Saylor *et al*., 2021). The overall mean IVOMD of Guatemala grass recorded from the current study (57.03%) was higher than the value reported by (Andualem and Hundessa, 2022) who reported the mean IVOMD of the same grass species was 48.0%. The variation could be due to the difference in agro ecology, harvesting stage, management practice and soil properties.

Table 3. The effect of agro-ecologies and harvesting stage on chemical composition and in vitro organic matter digestibility

Factors	**Parameters**							
	DM (%)	**Ash (%)**	**CP (%)**	**NDF (%)**	**ADF (%)**	**ADL (%)**	**ME(MJ/Kg)**	**IVOMD (%)**
Agro-ecologies								
Mid	91.74	12.23	10.49[a]	63.87	38.37	5.35	8.18	57.45[a]
Low	91.63	12.74	8.68[b]	62.58	38.09	5.49	8.12	56.60[b]
Mean	91.68	12.49	9.59	63.22	38.23	5.42	8.15	57.03
Harvesting Stage								
90 days	91.62	12.66	11.4[a]	62.10[c]	36.95[c]	4.46[b]	8.48[a]	59.50[a]
120 days	91.69	12.63	9.47[b]	62.99[b]	38.41[b]	5.80[a]	8.04[b]	56.18[b]
150 days	91.74	12.16	7.83[c]	64.57[a]	39.33[a]	5.99[a]	7.93[c]	55.40[c]
Mean	91.68	12.49	9.59	63.22	38.23	5.42	8.15	57.03
Source of Variation								
Agro-ecologies	0.3358	0.0556	<0.0001	0.1232	0.2783	0.1803	0.1121	0.0055
Harvesting stage	0.6984	0.2196	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Agro-ecologies *harvesting stage	0.5996	0.5391	0.0054	0.0678	0.0011	0.0486	0.8947	0.6702
SEM	0.054	0.134	0.303	0.232	0.198	0.107	0.0.38	0.286
CV	0.438	7.899	23.277	2.684	3.807	14.590	3.464	3.696

The means in each column with different superscripts are significantly different at p<0.05. NS=Non-Significant, DM=dry matte, CP=Crude Protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, ADL=Acid detergent lignin, ME=Metabolizable energy AE = agro-ecologies, HS= Harvesting Stage, IVOMD=In vitro organic matter digestibility, and *** =significantly different at p<0.001

4.3 Correlation among morphological parameters of Guatemala grass

The linear correlation coefficient between morphological characteristics dry matter yield (DMY) is shown in Table 4. The number of tillers per plant (NTPP), number of leaves per plant (NLPP), number of nodes per plant (NNPP), internode length (INL), and dry matter yield (DMY) are all strongly linked positively with plant height (PH). Similarly, a strong and positive correlation was found between the number of tillers per plant (NTPP) and the number of Leafs per plant (NLPP) (r=0.897), number of nodes per plant (NNPP) (r=0.920), internode length (INL) (r = 0.888), and dry matter yield (DMY) (r = 0.936). DMY had a positive correlation (P<0.05) with all morphological characteristics of Guatemala grass. The positive association between dry matter yield with morphological parameters (plant height and leaf length per plant) may result from better competition for radiant energy with extended days to harvest. The current result indicates that plant height was strongly correlated with most of the morphological characteristics. This result agrees with (Andualem and Hundessa, 2022) and (Mijena *et al.*, 2022) for Guatemala grass and Brachiaria grass respectively.

Table 4: Pearson's correlation coefficients between morphological parameters

	PH	NTPP	NLPP	NNPP	INL	DMY
PH	1	.906**	.892**	.957**	.936**	.927**
NTPP		1	.897**	.920**	.888**	.936**
NLPP			1	.920**	.852**	.874**
NNPP				1	.935**	.942**
INL					1	.908**
DMY						1

**. Correlation is significant at the 0.01 level (2-tailed); PH=plant height, NTPP=number of tillers per plant, NLPP = number of leafs per plant, NNPP=number of nodes per plant, INL=internode length, and DMY=dry matter yield.

4.4 Correlations between Chemical Composition Parameter and IVOMD of *Guatemala* Grass

The relationship between nutritional composition and IVOMD of Guatemala grass are shown in Table 5. The current result revealed both positive and negative relationships. The DM content correlated positively with NDF (r = 0.041), ADF (r= 0.003), and ADL (r = 0.130). However, it was negatively associated with ash (r = -0.07), CP (r = -0.051), ME (r = -0.070), and IVOMD (r = -0.088). The positive correlation of Ash content with CP, ME, and IVOMD might be due to improper sample handling or laboratory analysis error. The ash content was correlated negatively with NDF (r = -0.130), ADF (r = -0.290), and ADL (r = -0.091). The CP content was significantly correlated positively with ME (r = 0.710) and IVOMD (r = 0.802), but it was correlated negatively with NDF (r = -0.415), ADF (r = -0.170), and significantly with ADL (r = -0.559). The direct positive correlation between CP content with IVOMD might be due to high crude protein concentration was obtained at the early harvesting stage. In line with this study, (Andualem and Hundessa, 2022) reported that the CP content was correlated positive with IVOMD (r=0.89), but it was correlated negative with NDF (-0.94), ADL (r=-0.84), cellulose (r=-0.92) and OM (r=-0.91). The same author suggested that the higher CP could supply an adequate protein base for microbial growth and improves digestibility.

The NDF content correlated positively with ADF (r = 0.259) and ADL (r = 0.395), but it was negatively associated with ME (r = -0.493) and IVOMD (r = -0.484). The result indicated that the negative impact of NDF on IVOMD is related to increasing ADL content with an advanced stage of maturity and increasing NDF content by itself. The ADF content was significantly correlated positively with ADL (r = 0.659), but it was significantly correlated negatively with ME (r = -0.515) and negatively with IVOMD (r = -0.503). The ADL content was significantly correlated with ME (r = -0.815) and IVOMD (r = -0.805). ME content was strongly positive and significantly correlated with IVOMD (r = 984). The analysis shows that ADF and ADL are positively correlated ($P<0.05$) with each other’s which is in concur with the finding of (Asmare et al., 2017). NDF, ADF, and ADL were negatively correlated with ME, IVOMD, CP, and ash. This indicates that there was a negative relationship between forage quality and growth stage.

Table 5. Pearson's correlation coefficients between nutritional qualities of Guatemala grass

Correlations

	DM (%)	**Ash (%)**	**CP (%)**	**NDF (%)**	**ADF (%)**	**ADL (%)**	**ME(MJ/Kg)**	**IVOMD (%)**
DM (%)	1	-.075	-.051	.041	.003	.130	-.070	-.088
Ash (%)		1	.394**	-.130	-.290*	-.091	.157	.248
CP (%)			1	-.415**	-.170	-.559**	.710**	.802**
NDF (%)				1	.259	.395**	-.493**	-.484**
ADF (%)					1	.659**	-.515**	-.503**
ADL (%)						1	-.815**	-.805**
ME (MJ/Kg)							1	.984**
IVOMD (%)								1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed), DM=Dry Matter, CP=Crude Protein, Ash, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber, ADL=Acid Detergent Lignin, IVOMD=Invitro Organic Matter Digestibility, ME=Metabolizable Energy.

4.4 Methane and Gas production Characteristics

Harvesting stage found to have significant (*P*<0.05) effect on total gas production during the first two incubation period (3hr and 6hr). The interaction of harvesting stage and agro-ecologies were found to have significant (*P*<0.05) effect on total gas production during 3hr, 24hr and 48hr. As indicated in Table 6, agro-ecologies, harvesting stage and their interaction have non-significant effect on total methane (CH4) produced after 72hr of incubation. The in-vitro gas volume between the two agro-ecologies revealed significant variation during 3hr, 6hr, 12hr and 24hr of incubation period. During this incubation period, significantly (*P*<0.05) higher volume of total gas was produced from low agro-ecologies. On the other hand, there is non-significant variation between the two agro-ecologies during 48hr, and 72hr of incubation. Similarly, agro-ecologies found to have non-significant (*P*>0.05) variation on methane production after 72hr of incubation. As incubation period increases, the volume of total gas production increases (Figure 3). The least gas volume was produced during early stage of harvesting (90 days) at 3hr and 6hr with the mean value of 2.89±0.44 and 8.83±0.75 respectively.

Table 6. Methane and gas production characteristics of Guatemala grass (ml/200gm DM) after 3, 6, 12, 24, 48, 72h incubation period in the study area.

Factors	Incubation period (hrs.)						Gas production characteristics				CH4 (ml)
	3hr	6hr	12hr	24hr	48hr	72hr	a	b	c	a+b	72hr
Agro-ecologies											
Lowland	5.74±0.57^{a}	11.18±0.58^{a}	20.33±1.24^{a}	30.52±1.75^{a}	49.26±2.59	59.18±2.52	29.64	65.46	0.021	95.10	6.25±0.62
Midland	4.00±0.45^{b}	8.74±0.82^{b}	15.63±1.04^{b}	25.63±1.47^{b}	45.33±2.03	55.25±2.18	28.92	77.53	0.017	106.44	4.94±0.49
Harvesting Stage (days)											
90	2.89±0.44^{b}	8.83±0.75^{b}	16.77±1.20	28.67±1.75	47.22±2.69	55.22±2.83	24.00^{b}	53.55^{b}	0.025^{a}	77.55^{b}	4.52±0.55
120	5.94±0.72^{a}	9.39±0.90^{ab}	17.00±1.70	26.67±2.69	47.55±3.33	57.44±3.34	32.69^{a}	90.05^{a}	0.015^{b}	122.74^{a}	6.11±0.71
150	5.78±0.52^{a}	11.67±0.95^{a}	20.16±1.51	28.88±1.58	47.11±2.67	59.00±2.57	31.14^{ab}	70.88^{ab}	0.017^{ab}	102.03^{ab}	6.16±0.78
Overall mean	4.87±0.37	9.96±0.52	17.98±0.87	28.07±1.18	47.29±1.65	57.22±1.67	29.28	71.49	0.019	100.77	5.60±0.40
Source of variation											
Agro-ecologies	0.0048	0.0116	0.0037	0.0280	0.2243	0.2332	0.7765	0.2992	0.1676	0.3785	0.1037
HS	0.0001	0.0394	0.1449	0.6536	0.9930	0.6373	0.0176	0.0430	0.0124	0.0210	0.1702
Agro-ecologies x HS	0.0230	0.0831	0.0622	0.0129	0.0304	0.0593	0.8830	0.0385	0.0647	0.0739	0.5914

CH4: methane; HS: Harvesting Stage

All the parameters of gas production characteristics of the Guatemala grass were not significantly ($P>0.05$) differed across the two agro-ecologies. On the other hand, different harvesting stage found to have significant ($P<0.05$) effect on all the parameters of gas production characteristics of the grass. In concur with the current study result, Andualem and Hundessa, (2022) reported that the fruits and pods exhibited the highest gas production from the soluble fraction (a) compared to the leaves among the indigenous legume fodder trees and shrubs species in the lowland. Moreover, Bezabih *et al*., (2014) reported that the parameters of gas production kinetics showed large variation between species, suggesting differences in the rate and extent of fermentation characteristics of the feeds. The discrepancy of the current study with previously reported studies could be due to variation in plant species used for the trial. The gas production characteristics reported for the soluble fraction (a), potentially degradable fraction (b), and the potential of gas production (a+b) reported for Guatemala grass was lower than the value reported by (Mosisa *et al*., 2021) for selected grass from southern Ethiopia. According to Robinson *et al*., (2006) the inter laboratory variation might explain the disparity of the results of the gas production characteristics among studies.

Significantly higher gas production parameters were recorded during intermediate harvesting stage followed by late harvesting stage. During the intermediate harvesting stage (120 days), the higher value of gas production observed from potentially degradable fraction (b) with the mean value of 90.05 compared to late and early harvesting stage. The higher value of gas production observed from potentially gradable fraction (b) could be due to the substantial cell wall constituent of the grass, which resists degradation at an early stage due to its ample ADL and makes plenty of substrate available for fermentation as incubation progresses (Abraham *et al*., 2023). The rate at which the substrates are digested in the rumen is as critical as the extent of digestion as it determines the DM intake of the ruminants (Uwineza *et al*., 2023). Abraham *et al*., (2023) reported optimal rate of fermentation for the leguminous plant L. spartum, despite the abundant wall constituents. Lignin is the key element that limits cell-wall digestibility, but cross-linkage of lignin and wall polysaccharides by ferulic acid bridges may be a prerequisite for lignin to exert its affect (Jung and Allen, 1995). Lignin composition and p-coumaric acid in the wall are less likely to affect digestibility.

Methane production of Guatemala grass exhibited no significance difference between the two agro-ecologies and harvesting stage, which could be due to their non-significance variation of gas production from potentially degradable fraction (b). The methane volume of Guatemala grass of the current study (5.60 ml) was comparable with the finding reported by Andualem and Hundessa, (2022) who reported 5.50ml value for selected indigenous legume fodder tree and shrubs in the semi-humid condition of the southern Ethiopia. On the other hand, Acacia tortilis leaf and Acacia Senegal leaf reported in the mid rift valley of Ethiopia was 7.08ml and 8.04ml which were higher than the current study (Sisay *et al*., 2017). The variation could be due to the difference in plant species, agro-ecologies, and season of harvesting. The mean total gas production volume increased steadily from 4.87ml after 3hr of incubation to 57.22ml after 72hr of incubation time (Figure 3 and 4).

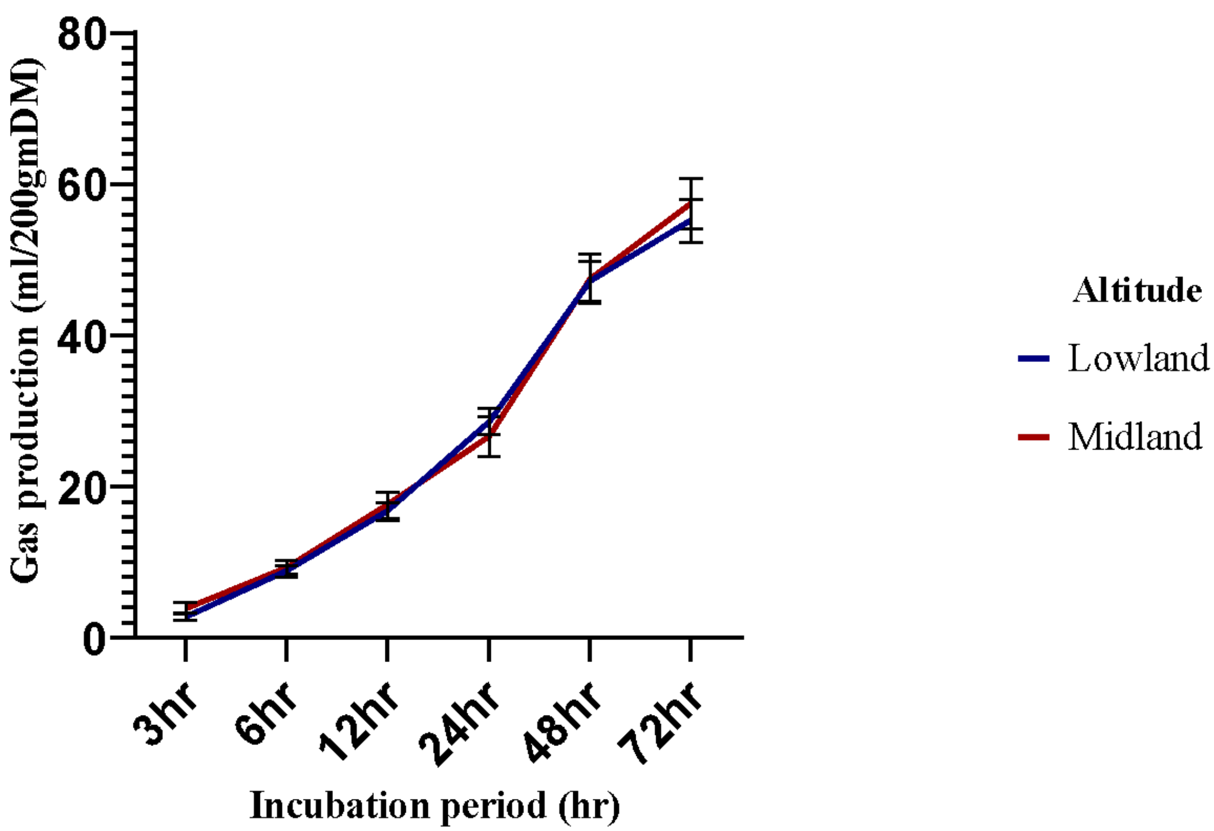

Figure 3. In-vitro total gas production from Guatemala grass at different agro-ecologies.

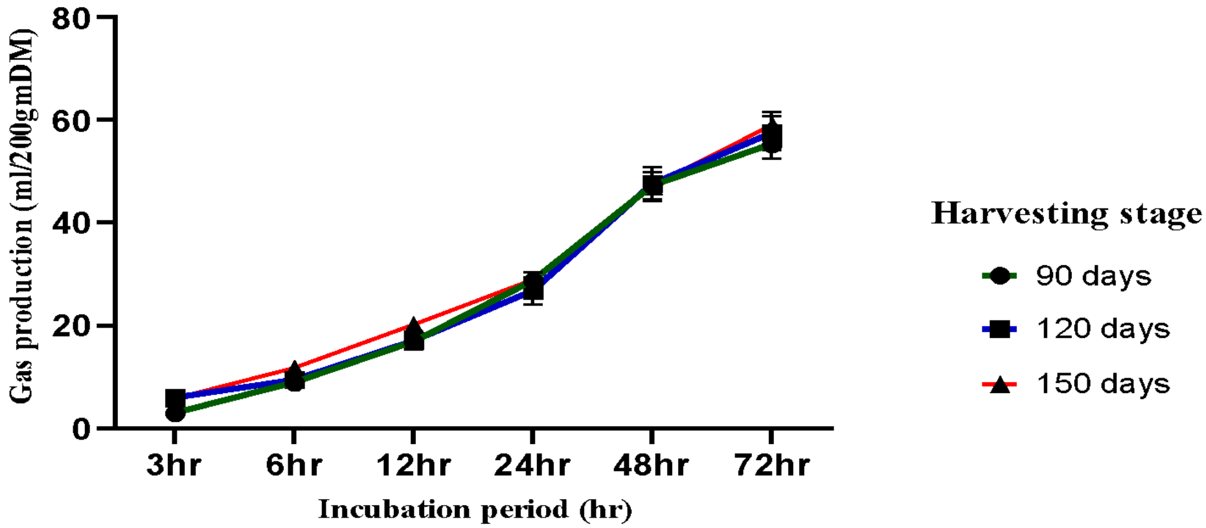

Figure 4. In-vitro total gas production from Guatemala grass at different harvesting stage.

The volume of total gas produced increased linearly with incubation time, in line with the observation that forage diets with a high in vivo passage rate (and thus less resident time in the rumen) are likely to produce less gas than those with a low passage rate (Bezabih *et al.*, 2014). Fibre quality and particle size of grasses are among the main factors that affect in vivo digestibility and passage rate of digesta, and thus may be targeted in the manipulation of the nutrition of ruminants (Owens and Basalan, 2016).

4.5 The Correlation between Gas Production Characteristics and Incubation Time

Methane production showed a significant positive correlation with incubation time after three hours. Moreover, it exhibited positive significant correlation with (b) and (a+b) unlike (a) which exhibited positive non-significant correlation. Fiber degradation has a substantial contribution to methane emission explained by the positive significant correlation of methane production with (b). Furthermore, the positive correlation of methane production with the incubation time after 3hr justified the substantial contribution of gas production from potentially degradable fraction (b) to methane emission.

As shown in table 7, soluble fraction (a) had non-significant ($p>0.05$) and negative correlation with different incubation time. In contradict with this result Andualem and Hundessa, (2022) reported that high significant correlation values of (a) with incubation time before 6hrs account for the high rate of gas production from the soluble fraction. On the other hand, potentially degradable fraction (b) had a significant ($P<0.05$) positive correlation with all incubation period and methane production. Likewise, (c) had a positive significant correlation with incubation time at 12hr (r=0.450) and 24hr (0.347). The positive significance correlation of (c) between 12hr and 24hr of incubation time suggests that the optimum rate constant of gas production of b (c) is due to the synergy between the optimal microbial population and the abundant substrate. The potential gas production (a+b) had strong and positive correlation with potentially degradable fraction (b) (r=0.996), CH_4 (r=0.551) and all different incubation time. As shown in Table 7, there is a positive and non-significant correlation between c and early incubation time (3hr, r= 0.089) and late incubation time (72hr, r=0.211). This positive non-significant correlation of (c) with early (3hr) and late (72hr) incubation time suggests the low rate of gas production of b (c) due to the impact of low microbial population and exhausted substrate respectively.

Table 7. Pearson correlation analysis of the gas production characteristics and incubation time (3h, 6h, 12 h, 24 h, 48 h and 72 h) of the Guatemala grass.

Correlations											
	CH4	**3hr**	**6hr**	**12hr**	**24hr**	**48hr**	**72hr**	**A**	**b**	**c**	**a+b**
CH4	1	.308*	.441**	.506**	.430**	.510**	.568**	-.192	.568**	.248	.551**
3hr		1	.789**	.601**	.413**	.415**	.475**	-.200	.475**	.089	.458**
6hr			1	.757**	.561**	.547**	.624**	-.094	.624**	.264	.616**
12hr				1	.888**	.832**	.830**	-.051	.830**	.450**	.826**
24hr					1	.933**	.879**	-.007	.879**	.347*	.878**
48hr						1	.970**	-.021	.970**	.249	.969**
72hr							1	-.048	1.000**	.211	.996**
a								1	-.048	.062	.038
b									1	.211	.996**
c										1	.216
a+b											1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.6 The Correlation between Nutrients, Total Gas and Methane Production

Dry matter content had neither positive nor negative significant correlation with all chemical composition, IVOMD, total gas production and methane (Table 8). On the other hand, ash had a strong negative correlation with ADF (r=-0.290). Crude protein had strong and negative correlation with NDF (r=-0.415) and ADL (r=-0.559), whereas strong positive correlation with ME (r=0.710) and IVODM (r=0.802). Furthermore, CP content had weak and negative correlation with total gas (r=-0.048) and CH4 (r=-0.224). In line with this study, Bezabih et al. (2014) reported that CP had strong negative correlation with NDF (r=-0.65) and ADF (r=-0.58). NDF showed strong positive correlation with ADL (r=0.395), whereas strong negative correlation with ME (r=-0.493) and IVOMD (r=-0.484). Likewise, ADF exhibited strong negative correlation with ME (r=-0.515) and IVOMD (r=-0.503), while strong positive correlation with ADL (r=0.659). ADL had significant inverse correlation with ME (r=-0.815) and IVOMD (r=-0.805), whereas positive correlation was found between ADL and CH4 (r=0.284). ME showed a strong positive correlation with IVOMD (r=0.984), week negative correlation with total gas (r=0.068) and CH4 (r=-0.282). Total gas produced had a strong and positive correlation with CH4 (r=0.517). Methane was a constituent of gas produced during in vitro microbial fermentation, explaining its positive correlation with total gas production. Various studies substantiated the positive correlation of methane and gas volume (Bezabih *et al.,* 2014; Andualem and Hundessa, 2022). Over the incubation period, the chemical components crude protein, metabolizable energy and in-vitro organic matter digestibility degraded steadily, explaining their inverse correlation with total gas and methane production. In line with this study result, Huang *et al.,* (2019); Souhil *et al.,* (2022) reported that the negative relationship between the cell wall component degradation and gas volume.

Table 8: Pearson correlation (r) analysis among the nutrients, gas and methane production of Guatemala grass.

Correlations										
	DM	ASH	CP	NDF	ADF	ADL	ME	IVOMD	Total gas	CH4
DM	1	-.075	-.051	.041	.003	.130	-.070	-.088	.016	-.008
ASH		1	.394**	-.130	-.290*	-.091	.157	.248	-.180	-.255
CP			1	-.415**	-.170	-.559**	.710**	.802**	-.048	-.224
NDF				1	.259	.395**	-.493**	-.484**	.210	.201
ADF					1	.659**	-.515**	-.503**	.252	.206
ADL						1	-.815**	-.805**	.167	.284*
ME							1	.984**	-.068	-.282*
IVOMD								1	-.083	-.278*
Total gas									1	.517**
CH4										1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

5. CONCLUSION AND RECOMMENDATION

The findings of this study indicated that harvesting stages significantly influences the morphological traits and dry matter yield of Guatemala grass. As the harvesting stage is extended, there is an increase in all morphological parameters and DMY. Morphological traits like plant height, number of nodes per plant, and DMY are also affected by differences in agro-ecologies. On the other hand, except for CP content and IVOMD, agro-ecological variations have no significant effect on the chemical composition parameters and ME of the grass. Additionally, besides ash and DM, the chemical composition parameters and IVOMD are affected by the stage of harvesting. With increased harvesting stage, NDF, ADF and ADL increase, while CP, ME, and IVOMD decrease. Guatemala grass, when incubated *in vitro*, produces a significant amount of gas, with the volume and methane production varying depending on the agro-ecologies and stage of harvest.

Significantly higher ($p<0.05$) gas production was produced at low agro-ecologies compared to mid agro-ecologies, particularly during the 3 to 24-hour incubation period. In the initial 3 and 6 hours of incubation, a significantly greater volume of total gas was generated from grass harvested in its later maturity stages. Additionally, there is a linear increase in the volume of total gas produced as the duration of the incubation period extends. Gas production from the degradable portion (b) of the Guatemala grass had a substantial impact on the overall gas volume throughout the incubation period and on methane emissions after 72 hours. The CP content in forage grass shows a strong negative correlation with fiber content, while it demonstrates a strong positive correlation with IVOMD and ME.

Methane had a strong positive correlation with total gas production during *in vitro* microbial fermentation. Guatemala grass harvested from mid agro-ecologies and harvested at early harvesting stages are superior in CP, IVOMD and ME. The proximate composition, IVOMD and gas production potential of Guatemala grass varied significantly with agro-ecologies and harvesting stage. Harvesting Guatemala grass at the proper growth stage is crucial for forage management. Intermediate to later harvesting date are recommended for optimum biomass yield and plant morphological traits, whereas early (90 days) to intermediate harvesting date (120 days) are recommended for crude protein content and IVOMD.

The current study revealed that Guatemala grass performed well both at midland and lowland agro-ecologies in southern Ethiopia. Overall, Guatemala grass in this study area is characterized as moderate quality to support ruminant livestock. Therefore, it can be concluded that it has potential as an alternative ruminant feed in mid and lowland agro-ecologies in southern Ethiopia.

To fully utilize the potential of Guatemala grass, further research is needed to describe the changes in feeding values across different season and agro-ecologies on agronomic and chemical composition involving live-animal experiments are recommended.

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7. APPENDICES

Appendix 1: Field pictures of plant growth

Appendix 2: Field picture during observations of plant morphology


Appendix 3: Grass sample picture that prepared for oven dry

APPENDIX 4: ANOVA for PH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	10765.51333	2153.10267	145.41	<.0001
Error	12	177.68667	14.80722		
Corrected Total	17	10943.20000			

R-Square	Coeff Var	Root MSE	PH Mean
0.983763	3.801135	3.848015	101.2333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	514.13556	514.13556	34.72	<.0001
HS	2	10015.21333	5007.60667	338.19	<.0001
ALT*HS	2	236.16444	118.08222	7.97	0.0063

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	514.13556	514.13556	34.72	<.0001
HS	2	10015.21333	5007.60667	338.19	<.0001
ALT*HS	2	236.16444	118.08222	7.97	0.0063

APPENDIX 5: ANOVA for NTPP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	81.17333333	16.23466667	32.95	<.0001
Error	12	5.91246667	0.49270556		
Corrected Total	17	87.08580000			

R-Square	Coeff Var	Root MSE	NTPP Mean
0.932108	8.945580	0.701930	7.846667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	2.16320000	2.16320000	4.39	0.0580
HS	2	78.22773333	39.11386667	79.39	<.0001
ALT*HS	2	0.78240000	0.39120000	0.79	0.4744

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	2.16320000	2.16320000	4.39	0.0580
HS	2	78.22773333	39.11386667	79.39	<.0001
ALT*HS	2	0.78240000	0.39120000	0.79	0.4744

APPENDIX 6: ANOVA for NLPP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5501.932000	1100.386400	19.81	<.0001
Error	12	666.571200	55.547600		
Corrected Total	17	6168.503200			

R-Square	Coeff Var	Root MSE	NLPP Mean
0.891940	10.55072	7.453026	70.64000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	2.16320000	2.16320000	4.39	0.0580
HS	2	78.22773333	39.11386667	79.39	<.0001
ALT*HS	2	0.78240000	0.39120000	0.79	0.4744

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	2.16320000	2.16320000	4.39	0.0580
HS	2	78.22773333	39.11386667	79.39	<.0001
ALT*HS	2	0.78240000	0.39120000	0.79	0.4744

APPENDIX 7: ANOVA for DMY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	305.6588667	61.1317733	123.30	<.0001
Error	12	5.9493333	0.4957778		
Corrected Total	17	311.6082000			

R-Square	Coeff Var	Root MSE	DMY Mean
0.980908	7.980146	0.704115	8.823333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	13.6242000	13.6242000	27.48	0.0002
HS	2	291.9065333	145.9532667	294.39	<.0001
ALT*HS	2	0.1281333	0.0640667	0.13	0.8800

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	13.6242000	13.6242000	27.48	0.0002
HS	2	291.9065333	145.9532667	294.39	<.0001
ALT*HS	2	0.1281333	0.0640667	0.13	0.8800

APPENDIX 8: ANOVA for (DM %)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.45761481	0.09152296	0.54	0.7445
Error	48	8.12640000	0.16930000		
Corrected Total	53	8.58401481			

R-Square	Coeff Var	Root MSE	DM Mean
0.053310	0.448761	0.411461	91.68815

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	0.16006667	0.16006667	0.95	0.3358
HS	2	0.12247037	0.06123519	0.36	0.6984
ALT*HS	2	0.17507778	0.08753889	0.52	0.5996

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	0.16006667	0.16006667	0.95	0.3358
HS	2	0.12247037	0.06123519	0.36	0.6984
ALT*HS	2	0.17507778	0.08753889	0.52	0.5996

APPENDIX 9: ANOVA for (Ash %)

R-Square	Coeff Var	Root MSE	Ash Mean
0.146381	7.668861	0.957855	12.49019

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	3.53177963	3.53177963	3.85	0.0556
HS	2	2.87155926	1.43577963	1.56	0.2196
ALT*HS	2	1.14862593	0.57431296	0.63	0.5391

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	7.55196481	1.51039296	1.65	0.1659
Error	48	44.03933333	0.91748611		
Corrected Total	53	51.59129815			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	3.53177963	3.53177963	3.85	0.0556
HS	2	2.87155926	1.43577963	1.56	0.2196
ALT*HS	2	1.14862593	0.57431296	0.63	0.5391

APPENDIX10: ANOVA for (CP %)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	183.2399204	36.6479841	21.75	<.0001
Error	48	80.8755778	1.6849079		
Corrected Total	53	264.1154981			

R-Square	Coeff Var	Root MSE	CP Mean
0.693787	13.53509	1.298040	9.590185

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	44.4811130	44.4811130	26.40	<.0001
HS	2	119.1025148	59.5512574	35.34	<.0001
ALT*HS	2	19.6562926	9.8281463	5.83	0.0054

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	44.4811130	44.4811130	26.40	<.0001
HS	2	119.1025148	59.5512574	35.34	<.0001
ALT*HS	2	19.6562926	9.8281463	5.83	0.0054

APPENDIX11: ANOVA for (NDF %)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	82.8787944	16.5757589	13.82	<.0001
Error	48	57.5594889	1.1991560		
Corrected Total	53	140.4382833			

R-Square	Coeff Var	Root MSE	NDF Mean
0.590144	1.731944	1.095060	63.22722

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	22.51697963	22.51697963	18.78	<.0001
HS	2	56.38990000	28.19495000	23.51	<.0001
ALT*HS	2	3.97191481	1.98595741	1.66	0.2016

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	22.51697963	22.51697963	18.78	<.0001
HS	2	56.38990000	28.19495000	23.51	<.0001
ALT*HS	2	3.97191481	1.98595741	1.66	0.2016

APPENDIX 12: ANOVA for (ADF %)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	67.5962444	13.5192489	14.51	<.0001
Error	48	44.7360889	0.9320019		
Corrected Total	53	112.3323333			

R-Square	Coeff Var	Root MSE	ADF Mean
0.601752	2.524881	0.965402	38.23556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	1.12089630	1.12089630	1.20	0.2783
HS	2	51.68754444	25.84377222	27.73	<.0001
ALT*HS	2	14.78780370	7.39390185	7.93	0.0011

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	1.12089630	1.12089630	1.20	0.2783
HS	2	51.68754444	25.84377222	27.73	<.0001
ALT*HS	2	14.78780370	7.39390185	7.93	0.0011

APPENDIX13: ANOVA for (ADL %)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	26.19200926	5.23840185	36.02	<.0001
Error	48	6.97997778	0.14541620		
Corrected Total	53	33.17198704			

R-Square	Coeff Var	Root MSE	ADL Mean
0.789582	7.032573	0.381335	5.422407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	0.26881667	0.26881667	1.85	0.1803
HS	2	24.98589259	12.49294630	85.91	<.0001
ALT*HS	2	0.93730000	0.46865000	3.22	0.0486

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	0.26881667	0.26881667	1.85	0.1803
HS	2	24.98589259	12.49294630	85.91	<.0001
ALT*HS	2	0.93730000	0.46865000	3.22	0.0486

APPENDIX14: ANOVA for (ME)

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	5	3.15274815	0.63054963	28.16	<.0001
Error	48	1.07477778	0.02239120		
Corrected Total	53	4.22752593			

R-Square	**Coeff Var**	**Root MSE**	**ME Mean**
0.745767	1.835368	0.149637	8.152963

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
ALT	1	0.05867407	0.05867407	2.62	0.1121
HS	2	3.08908148	1.54454074	68.98	<.0001
ALT*HS	2	0.00499259	0.00249630	0.11	0.8947

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
ALT	1	0.05867407	0.05867407	2.62	0.1121
HS	2	3.08908148	1.54454074	68.98	<.0001
ALT*HS	2	0.00499259	0.00249630	0.11	0.8947

APPENDIX15: ANOVA for (IVOMD)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	181.0131426	36.2026285	31.88	<.0001
Error	48	54.5165111	1.1357606		
Corrected Total	53	235.5296537			

R-Square	Coeff Var	Root MSE	IVOMD Mean
0.768537	1.868672	1.065721	57.03093

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ALT	1	9.6182241	9.6182241	8.47	0.0055
HS	2	170.4782370	85.2391185	75.05	<.0001
ALT*HS	2	0.9166815	0.4583407	0.40	0.6702

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ALT	1	9.6182241	9.6182241	8.47	0.0055
HS	2	170.4782370	85.2391185	75.05	<.0001
ALT*HS	2	0.9166815	0.4583407	0.40	0.6702