

Influence of phosphorus sources and storage duration on physical characteristics and fibre fractions of *Stylosanthes guianensis* hay

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Abstract

Phosphorus is a primary nutrient essential for plant growth and development for regulation of various enzymatic activities while 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. However, most of the agricultural soils have inadequate amounts of P to support efficient biological nitrogen fixation. So there is a need to review P resources to better legume crop productivity and soil sustainability through increasing efficiency in legumes. A research was conducted to evaluate physical characteristics and fibre fractions of hay made from *Stylosanthes guianensis* as influenced by phosphorus sources and storage duration. The study was a 3 x 3 factorial arrangement laid out as split-plot design comprising of three phosphorus sources (SSP, poultry and control) as the main plot while the three storage duration (0, 2 and 4 months) the sub plot with three replicates. Result showed that physical characteristics evaluated as influenced by phosphorus sources were significant ($p < 0.05$) except for foreign materials. The unfertilized *Stylosanthes guianensis* hay (12.28) and those fertilized with poultry manure (12.96) recorded higher leafiness than those fertilized with SSP (10.39). The values for color were higher in the unfertilized legume hay (12.65). Odour values ranged from 14.14 in the legume hay fertilized with SSP to 14.77 in those fertilized with poultry manure. However, leafiness and colour were the only parameters influenced by the interaction of phosphorus sources and storage duration. The highest leafiness was recorded for *Stylosanthes guianensis* hay fertilized with poultry manure stored for zero month (14.00) and those fertilized with SSP and stored for two months (13.96). The highest and lowest Neutral detergent fibre (NDF) and Acid detergent lignin (ADL) values were recorded for poultry fertilized legume hay (51.55% and 8.41%) and those fertilized with SSP (46.58% and 7.83). The effect of storage duration on the fibre fractions of *Stylosanthes guianensis* hay followed a similar trend. NDF (52.53%), ADF (17.10%), ADL (7.50%), and hemicellulose (34.43%) were significantly higher for the legume hay stored for four months and lower in those stored for 0 months (48.53%, 16.17%, 7.30%, and 29.37%, respectively). The interaction of phosphorus sources and storage duration was only significant on NDF and hemicellulose. *Stylosanthes* hay stored for zero and two months are more palatable due to their high leaf content, while both SSP and poultry manure enhance hay leafiness during storage, this implies that the interaction of these factors could provide animals with sufficient nutrient uptake from the higher number of leaves recorded.

Keywords: Fibre fractions, legumes, *Stylosanthes*, hay, phosphorus

Influence des sources de phosphore et de la durée de stockage sur les caractéristiques physiques et les fractions de fibres du foin de *Stylosanthes guianensis*



Résumé

*Le phosphore est un nutriment primaire essentiel à la croissance et au développement des plantes pour la régulation de diverses activités enzymatiques, tandis que la carence en phosphore dans le sol a un impact négatif sur la production de légumineuses car il est nécessaire pour la transformation de l'énergie dans les nodules et l'amélioration de la fixation de l'azote. Cependant, la plupart des sols agricoles ont des quantités insuffisantes de phosphore pour soutenir une fixation biologique efficace de l'azote (Brown et al. 2013). Il est donc nécessaire de revoir les ressources en P pour améliorer la productivité des cultures de légumineuses et la durabilité des sols en augmentant l'efficacité des légumineuses. Une recherche a été menée pour évaluer les caractéristiques physiques et les fractions de fibres du foin fabriqué à partir de *Stylosanthes guianensis* en fonction des sources de phosphore et de la durée de stockage. L'étude était un arrangement factoriel 3 x 3 présenté sous forme de parcelle divisée comprenant trois sources de phosphore (SSP, volaille et contrôle) comme parcelle principale tandis que les trois durées de stockage (0, 2 et 4 mois) la sous-parcelle avec trois répétitions. Les résultats ont montré que les caractéristiques physiques évaluées comme étant influencées par les sources de phosphore étaient significatives ($p < 0,05$) sauf pour les matières étrangères. Le foin de *Stylosanthes guianensis* non fertilisé (12,28) et ceux fertilisés avec du fumier de volaille (12,96) ont enregistré une foliation plus élevée que ceux fertilisés avec du SSP (10,39). Les valeurs de couleur étaient plus élevées dans le foin de légumineuses non fertilisé (12,65). Les valeurs d'odeur variaient de 14,14 dans le foin de légumineuses fertilisé avec SSP à 14,77 dans ceux fertilisés avec du fumier de volaille. Cependant, le feuillage et la couleur étaient les seuls paramètres influencés par l'interaction des sources de phosphore et de la durée de stockage. La foliation la plus élevée a été enregistrée pour le foin de *Stylosanthes guianensis* fertilisé avec du fumier de volaille stocké pendant zéro mois (14,00) et ceux fertilisés avec du SSP et stockés pendant deux mois (13,96). Les valeurs les plus élevées et les plus basses de fibres détergentes neutres (FDN) et de lignine détergente acide (LDA) ont été enregistrées pour le foin de légumineuses fertilisé à la volaille (51,55 % et 8,41 %) et ceux fertilisés avec SSP (46,58 % et 7,83). L'effet de la durée de stockage sur les fractions de fibres du foin de *Stylosanthes guianensis* a suivi une tendance similaire. Le FDN (52,53 %), l'ADF (17,10 %), le LDA (7,50 %) et l'hémicellulose (34,43 %) étaient significativement plus élevés pour le foin de légumineuses stocké pendant quatre mois et plus faibles pour ceux stockés pendant 0 mois (48,53 %, 16,17 %, 7,30 % et 29,37 %, respectivement). L'interaction des sources de phosphore et de la durée de stockage n'était significative que sur le FDN et l'hémicellulose. Le foin de *Stylosanthes* stocké pendant zéro et deux mois est plus agréable au goût en raison de sa forte teneur en feuilles, tandis que le SSP et le fumier de volaille améliorent la feuillaison du foin pendant le stockage, cela implique que l'interaction de ces facteurs pourrait fournir aux animaux une absorption suffisante de nutriments du nombre plus élevé de feuilles enregistrées.*

Mots-clés : Fraction fibreuse, légumineuses, *Stylosanthes*, foin, phosphore

Introduction

Livestock production is one of the fastest-growing agricultural sub-sector in developing countries (Ewetola, 2018). In Nigeria, it contributes 6-8% of the GDP and 20-25% of agricultural GDP (Aishat, 2016). The demand for livestock and their by-products will continue to increase due to population growth and urbanization. However, prolonged yearly dry seasons, on the other hand, have a detrimental impact on plant performance, resulting in a restricted quantity and poor quality of available fodder at this time. This results in lower voluntary feed intake, nutrient utilization, and lower overall ruminant animal performance (Ojo *et al.*, 2020a). Ruminants relish forages which give them the energy needed to grow, reproduce and stay healthy. They depend on pasture for their nutrition than any other feed resource (Aderinola *et al.*, 2008). Hence, forages must be available for livestock consumption during the rainy and dry season to enhance sustainable livestock production. Hay production is an essential method of forage conservation during lush to provide feed resource when grasses become scarce. On the other hand, inclement weather frequently stymies hay production when forages are due for preservation, as the hay must be thoroughly dried for efficient preservation (Michael, 1999). This appears to have deterred farmers from producing and using hay for animal feed, particularly in the humid tropics, where silage production and use has an advantage over hay for farmers who can afford the necessary infrastructure (Ewetola *et al.*, 2021; Ojo *et al.*, 2020b; Usman *et al.*, 2021). Several animal manures have been described as beneficial for soil amendment and increasing the nutritional quality of tropical plants (Ewetola *et al.*, 2016; Ojo *et al.*, 2015b). Potential soil amendments for agricultural and pasture plants include swine manure (Ojo *et al.*, 2020a), cattle dung (Jimoh *et al.*, 2019), farmyard manure

(Ahmed *et al.*, 2012), and poultry droppings (Amisu *et al.*, 2019). As a result, organic manure fertilization of pastureland fits nicely into the context of nutrient recycling and environmental protection (Jimoh *et al.*, 2019) while improving the yield and quality of forages (Amisu *et al.*, 2019; Idowu *et al.*, 2020; Ojo *et al.*, 2020b). Moreover, studies on how the length of storage influence hay quality in southwestern Nigeria is relatively limited. This suggests the need for more studies in this front to identify how long hay can be stored in this region.

Stylosanthes is a vital fodder legume that has been widely utilized for livestock feed. It is a phosphorus-loving plant (Hanson and Heering, 1994; Nkaa *et al.*, 2014). It is native to South America and widely cultivated in South-East Asia, Africa, and Australia, where many cultivars had been developed to suit local conditions (Mannetje, 1992). *Stylosanthes guianensis* is particularly suited for forage in subhumid tropical and subtropical areas with a marked dry season. It is used for hay, cut-and-carry systems and pasture (Cook *et al.*, 2005). In this sense, understanding the better source of P for *stylosanthes* cultivation and the storage duration of the ensuing hay could help increase the availability of feed resources for ruminants during the off-season. Thus, the present study aims to determine the influence of different sources of P fertilizer and storage duration on physical characteristics and fibre fractions of hay produced from *S. guianensis*.

Materials and methods

Experimental site

The field experiment was carried out at Pasture unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria, located in the derived savannah zone of the southwest Nigeria on latitude 7° 10' N, longitude 3° 2'E and altitude of 76m above sea level while the

laboratory experiment took place at the Department of Pasture and Range Management laboratory, FUNAAB.

Land preparation

The land was ploughed and allowed to rest for two weeks before it was harrowed. The experimental land measuring 336m² (28mx12m) was mapped out and pegged, with the main plot (9mx2m) and the sub-plot (2mx2m). A boundary of 2m was set out between blocks Prior to planting, soil samples were randomly collected using diagonal method from the field at depth of 0–15cm using soil auger to represent the topsoil. The samples were bulked per replicate, mixed thoroughly and sub-samples were analyzed to determine the pre-planting nutrient status of the soil.

Sourcing and application of fertilizers

The soil amendments used were poultry manure and Single superphosphate (SSP). Poultry manure was sourced from the poultry unit, the Directorate of University Farms (DUFARMS) and SSP from a reputable agro-allied store at Igboora, Oyo state. The poultry manure and SSP were applied at 80kgP/ha. The application rate was calculated based on the fertilizers' chemical composition (i.e., P content).

Sourcing of sowing materials, scarification and sowing

Seeds of *S. guianensis* were sourced from the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University, Zaria. The legume seeds were scarified with hot water (80°C) a day before planting. (Olanite, 1997). The seeds were sown by broadcasting at the rate of 7kg/ha.

Experimental design

The study was a 3×3 factorial arrangement laid out in a split-plot design with an experimental area measuring 336m². The sources of phosphorus (SSP, poultry and control) were assigned to the main plot, while the storage duration (0, 2 and 4 months) adopted was assigned to the sub-plot. The inter plot and intra-plot spaces

were kept weed free throughout the experimental period by hand weeding.

Sample harvesting and hay production

Samples were harvested 10 weeks after sowing. The herbage samples (2 kg) collected on the field were packed into nets and labeled and conserved as hay. Drying was carried out conventionally by spreading the legume on the open floor for gradual dehydration under solar radiation.

Laboratory analysis

Fibre fraction such as Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined with the procedure of Van Soest *et al.* (1991). Cellulose content was taken as the difference between ADF and ADL while hemicellulose content was calculated as the difference between NDF and ADF.

Statistical analysis

Data collected were subjected to two-way analysis of variance (ANOVA), and the treatment means were separated using Duncan's Multiple Range Test of SAS (1999) package.

Results

Table 1 shows the effects of phosphorus sources and storage duration on the physical characteristics of hay produced from *stylosanthes guianensis*. The unfertilized *Stylosanthes guianensis* hay (12.28) and those fertilized with poultry manure (12.96) recorded higher leafiness than those fertilized with SSP (10.39). The values for color was higher in the unfertilized legume hay (12.65) and lower in those fertilized with poultry manure (11.78). Odour values ranged from 14.14 in the legume hay fertilized with SSP to 14.77 in those fertilized with poultry manure. However, no effect of phosphorus sources was observed on foreign materials in the legume hay. For storage duration, leafiness was similar at 0 (13.09) and two (2) months (13.88) but significantly lower at four (4) months (8.47). The values recorded for colour was

similar for 0 and 2 months, and the least was observed for the legume hay stored for four months. Odour and foreign materials were not influenced by storage duration. Only leafiness and colour were influenced by the interaction of phosphorus sources and storage duration (Table 2). The highest leafiness was recorded for *Stylosanthes guianensis* hay fertilized with poultry manure and stored for 0 months (14.00) and those fertilized with SSP and stored for (2) months (13.96). The legume hay fertilized with SSP and stored for 0 months, and those fertilized with poultry manure and stored for four (4) months recorded statistically similar values. The lowest leafiness was recorded for the unfertilized legume hay stored for four (4) months. Table 3 shows phosphorus sources and storage duration effects on the fibre fractions of hay produced from *Stylosanthes guianensis*. The highest and lowest NDF and ADL were recorded for poultry fertilized legume hay (51.55% and 8.41%) and those fertilized with SSP (46.58% and 7.83%). In contrast, ADF was higher in the legume hay fertilized with SSP (18.75%) and the least was recorded for the unfertilized hay

(17.63%). Phosphorus sources did not influence hemicellulose and cellulose. The effect of storage duration on the fibre fractions of *Stylosanthes guianensis* hay followed a similar trend. NDF (52.53%), ADF (17.10%), ADL (7.50%), and hemicellulose (34.43%) were significantly higher for the legume hay stored for four (4) months and lower in those stored for 0 months (48.53%, 16.17%, 7.30%, and 29.37%, respectively). There was no effect of storage duration on cellulose. The interaction of phosphorus sources and storage duration was significant on NDF and hemicellulose but not the legume hay's ADF, ADL, and cellulose content. The highest and lowest NDF was recorded for the unfertilized legume hay stored for 0 months (62%) and those fertilized with poultry manure and stored for 0 months (34%). However, the *Stylosanthes guianensis* hay fertilized with SSP and stored for 0 months (50%) and two (2) months (50%) recorded statistically similar values. Hemicellulose was ($p < 0.05$) higher in poultry fertilized legume hay stored for four (4) months (40%) and lower in those fertilized with the same manure and stored for 0 months (12%).

Table 1: Effects of phosphorus sources and storage duration on physical characteristics of hay produced from *Stylosanthes guianensis*

Factors	Leafiness	colour	odour	Foreign material
Phosphorus sources				
Control	12.28 ^a	12.65 ^a	14.67 ^b	8.33
SSP	10.39 ^b	11.92 ^b	14.13 ^c	10.00
Poultry	12.96 ^a	11.78 ^c	14.77 ^a	9.43
SEM	1.07	1.22	1.67 ^r	1.00
Storage duration				
0 months	13.09 ^a	12.30 ^a	17.00	8.50
2 months	13.88 ^a	14.18 ^a	16.43	7.00
4 months	8.47 ^b	9.14 ^b	13.33	6.90
SEM	0.79	0.96	1.13	1.00

^{a,b}: means with same superscript are not significant

SEM = Standard error of mean

Table 2: Interaction effects of phosphorus sources and storage duration on physical characteristics of hay produced from *Stylosanthes guianensis*

		Leafiness	colour	odour	Foreign material
Control	0 Months	8.00 ^e	13.00 ^b	12.67	10.00
	2 Months	7.00 ^f	12.33 ^c	11.33	11.67
	4 Months	5.00 ^g	10.00 ^f	10.00	9.00
SSP	0 Months	11.63 ^c	11.84 ^d	17.67	10.00
	2 Months	13.96 ^a	15.26 ^a	16.00	10.00
	4 Months	9.67 ^d	8.48 ^g	15.90	8.00
Poultry	0 Months	14.00 ^a	15.00 ^a	15.00	7.00
	2 Months	13.67 ^b	13.00 ^b	13.67	7.08
	4 Months	11.95 ^c	11.00 ^e	11.63	6.50
SEM		0.67	0.64	0.53	0.43

a,b,c, d,e, f, g: means with same superscript are not significant

SEM = Standard error of mean

Table 3: Effect of phosphorus sources and storage duration on the fibre fraction (%) of hay from *Stylosanthes guianensis*

Factors	NDF	ADF	ADL	Hemicellulose	Cellulose
Phosphorus sources					
Control	49.00 ^b	17.63 ^c	8.17 ^b	29.17	7.67
SSP	46.58 ^c	18.75 ^a	7.83 ^c	25.53	8.92
Poultry	51.55 ^a	18.08 ^b	8.41 ^a	31.67	7.68
SEM	2.05	1.70	0.97	2.73	1.95
Storage duration					
0 months	48.53 ^c	16.17 ^c	7.30 ^c	29.37 ^c	7.47
2 months	51.00 ^b	16.25 ^b	7.47 ^b	31.83 ^b	7.50
4 months	52.53 ^a	17.10 ^a	7.50 ^a	34.43 ^a	7.45
SEM	1.36	1.18	0.62	1.82	1.29

a,b,c: means in the same column with different superscript are significantly different (p<0.05)

SEM: Standard error of mean

NDF = Neutral detergent fibre

ADF = Acid detergent fibre

ADL = Acid detergent lignin

Table 4: Interaction effects of phosphorus sources and storage duration on the fibre fractions (%) of hay from *Stylosanthes guianensis*

Factors		NDF	ADF	ADL	Hemicellulose	Cellulose
Control	0 Months	62.00 ^a	23.00	4.67	37.00 ^b	16.33
	2 Months	48.00 ^d	28.33	6.33	17.67 ^h	20.00
	4 Months	52.00 ^b	15.00	8.00	35.00 ^c	5.00
SSP	0 Months	50.00 ^c	18.00	4.67	30.00 ^d	11.33
	2 Months	50.00 ^c	19.00	9.07	29.00 ^e	7.93
	4 Months	43.00 ^f	19.00	8.00	22.00 ^f	9.00
Poultry	0 Months	34.00 ^g	20.00	10.00	12.00 ⁱ	8.00
	2 Months	46.00 ^c	22.00	11.67	22.00 ^g	8.33
	4 Months	55.00 ^{ab}	13.00	8.63	40.00 ^a	3.37
SEM		2.22	2.16	1.62	2.12	2.87

a,b,...,f: means in the same column with different superscript are significantly different (p<0.05)

SEM: Standard error of mean

NDF = Neutral detergent fibre

ADF = Acid detergent fibre

ADL = Acid detergent lignin

Discussion

In the tropics and subtropics, legumes as an essential component of ruminant feed are widely acknowledged, especially in the dry season (Li *et al.*, 2017; Ojo *et al.*, 2015a). Forage nutritive value is essential in appraising the suitability of fodder for livestock feeding. When forages are conserved as hay, visual examination of the conserved fodder could help determine the possibility of livestock deriving sufficient nutrients from it. In this sense, factors such as colour, leafiness, proportion and coarseness of stems, odour, moulds, and the presence of foreign materials are considered (Salama and Zeid 2016; Marsalis *et al.* 2009). In this study, leafiness was higher in the unfertilized *Stylosanthes guianensis* hay and those fertilized with poultry manure than in the legume fertilized with SSP. This has the consequence that the former is of higher quality than the latter in terms of leafiness. The result further implies that the unfertilized and poultry manure fertilized hay will have higher nutrient content because most nutrients in plant tissues are found in leaves (Bosworth and Hudson 2017; Corvallis 2016).

The hay stored for 0 and two (2) months are better than those produced at four (4) months storage because the former had more leaves. Hay leafiness is related to palatability (Marsalis *et al.*, 2009), and leaves are more digestible than stems. Hence, the *Stylosanthes* hay stored for 0 and two (2) months are more palatable due to their high leaf content. Furthermore, both SSP and poultry manure enhanced hay leafiness at 0 and 2 months' storage, respectively. This implies that the interaction of these factors could provide animals with sufficient nutrient uptake from the higher number of leaves recorded. Although hay colour does not indicate good quality (Ewetola, 2018), it is an index of vitamin A that confers proper curing

(Marsalis *et al.*, 2009). Hay colour may signify overheating (i.e., bleaching) or microbial activity that leads to heat generation due to microbial respiration in the process of drying (Ewetola, 2018). This study shows that hay colour was in the order control > SSP > poultry. This suggests that the phosphorus sources adopted in this study did not enhance hay colour compared to the control. Notably, the mean hay colour across the treatment suggests bleaching at the surface, which may decline its acceptability by animals. Concerning storage duration, the results indicate that hay colour can be retained for up to two (2) months, beyond which the colour will deteriorate into golden yellow, possibly due to microbial activities (Bosworth and Hudson, 2017). The study found that hay color was better at the interaction level, though with little bleaching, in the *stylosanthes* fertilized with SSP and poultry manure. Hence, hay from these treatments would be more acceptable by animals than others.

Odour depicts the kind of fragrance that will be perceived by animals when consuming cured hay. It is primarily affected by the moisture content in the forage at bailing (Rocateli and Zhang, n.d.). The unfertilized and P-fertilized legume hay produced in this study had the smell of newly mown hay. This means that the hays are generally of good quality. However, hays that smell mildew or off-odor will have lower quality and not be readily acceptable by animals. The NDF is a cell wall content that comprises cellulose, hemicellulose, lignin, silica, and minerals (Hancock *et al.*, 2014). A forage quality index is directly related to animals' dry matter intake (Ojo *et al.*, 2017). In this study, the higher NDF recorded for the legume hay fertilized with poultry manure is below 65%, the value generally known to impairs forage intake by ruminants (Van Soest, 1994; Muia, 2000). However, the NDF values recorded across

the treatments are higher than 39.1% reported for 'Stylo 184' by (Kiyothong and Wanapat, 2004).

The legume hay stored for four (4) months had higher NDF than those stored for 0 and two (2) months, respectively. However, the NDF values across the storage period are higher than the range of 38.48% to 41.06% reported for *Medicago lupulina*, *Trifolium repens* and two other legumes in Turkey. Further, the values are higher than 44.38% reported for *Vicia sativus* by Tuna *et al.* (2004). The observed differences may be related to variation in growing sites, climate, fertilization, and the legume crops utilized in these studies. Nevertheless, all the NDF values recorded for the interaction of the studied factors are sufficient to promote forage intake by ruminants. The ADF of forage is related to digestibility. Notably, forages with low digestibility will lack the potential to meet livestock nutrient requirements, necessitating supplementation, which increases production costs (Hancock, 2014). The SSP fertilized legume hay had a higher ADF than other treatments, which will reduce its digestibility when consumed by animals. Further, ADF increases with increasing storage duration, depicting a decline in quality as storage advances. However, the ADF values recorded in this study rank the legume hay as prime (<31% ADF) according to the American Forage and Grassland Council (Marsalis *et al.*, 2009). Lignin, a non-digestible cell wall component prevents microbial enzymes from breaking down cellulose and hemicellulose in the rumen (Hancock, 2014). Therefore, the higher lignin recorded for the legume hay fertilized with poultry manure implies lower digestibility. This may impair the ability of the conserved forage hay to provide ruminants with sufficient energy following consumption. The results also show that lignin content in the Stylosanthes hay increases with storage

duration. Hence, further research is needed to examine possible ways of reducing lignin build-up during hay storage. Hemicellulose is a structural carbohydrate that is only slightly digestible in the rumen of animals, though slowly. Therefore, the high hemicellulose content recorded at four (4) months storage may further slow down its digestion and possibly reduce, if not prevent, animals from deriving energy from it. Moreover, poultry manure fertilized legume hay stored for four (4) months is less digestible than other treatments due to high lignin content. However, the same treatment stored for 0 months could better provide animals with energy.

Conclusion

The study showed that there were variation effects from the fertilizer and storage duration as stylosanthes hay stored for 0 and two (2) months were more acceptable due to their high leaf content, while both single super phosphate and poultry manure enhance hay leafiness at 0 and 2 months' storage, respectively. This implies that the interaction of these factors could provide animals with sufficient nutrient uptake from the higher number of leaves recorded. However, poultry manure fertilized legume hay stored for four (4) months is less digestible than other treatments due to high lignin content as results also showed that acid detergent fibre and lignin content in the stylosanthes hay increases with storage duration depicting a decline in quality as storage advances.

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