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**TOPIC: INFLUENCE OF PHOSPHORUS FERTILIZER AND STORAGE DURATION ON THE PROXIMATE COMPOSITION OF HAY MADE FROM STYLOSANTHES GUIANENSIS.**

**INTRODUCTION**

Ruminants production has been faced with the limitation of high quality and quantity forage due to prolonged annual dry season that negatively affects plant's performance.In order to mitigate poor ruminant nutrition problems, Olanite (2002) and Ojo *et al*.(2015a) suggested that the use of sown and purposely managed pastures could helpraise ruminant productivity.Livestock are an important livelihood strategy in most developing countries (Otte *et al*. 2005; Otieno *et al*. 2006; Rao *et al*. 2009) by providing meat, milk, manure, hides, draught power and collateral (financial asset, insurance). Smallholder cattle farming is the major source of livelihood for over 54% of poor people in sub-Saharan Africa (IFPRI 2007) and over 70% of the population in Pakistan is directly involved with livestock as a primary source of food and/or income (Devendra and Thomas 2002; Thomas *et al*. 2002). Furthermore smallholder livestock farms are the major meat and milk producers in developing countries of Asia and Africa. Despite their importance, livestock are usually undernourished due to lack of feeds of sufficient quality and quantity; the consequences of which are low production, increased disease susceptibility, higher mortality rates and reduced fertility (Xue *et al*. 2005).

Nigeria is one of the four leading livestock producers in Sub-Sahara Africa. In the early 1990s,estimated livestock population in Nigeria was 14 million cattle, 23 million goats and 13 millionsheep (RIM, 1990). However, these figures have since increased to 15.2 million cattle, 28 milliongoats and 23 million sheep (FAO, 2006). Livestock productivity is low because of poor nutrition,which is primarily derived from natural pastures and limited amounts from crop residues. Nativepastures are the most widely available low cost feeds for ruminants in the tropics (Tchinda *et al*.,1993). The native pastures deteriorate rapidly as the season advance towards the dry season.Forage legumes can be defined as members of the Fabaceae(Leguminosae) that have plant parts other than separated grainthat are used to feed ruminant livestock (Barnes *et al.*, 1995;Graham and Vance, 2003). They are generally grazed or offered

as silage or hay, and can be grown as monocultures or mixtureswith other species, most commonly grasses. Fabaceae areextremely diverse and widespread, with an estimated 16,000 to

17,000 species in 750 genera (Allen, 1981). The use of herbaceous or tree legumes have been reported (Ezenwa and Aken'ova, 1988; Bamikole and Ezenwa, 1999). Legumes are able to return soil fertility back by converting natural atmospheric air nitrogen and deposit it into the soil. They are rich in protein which is usually the most limitingnutrients in tropical animal diets (Andrea and Pablo, 1999). A sustainable way of improving the feeding value of poor quality pastures is throughsupplementation with forage legumes (Andrea and Pablo, 1999). The capability to fix nitrogen intothe soil and as such, enhancing the crude protein content of forage with resultant increase in yieldand feed quality, make legumes an integral part of pastures (Aribisala, 2003; Sanginga andMulongoy, 1992).Forage quality can be defined as the extent to which a forage has the potential to produce a desired animal r*es*ponse (Ball *et al.*, 2001) .Animal production is greatly influence by the quality of the forage on offer rather than the quantity, as such,tropical forage legume are rich in protein which is usually the most limiting nutrient in tropical animal diet.

Phosphorus is a primary nutrient essential for plant growth and development and important for regulation of various enzymatic deficiency of phosphorus in soil has an adverse impact on legume production as it is required for energy transformation in nodules and enhanced N-fixation (Rotaru and Sinclair 2009; Udvardi and Poole 2013; Yadav *et al.* 2017). Phosphorus also plays a crucial role in the development of the symbioticrelationship between legumes and bacteria as a certain amount of P is requiredto carry out biological nitrogen fixation (BNF) (Oliveira *et al.* 2002; Rotaru andSinclair 2009). There is considerable evidence that nodulated legumes require moreP than nonsymbiotic plants grown solely on a mineral N source (Rotaru and Sinclair

2009; Sulieman and Schulze 2010).A large amount of P is required for metabolic pathways of energy transfer that takes place during nodule functioning (Hernandez *et al.* 2009; Cabeza *et al.* 2014a, b).But most of the agricultural soils have inadequate amounts of P to support efficient

BNF (Brown *et al.* 2013). The inadequacy of P in soil is mainly due to its retention as adsorbed P on the surface of soil particles and associated with amorphous aluminum (Al) and iron (Fe) oxides (Mitran and Mani 2017). About 90% of the inorganic P fertilizers are used in agriculture crop production produced from high-grade rock phosphates which expected to be depleted shortly within 30–50 years (Abrol and Palaniappan 1988; Cordell and Drangert 2009). So there will be possibilities of less vegetative growth and production of legumes as P availability expected to decrease shortly as the growth of the N-fixing legumes severely affected under P-deficient condition due to poor nodule functioning (Sulieman and Tran 2015; Dhakal *et al.*2016).So there is a need to improve P resources to better legume crop productivity and soilsustainability through increasing efficiency in legumes.

Laboratory analyses are used to determine the nutritive value of forages (Ball *et al.*, *2001*). The concentration of chemical componenets in forage varies depending forages on some factors such as plant species (Ramirez *et al.* 2004),fertilization (Tϋrk *et al.*, 2007)harvesting date (Ball *et al.*, 2001),soil properties and other enviromental variable (Kulik, 2009).Therefore,this study is to evaluate the influence of phosphorus fertilizer as well storage duration on the proximate composition of hay made from *Stylosanthes guianensis.*

**1.1 Broad objective**

To evaluate the influence of phosphorus fertilizer and storage duration on the proximate composition of hay made from *Stylosanthes guianensis.*

**1.2 Specific objectives**

To determine:

1. The influence of phosphorus fertilizer (poultry manure, single super phosphate (SSP) and control) on proximate composition (Dry Matter(DM), Crude Protein(CP), Ether Extract(EE) and Ash) of hay made from *Stylosanthes guianensis.*
2. The influence of storage duration (0, 2 and 4 months) on proximate composition (DM, CP, EE and Ash) of hay made from*Stylosanthes guianensis.*

**2.0 LITERATURE REVIEW**

**2.1 Description and morphology of Stylosanthes *guianensis***

Stylo (*Stylosanthes guianensis*)is a tropical legume shrub widely grown for forage throughout the tropics andSubtropics.Stylo is a short-lived, erect or semi-erect perennial legume that can reach a height of 1-1.5 m. Stylo has a strong taproot that is nodulated. The stems are many-branched and may be woody at the base. Stylo does not twin, unlike other legumes. Stylo is a leafy species that remains green under dry conditions. The leaves are trifoliolate with elliptical to lanceolate leaflets, 0.5-45 mmlong x 20 mm broad. The inflorescence is a densely flowered spike, with up to 40 flowers/head. Flowers are yellow to orange with black or red stripes. The fruit is a one-seeded pod, 2-3 mmlong x 1.5-2.5 mm wide. The seed are very small, pale brown or purple in colour (US Forest Service, 2014; Cook *et al.*, 2005; Mannetje, 1992). There are 7 varieties of stylo, notably var.*guianensis* (common stylo) and var. *intermedia* (fine stem stylo) (Mannetje, 1984). Fine stem stylo has finer and shorter stems than the common stylo (1-2 mm in diameter and only 30 cm in height). Its inflorescence is a denser cluster and the seeds are yellowish brown (Cook *et al.*, 2005; Mannetje, 1984).

**2.2 Distribution of *Stylosanthes guianensis***

*Stylosanthes guianensis* is native to Central and South America, where it is used in the manner of alfalfa. It was naturalized in many tropical and subtropical areas where it became a popular legume forage (Cook *et al.*, 2005; Mannetje, 1992). However,its cultivation largely ceased after an outbreak of anthracnose in the 1970s and only resumed in the 1990s, after anthracnose resistant lines were developed and released commercially.Stylo is found from 20°N to 32°S, and from sea level up to an altitude of 2000 m (Cook *et al.*, 2005; Mannetje, 1992). Stylo can grow in places where annual rainfall ranges from 700 to 5000 mm, but it does better between 1000 and 2500 mm for common stylo and between 600 and 1800 mm for fine stem stylo. Stylo is a warm season growing legume that thrives in places where annual temperatures are between 23 and 27°C. However, stylo can survive light frost (0°C) and can remain productive down to 15°C. Fine stem stylo has more frost tolerance than common stylo. Stylo does well in most soils from sands to light clays (including those that are relatively infertile or deprived of P) provided they are well-drained. Soil pH ranging from 4 to 8.3 is acceptable to var. *Guianensis,* which also has some tolerance of Al and Mn. Fine stem stylo prefers neutral soils. Stylo is no salt tolerant. It is a full light species (Cook *et al.*, 2005).

**2.3 Management of Stylosanthes *guianensis***

**2.3.1 Establishment**

Stylo can be sown alone or mixed with companion species. In Australia, it is often oversown in native grasslands (Partridge,2003). Stylo can be sown in plots (7-12 seeds/plot) and should not be buried as the seeds are very small. Stylo can be broadcast when overseeded in grassland. In humid areas, stylo can be sown at any time provided that there is no dry period during its establishment. In drier parts, it should be sown as soon as possible after the start of the rainy season, and at least two months before the rain stops (Husson *et al.*, 2008). Stylo is a high yielding forage legume that can produce 10-20 t DM/ha depending on soil fertility (Cook *et al.*, 2005).

Stylo can be mixed with tropical grasses such as *Brachiaria* spp., *Andropogon gayanus*, *Chloris gayana*, *Digitaria eriantha*,*Heteropogon contortus*, *Hyparrhenia rufa*, *Melinis minutiflora*, *Pennisetum purpureum* or *Setaria sphacelata*. It can be out shaded when it is sown with Guinea grass (*Megathyrsus maximus*) (Cook *et al.*, 2005). Stylo is not often sown with other legumes but it can be intercropped with rice, maize or cassava, depending on soil fertility (Husson *et al.*, 2008).

**2.3.2 Grazing**

Stylo can be grazed but it is sensitive to heavy grazing. It should not be grazed until 6-8 weeks after sowing. Rotational grazing is preferable with 4-8 week rest intervals (Skerman *et al.*, 1990). Stylo has been used to improve the nutritive value of natural grasslands in Australia (Partridge, 2003).

1. **Cut-and-carry system**

Stylo can be easily cut and then fed fresh to livestock. It is, however, not very palatable when young and it is advised to wilt it to soften its bristles before offering it to the animals (Skerman *et al.*, 1990).

1. **Hay and silage**

Stylo can make valuable hay but should be handled carefully so that it does not shed its leaves. For sward longevity, stylo should not be cut below 20 cm and no more than once a year (Skerman *et al.*, 1990). Stylo may be used as silage when ensiled with salts and molasses (FAO, 2014).

**2.4 Environmental impact of Stylosanthes *guianensis***

**2.4.1 Soil improver**

Stylo is a N-fixing legume that readily nodulates and improves soil N mineral status. It is able to extract P from soils that are very poor in this nutrient and it is tolerant of low Mo levels. In Laos, stylo fallow increased rice yield and decreased weed biomass (Saito *et al.*, 2006). In Nigeria, a stylo fallow preceding a maize crop resulted in a yield of maize similar to that obtained with the addition of 45 kg N/ha (Tarawali, 1991).

**2.4.2 Weed controller**

Stylo was reported to control weeds such as *Striga asiatica*, *Rottboellia exaltata*, *Borreria alata*, *Boerhavia diffusa* and *Imperata cylindrica* (Husson *et al.*, 2008).

**2.5 Nutritional attributes *Stylosanthes guianensis***

The palatability of Stylosanthes *guianensis* increases with maturity. Fresh young stylo is not very palatable to livestock, possibly due to the bristles borne on the stems (Skerman *et al.*, 1990; FAO, 2014). Stylo has a low palatability during the rainy season, but is readily eaten in the dry season. If kept short, it does not become woody but remains leafy and palatable (Göhl,1982). Satisfactory intake was observed in zebu cattle when stylo was fed fresh as a supplement (Pen *et al.*, 2013). Stylo seems to be more palatable when it is wilted before being stall-fed to cattle (Skerman *et al.*, 1990; FAO, 2014).*Stylosanthes guianensis* has a variable protein content, which is usually moderate (about 14% DM) but can be as low as 6% or exceed 20% DM. The fibre content is quite high (more than 25% DM crude fibre). Stylo contains condensed tannins (Baloyi *et al.*, 2001; Thang *et al.*, 2010). Stylo is a valuable forage legume for ruminants, usually fed as hay (or sun-dried), cut-and-carry forage, or grazed. Like other legumes, *Stylosanthes guianensis* is often used as a supplement during the dry season to improve the nutritive value of low quality forages, including crop residues or by-products such as rice straw (Thang *et al.*, 2010), maize stover (Said *et al.*, 1993),or other locally available forages (Matizha *et al.*, 1997; Akinlade *et al.*, 2002; Kiyothong *et al.*, 2004; Pen *et al.*, 2013).

The organic matter digestibility of Stylosanthes *guianensis* ranges between 51 and 67% which is low compared to other tropical legumes (Gardener *et al.*, 1982; Mupangwa *et al.*, 2000; Magalhaes *et al.*, 2003). It has a high proportion of moderately soluble proteins compared with other tropical legumes (Magalhaes *et al.*, 2003).

In Thailand, *Stylosanthes guianensis* hay used in association with cassava hay in moderate amounts (1-2 kg/d), as a supplement for dairy cows fed a basal forage of moderate quality reduced significantly the amount of concentrate without altering milk production (Kiyothong *et al.*, 2004). Stylo has a relatively high fibre content, and thus a low energy value estimated at 7.2-7.6 MJ/kg DM (Bai Changjun *et al.*, 2004), which prevents it from being a major ingredient for poultry diets. However, small amounts of stylo leaf meal have been tested to partially replace other ingredients such as bran. In Nigeria, with starter broiler chicks, growth performance was

depressed by the inclusion of 5% stylo leaf meal, although this result was not constant (Onwudike *et al.*, 1979a; Onwudike *et al.*, 1979b). In growing and finishing broilers, levels of 5% seemed safe, and higher levels have been offered successfully in Nigeria, China and India (Onwudike *et al.*, 1979b; Bai Changjun *et al.*, 2004; Krishna *et al.*, 2008). A Chinese trial showed a trend towards lower performance with increasing stylo from 3 to 6% and 9% (Bai Changjun *et al.*, 2004). In layers, good quality stylo meal (more than 20% protein) did not significantly decrease laying rate when used below 10% of the diet (Onwudike etal., 1978).In general, stylo leaf meal should preferably be limited to 2-5% in broilers, and feeding stylo to young birds should be avoided.Stylo was fed successfully at a higher rate to other bird species: 8-12% to ducks and 15-20% to geese (Bai Changjun *et al.*,2004). When formulating diets with stylo, special care should be taken to ensure correct energy levels and amino acid balance.

In less intensive production systems, stylo may be fed at higher levels and used as green forage for broilers or layers (Gupta etal., 1992).Fresh stylo is frequently used as a cut-and-carry fodder for rabbits by smallholders in Asia (Phaikaew *et al.*, 2004), and Africa(Nigeria, Odeyinka *et al.*, 2007). Stylo with a moderate to low protein content (less than 15% DM) used as sole forage is not able to support maintenance or growth (Adegbola *et al.*, 1985; Raharjo *et al.*, 1986). On the contrary, when the protein level is high (19-20% DM), stylo can be used as sole feed for growing rabbits. In Nigeria, fresh *Stylosanthes guianensis* forage provided better growth rate (8.0 g/d *vs.* 6.7 g/d) than *Lablab purpureus* forage, or the fresh leaves of Mexican sunflower (*Tithonia diversifolia* Hemsl.) (Omole *et al.*, 2007). This difference in ability of stylo forage to sustain growth is probably related to the low protein digestibility in mature plants (54%, Raharjo *et al.*, 1985) compared to that of young forage (protein digestibility 70%, protein 20% DM, when cut every 40-45 days, Omole *et al.*, 2007), which is slightly higher than that observed dehydrated alfalfa of the same protein content (Perez *et al.*, 1998). The low protein level associated with a low protein digestibility exacerbates the deficiency of *Stylosanthes guianensis* in supplying sulphur-containing amino acids and lysine: 56% and 86% of requirements, respectively (Lebas, 2004).As a source of protein and fibre, *Stylosanthes guianensis* can be used profitably to supplement other less fibrous fresh foragessuch as water spinach (Khuc Thi Hue *et al.*, 2006) or to supplement concentrate diets (Hongthong Phimmasan *et al.*, 2005a;Jin *et al.*, 2007; Iyeghe-Erakpotobor *et al.*, 2008).Dried stylo has been used safely in balanced diets for growing rabbits and breeding does. Inclusion levels were 25-30% for growth and reproduction (Fomunyam *et al.*, 1984), or 40% for growth (Harsris *et al.*, 1981). Stylo is a source of calcium but its low phosphorus content requires supplementation with minerals or ingredients rich in phosphorus, such as wheat bran.

**2.6 Importance of Stylosanthes *guianensis***

*Stylosanthes guianensis* cv. Cook is one of the forage legumes well suited to the sub humid tropical zone with a marked dry season (Heuzé, *et al.*, 2015). Bogdan (1977) and Mannetje and Lones (1992) pointed out that *S. guianensis* is a tropical herbaceous perennial legume, primarily used for pasture in humid tropical regions. Mannetje and Lones (1992) further stated that over sowing of *S. guianensis* improves the quality of tropical rangeland and it can also be used as a cover crop, green manure crop and as fallow crop. The crop has been reported to be palatable to livestock when mature and can be easily established on very low fertile soils. Thang *et al.* (2010) stated that *S.guianensis* is used as a supplementary feed during dry season to improve the nutritive value of low quality crop residues. Heuzé, *et al.* (2015) reported yield of 10 - 20 t DM ha-1for *S. guainensis*. Magalhaes *et al.* (2003) found that the Organic Matter Digestibility (OMD) of *S. guianensis* was in the range of51 - 67% by goats. Dry matter and crude protein digestibilities in West Africa Dwarf sheep were reported to be 71.82 and 71.80% respectively (Ogunbode and Akinlade, 2012). It has become important to evaluate forage yield and quality of Stylo (*S.guianensis cv.* cook) at different stages of growth to determine the optimum stage of growth for which the forage crop could be harvested for livestock feeding either as pasture, hay or silage.

**2.7 Sources of Available Phosphorus for Leguminous Crops**

A number of phosphatic fertilizers are available based on their solubility (Ghosal and Chakraborty 2012). The available phosphate can be defined by their solubility either in water or in neutral or alkaline ammonium citrate (Ghosal and Chakraborty 2012). It varies from country to country; some are using water to extract available P from fertilizer or by dissolving it in citrate or both. These definitions are not always adequate for evaluation of fertilizer availability for alkaline and calcareous soils. In calcareous soil, where pH is in the higher range, water solubility of P is hindered (Leytem and Mikkelsen 2005). Some of the highly water-soluble phosphate fertilizers are monocalcium phosphates, phosphoric acids, ammonium ortho- and polyphosphates, etc., whereas calcium metaphosphates, di- and tricalcium phosphates, and basic slag are not soluble in water but are citrate soluble (MacKay *et al.* 1990; Yadav *et al.* 2017). Apatites are major components of source rock phosphate that are insoluble even in ammonium citrate (Chien *et al.* 2011).Phosphatic fertilizers are either ordinary superphosphate (approximately 16% P205) or concentrated superphosphate (43–46% P205 approximately); both are predominantly monocalcium phosphates (Ca [H2P04]2) with relatively small amounts of iron

and aluminum phosphates and dicalcium phosphate (CaHP04). Orthophosphoric acid as a phosphate (55% P205) fertilizer is very effective in calcareous and alkaline soils where the Ca content is large enough to prevent undesirable acidification. The solubility of ammonium phosphate fertilizers is higher than superphosphate fertilizers. The N and P content of fertilizer grade monoammonium phosphate (MAP) and diammonium phosphate (DAP) is approximately 12% and 18% N and 61% and 46% P205, respectively. These fertilizers are industrially attractive having a high nutrient content, the low tendency for caking, and low hygroscopicity. Whereas the nitric phosphate fertilizers are highly hygroscopic and citrate soluble which contains 4–13% P and 14–20% N. The nitric phosphate fertilizers are effective in neutral, alkaline, and calcareous soils as a P source to plants is a function of the ratio of water- to citrate-soluble phosphate.

**2.8 Phosphorus Use Efficiency in Legumes**

The PUE is low in agriculture soils. When P is applied to the soil through a source of fertilizer or organic manure, it undergoes several biochemical reactions which remove phosphate ions from the soil solution (Kruse *et al.* 2015). It is measured that only 15–30% of applied fertilizer P is taken up by crops in the year of its application (Swarup 2002; Syers *et al.* 2008). However, the remaining 70–90% becomes part of the soil P pool, which is fixed but subsequently released to the crop over the following months and years (Roberts and Johnston 2015). Improving the PUE for growth in legume crops requires enhanced P acquisition from the soil and enhanced use of P in processes that lead to faster growth and a greater allocation of biomass to the harvestable parts (Kruse *et al.* 2015). In biomass calculations, measurements are often restricted to the above ground portion of plant parts in leguminous crops. The PUE is the amount of total biomass produced per unit of P uptake (Hammond *et al.* 2009; Varma and Meena *et al.* 2016). Intraspecies and large genotypic differences for PUE are well known for different legumes such as cowpea (*Vignaunguiculata* L.; Sanginga *et al.* 2000), soybean (*Glycine max* L.; Furlani *et al.* 2002; Jemo *et al.* 2006), faba bean (*Vicia faba* L.; Daoui *et al.* 2012), and common bean (*Phaseolusvulgaris* L.; Vadez *et al.* 1999).

**2.9 Adaptive Strategies to Overcome Phosphorus Deficiency**

**for Better Nitrogen-Fixation and Legume Productivity**

There is a need to develop some adaptive strategies which can help to conserve the supply of P under the deficient condition and enhance legume productivity (Veneklaas *et al.* 2012; Meena *et al.* 2015d). The adaptive response of nodule metabolism to P deficiency is crucial to improving symbiotic efficiency under P-deficient situations (Esfahani *et al.* 2014). There are a number of adaptive strategies such as P-homeostasis in nodule, increasing P acquisition, upgrading N-fixation per unit of nodule mass, and consumption per unit of nodule mass which compensate for the reduction in the number of nodules (Vance *et al.* 2003; Lopez-Arredondo *et al.* 2014; Sulieman and Tran 2015). However, the molecular mechanism is including maintenance of the P-homeostasis in nodules for rhizobia-legume symbiosis emerging as a main adaptive strategy for P-deficient soil (Sulieman and Tran 2015).The main concept of such strategies is to conserve more P concentration in the nodule which can maintain a high rate of N-fixation (Graham 1992; Nogales *et al.*2000; Dhakal *et al.* 2016). There are several ways to P stabilization in the symbiotic tissue such as including higher P allocation to nodules, the formation of a strong P sink in nodules, direct P acquisition via nodule surface and P remobilization from organic-P containing products (Sulieman and Tran 2015). Several studies have shown that symbiotic N-fixation could continue without any disturbance if total plant P is estimated to be allocated toward nodule up to 20% (Jebara *et al.* 2005; Tajini *et al.* 2009). Nodules represent a preferential strong sink for P incorporation during P starvation among the other plant parts (Le Roux *et al.* 2008; Hernandez *et al.* 2009). Formation of cluster root and mycorrhizas also plays a key role in

N-fixation by increasing root surface area and exudation of an organic acid and hence enhanced P acquisition during low P supply (Schulze *et al.* 2006; Tajini *et al.* 2009). Remobilization of organic P within the plant by encoding acid phosphatase (Qin *et al.* 2012; Zhang *et al.* 2014) is also an important biochemical and physiological adaptive strategy to P deficiency.

**3.0 MATERIALS AND METHODS**

**3**.**1** **Experimental site**

The field work will be carried out at the Pasture unit of the Directorate of University Farms (DUFARM), while the laboratory analysis will carried out at the laboratory of the Department of Pasture and Range Management both at Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria.

**3.2 Land preparation**

The land will be cleared manually using cutlasses and hoe, and stumps will be removed on the total area, which will be allowed to rest for two weeks before making seed bed. A total experimental land measuring 336 m2 (28 m x 12 m) will be mapped out and pegged, having the main plot measuring (9 m x 2 m) and the sub-plots of (2 m x 2 m). A boundary of 2 m will be made between blocks.

**3.2.1 Soil Sample Collection and Analysis**

After land preparation and before sowing, soil sample will be randomly collected from representative spots of the entire experimental field by using diagonal sampling method. The samples will be collected at the depth of 0-15cm using soil auger. The samples will be bulked per replicate, mixed thoroughly and sub- samples will be taken for analysis to determine the pre- sowing nutrients status of the soil.

**3.2.2 Sourcing of Fertilizers, Analysis and Application**

Fertilizers to be used are poultry manure and SSP. The poultry manure will be sourced from the poultry unit of Directorate of University Farms (DUFARMS) and SSP from an agro-allied store in Igboora, Oyo state. Sub samples will be taken from the total manure (Poultry) collected from the farm and other fertilizers, which will be thoroughly mixed and air dried for laboratory analysis in order to determine the phosphorus content of the manure. The rate of application for the fertilizers will be 80kgP/ha. The quantity of each manure applied will be calculated based on the chemical composition (i.e. phosphorus) content of the fertilizers.

**3.2.3 Sourcing of sowing materials, scarification and sowing.**

Seeds of *Stylosanthes guianensis* will be sourced from the National Animal Production Research Institute (NAPRI) Shika,Ahmadu Bello University, Zaria. A day before planting, the legume seeds will be scarified, using hot water method, the seeds will be placed in a clothed bag and immersed in hot water (80°C) for 3-4 minutes. The seeds will be thereafter air-dried before sowing (Olanite, 1997).

The seeds will be sown by drilling based on the treatment allotment at a seed rate of 7kg/ha. Drilling of the seeds will be achieved by sowing the seeds along each row within the plots at spacing of 50 cm apart.

**3.3 Experimental design and plot measurement**

The study will be a 4 x 2 factorial arrangement laid out as a split – plot design with an experimental area measuring 336m2. The sources of phosphorus will be assigned to the main plot, while the storage duration adopted will be assigned to sub-plots.

**3.4 DATA COLLECTION**

**3.4.1 Harvesting of forage materials**

Samples will be harvested at 10 weeks after sowing, Sub samples of 1kg will be taken from the herbage, weighed and packed for hay production.

**3.4.2 Hay production**

Sub samples (2kg) form harvested biomass will be gently packed in nets for hay production which will be labelled accordingly. Drying will be done conventionally i.e. spreading the legume on the open floor for gradual dehydration with the aid of solar radiation.

**3.4.3 Storage and sampling of hay**

The hay will be stored in a cool dry condition for the period of four (4) months. The hay produced will be sampled at intervals of (0,2,4 months). Samples will be taken to determine the proximate composition of hay.

**3.5 Chemical analysis**

The dried sample will then be mill to pass through a 1mm sieve screen. The sample will then be analyze for:

1. **Proximate composition:** The dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash will be determined according to A.O.A.C(2000).

**3.6 Statistical analysis**

Data collected will be analyzed using two-way analysis of variance.

**3.6.1 Statistical model**

Yijkl = µ + Mi + Sj + (MS)ij  +*ɛ*ijkl

Where;

Yijkl  = Observation value of the dependent variable

µ = Population mean

Mi = Effect of the varing sources of phosphorus fertilizer

Sj = Effect of storage duration.

(MS)ij = Interaction effect of varying phosphorus sources and the storage duration.

*ɛ*ijkl = Random residual error

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