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RESEARCH ARTICLE

Productivity and nature of compatibility in mixed pasture of *Brachiaria decumbens* cv. Kluang comel and *Stylosanthes guianensis* CIAT 184 under different planting ratio

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ABSTRACT

This study aimed to evaluate the productivity and compatibility of *Brachiaria decumbens* cv. Kluang Comel (Kluang Comel) and *Stylosanthes guianensis* CIAT 184 (Stylo 184) under varying planting ratios. Five treatments with different ratios of Kluang Comel and Stylo 184 were tested (100G:0L, 75G:25L, 50G:50L, 25G:75L and 0G:100L). Forages were manually harvested from each polybag after seven weeks following the initial cut-back, and samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) content. Results showed that the 50:50 planting ratio of Kluang Comel and Stylo 184 yielded the highest biomass per hectare. The 75:25 planting ratio favored Kluang Comel, producing the highest leaf percentage in the grass component, while the 25:75 ratio produced the highest leaf percentage in the legume component. Increased crude protein content was observed in the 50:50 and 25:75 ratios due to the higher proportion of legume. Additionally, the 25:75 ratio yielded the highest calcium percentage. In conclusion, the 50:50 mixture of Kluang Comel and Stylo 184 is the most advantageous planting ratio, optimizing both yield and protein content. Further research is recommended to assess the longevity and nitrogen fixation capacity of the legume component to support long-term productivity in forage systems.

1. INTRODUCTION

Out of 3.4 billion hectares of the world's permanent grasslands, the tropics account for 1.3 billion hectares as either wild or cultivated fodder plants (Thomas 1995). At the same time, animal production from pastures is generally low in most developing countries compared to developed countries, which indicates insufficiency in nutrients that were provided by pastures in the most tropics (Sakadevan and Nguyen 2017). The concept of combining grasses and legumes in a pasture has been practiced quite over a long period of time especially by the western farmer. The grass-legume mixture is a kind of pasture that is raised by combining both grass and legume plant in the same area. Such examples are *Pannicum maximum* (guinea grass) with *Stylosanthes guianensis* (stylo), *Brachiaria humidicola* with *Arachis pintoi* and many others.

In traditional farming practices without incorporating mixed cropping of grasses and legumes, farmers have relied primarily on natural pastures, monocultures, crop residues, browse trees, and other supplementary feed sources. However, these sources can often limit animal growth due to various constraints. For example, tropical grasses typically contain only 8% or less crude protein, which is insufficient for optimal animal production (Humpreys 1991). Adequate protein intake is crucial for beef and dairy cattle to produce desirable quantities of meat and milk, thereby enhancing profitability for farmers.

Crop residues also present challenges; many contain high levels of lignin and hemicellulose rather than cellulose, making them less suitable and inefficient for ruminal digestion. Consequently, pre-treatment is often necessary to improve their effectiveness as animal feed. The practice of integrating grass-legume mixtures offers a promising alternative, as it not only increases the protein content in feed but also contributes to improved soil fertility. Additionally, grass-legume mixtures achieve greater land use efficiency than monocultures without requiring additional investments (Alemu et al., 2007). This approach can be economically advantageous for farmers, as it supports both animal growth and production without increasing operational costs.

However, there is little information available on the planting ratio in the establishment of grass-legume mixtures in the tropics. The identification of grass-legume mixtures that are compatible will aid in mitigating the issue and enhancing animal growth. This study aimed to determine the productivity and compatibility between the grass *Brachiaria decumbens* cv. Kluang Comel (Kluang Comel) and the legume *Stylosanthes guianensis* CIAT 184 (Stylo 184) under different planting ratios.

2. MATERIALS AND METHODS

2.1. Experimental Location

The study was conducted at the Universiti Putra Malaysia. The independent variables for this experiment were species of grass, soil condition, fertilizer types, fertilizer dosage, planting methods, amount of watering and lighting. The dependent variables were plant height, plant dry matter yield, number of tillers from each plant, and the chemical composition.

2.2. Experimental design

The experiment consisted of five treatments of different ratio of mixing between Kluang Comel and Stylo 184. The planting ratios of Kluang Comel and Stylo 184 in each treatment were: T1 (100:0 grass-legume planting ratio), T2 (75:25 grass-legume planting ratio), T3 (50:50 grass-legume planting ratio), T4 (25:75 grass-legume planting ratio), and T5 (0:100 grass-legume planting ratio). It was conducted using a complete randomized design and carried out in five replicates for each treatment.

2.3. Preparation of Soil Medium

The medium was prepared by thorough mixing of soil, compost and sand in the ratio of 3:2:1 respectively. Equal amounts of soil medium were filled into each of the polyethylene bags of size 0.24 m (height) x 0.6 m (length) x 0.6m (width).

2.4. Establishment of *Brachiaria decumbens* cv. Kluang Comel and *Stylosanthes guianensis* CIAT 184

Kluang Comel was propagated by rootstock as the seed is not viable while Stylo 184 was propagated by seeds. To break the dormancy, Stylo 184 seeds were initially treated with hot water at 80°C for 3 minutes (Baba et al., 2011). Kluang Comel and Stylo 184 were cut back after 7 weeks of establishment at 10 cm height from the ground.

2.5. Fertilizer application

Basal dressing was applied at 60:30:30 kg/ha of nitrogen (N), phosphorus (P), and potassium (K) for grass and 30:30 kg/ha of P and K for legume and grass legume mixture. The fertilizers were urea as N source, Triple superphospate (TSP) as P source and Muriate of potash (MOP) as a source of K.

2.6 Harvest and sample preparation for analysis

The forages were harvested manually from each poly bag individually using secateurs at the height of 5 cm above the ground after 7 weeks from the first cut back. The samples from each poly bag were hand separated into leaf blade and stem (including leaf sheath and inflorescence) components and weighed to determine leaf stem ratio (Smart et al., 2001).

2.7 Sample Analysis

The samples were placed in paper bags and put in the oven for dry matter measurement and determination of leaf to stem ratio. The samples were analysed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). CP was calculated as N × 6.25. NDF, ADF and ADL were determined according to Goering and Van Soest (1970). Hemicellulose and cellulose were calculated as NDF – ADF and ADF – ADL, respectively. Calcium and P percentage was measured using an atomic absorption spectrophotometer (AAS).

2.8 Statistical analysis

All the data were subjected to the analysis of variance (ANOVA) using Statistical Analysis Software (SAS) package. Duncan's multiple range test was used to identify differences among treatment means. Differences were considered significant if $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1 presents the physical attributes of Kluang Comel and Stylo 184 at various planting ratios. Significant differences ($p<0.05$) in dry matter yield were observed among the mixtures of Kluang Comel and Stylo 184 at various planting ratios. The maximum dry matter yield was recorded in T3 (2.384 tons per hectare) in comparison to T1 (1.211 tons per hectare), T5 (1.330 tons per hectare), T2 (2.070 tons per hectare), and T4 (1.767 tons per hectare). Furthermore, there were notable differences ($p<0.05$) in plant height among the mixtures of Kluang

Comel and Stylo 184 at various planting ratios for both grass and legume. The grass height was lowest in T4 at 26.21 cm, in contrast to T1 at 31.82 cm, T2 at 31.23 cm, and T3 at 29.41 cm. Conversely, the height of the legume in T2 was shortest at 43.83 cm, in contrast to T5 at 49.21 cm, T4 at 47.61 cm, and T3 at 47.01 cm. A notable difference ($p<0.05$) in leaf percentage was observed between the Kluang Comel and Stylo 184 mixtures at various planting ratios for both grass and legume. The leaf percentage of grass was highest in T2 (61.82%) compared to T1 (58.02%), while no significant difference was observed between T3 (54.53%) and T4 (55.62%). Conversely, the leaf percentage of legumes was highest in T2 (60.46%) compared to T3 (55.22%), while T4 (50.02%) and T5 (50.88%) exhibited similar percentages.

Table 2 illustrates the chemical composition of the mixture of Kluang Comel and Stylo 184 at various planting ratios. The T5 (15.99%) exhibited a significantly higher crude protein percentage ($p<0.05$) compared to T4 (13.79%), T3 (13.01%), T2 (11.86%), and T1 (11.36%). The NDF percentage was markedly elevated ($p<0.05$) in T1 (74.3%) relative to T3 (68.3%), T4 (64.36%), and T5 (60.22%). However, the NDF percentage in T1 was not significantly different from that in T2 (71.8%). The ADF percentage was markedly elevated ($p<0.05$) in T5 (41.41%) relative to T1 (32.51%), T2 (35.92%), and T3 (38.3%). The ADF percentage in T5 did not differ significantly from that in T4 (41.06%). No significant difference ($p\geq0.05$) was observed in the ADL percentage among the mixtures of Kluang Comel and Stylo 184 at various planting ratios. The ADL percentage reached its peak in T5 at 5.84%, surpassing T1 at 5.43%, T2 at 5.5%, T3 at 5.576%, and T4 at 5.604%. The Ca percentage in T5 (1.916%) was significantly greater ($p<0.05$) than in T1 (0.229%), T2 (0.588%), and T3 (0.649%). Nevertheless, the Ca percentage in T5 did not significantly differ from that in T4 (1.024%). The P percentage was highest ($p<0.05$) in T5 at 0.49%. Nonetheless, no substantial differences were observed in T2 (0.418%), T3 (0.422%), and T4 (0.427%). The P percentage in T1 was the lowest at 0.376%.

Table 1: Physical characteristics of *Brachiaria decumbens* cv. Kluang Comel and *Stylosanthes guianensis* CIAT 184 at different planting ratios.

Parameters	Treatment*					*p*-Value
	T1	T2	T3	T4	T5	
Dry matter yield, tonne/ha	1.21^{d}	2.07^{b}	2.38^{a}	1.77^{c}	1.33^{d}	<0.05
Plant height (grass), cm	31.82^{a}	31.23^{a}	29.41^{b}	26.21^{c}	n/a	<0.05
Plant height (legume), cm	n/a	43.83^{c}	47.01^{b}	47.61^{b}	49.21^{a}	<0.05
Leave percentage (grass), %	58.02^{b}	61.82^{a}	54.53^{c}	55.62^{c}	n/a	<0.05
Leave percentage (legume), %	n/a	60.46^{a}	55.22^{b}	50.02^{c}	50.88^{c}	<0.05

*T1, 100:0 grass-legume planting ratio; T2, 75:25 grass-legume planting ratio; T3, 50:50 grass-legume planting ratio; T4, 25:75 grass-legume planting ratio; T5, 0:100 grass-legume planting ratio.

a,b,c,dMeans with different superscripts in the same row indicate significant difference at $p<0.05$.

Table 2: Chemical composition of *Brachiaria decumbens* cv. Kluang Comel and *Stylosanthes guianensis* CIAT 184 at different planting ratios.

Parameters	Treatment*					*p*-Value
	T1	T2	T3	T4	T5	
Crude protein, %	11.36^{c}	11.86^{c}	13.01^{b}	13.79^{b}	15.99^{a}	<0.05
NDF, %	74.30^{a}	71.80^{a}	68.30^{b}	64.36^{bc}	60.22^{c}	<0.05
ADF, %	32.51^{c}	35.92^{b}	38.30^{b}	41.06^{a}	41.41^{a}	<0.05
ADL, %	5.43	5.50	5.58	5.60	5.84	≥0.05
Calcium, %	0.229^{d}	0.588^{c}	0.649^{c}	1.02^{b}	1.92^{a}	<0.05
Phosphorus, %	0.376^{c}	0.418^{b}	0.422^{b}	0.427^{b}	0.490^{a}	<0.05

*T1, 100:0 grass-legume planting ratio; T2, 75:25 grass-legume planting ratio; T3, 50:50 grass-legume planting ratio; T4, 25:75 grass-legume planting ratio; T5, 0:100 grass-legume planting ratio.

a,b,c,dMeans with different superscripts in the same row indicate significant difference at $p<0.05$.

3.1 Physical characteristics

The mixture of Kluang Comel and Stylo 184 at the planting ratio of 50:50 (Treatment 3) produced the highest yield per hectare which is 2.384 ton per hectare. Similarly, Baba et al. (2011) reported significantly higher yield in guinea-centro mixture at 2:2 planting ratio as compared to the guinea grass monoculture, and significantly lower yield in *Centrosema pubescens* monoculture compared to guinea-centro mixture. The higher dry matter yields of grass compared to legumes in monocultures and mixtures could be due to vigorous nature of grass growth and its ability to rapidly utilize the N in the soil which is released following cultivation (Tessema and Baars 2006). The increased yield advantage achieved at equal seed rates could be due to the improved soil fertility produced by the legume and increased biological efficiency of the mixture, through efficient use of light, water and nutrients (Cong et al., 2015; Rani et al., 2019). Although no data on soil quality was obtained from this experiment, past and recent literature agreed that intercropping of various crops with legumes showed significant improvement in terms of carbon and N content of soil (Cong et al., 2015; Choudhary et al., 2018; Silva et al., 2022). Thus, superior yield from Treatment 3 could be singled out to the N fixation by the presence of Stylo 184.

As stated by Suparjo and Shokri (2005), leaf percentage is one of the criteria for evaluating the quality of pasture with a higher proportion of leaf indicating better nutritive value. From this experiment, both Kluang Comel and Stylo 184 had the most leaves when planted using a 75:25 planting ratio (Treatment 2). This finding agrees with a study by Abusuwar and Omer (2011), whereby intercropping of *Sorghum sudanese* and *Clitoria ternatea* resulted in the highest leaf-to-stem ratio for both plants. Although the dry matter yield was higher in Treatment 3 (50:50), the higher leaf-to-stem ratio in Treatment 2 (75:25) could be contributed by the higher leaf-to-stem ratio of grass than legumes (Table 1).

3.2 Molecular identification of isolated bacteria

Higher crude protein was observed in the 50:50 (Treatment 3) and 25:75 (Treatment 4) planting ratios of Kluang Comel and Stylo 184 compared to the other mixture due to the higher population of legume. However, the crude protein content was not improved in the grass due to time constraint, thus the root nodules in the legume are not yet developed to fix the N cycle in the soil. The higher crude protein content was associated with older plants due to more efficient N fixation resulting from better developed root nodules as the plant became more strongly established (Guy et al., 2018; Reinsch et al., 2020).

The neutral detergent fiber (NDF) percentage was significantly higher in the 75:25 planting ratio mixture of Kluang Comel and Stylo 184, reaching 71.8%. As the proportion of legumes in the mixture increased, the NDF percentage of the mixed pasture decreased. NDF represents the total fiber content in the forage and is an important factor influencing forage intake by ruminants (Anderson et al., 1991; Ball et al., 2001; Undersander et al., 2002; Sheaffer et al., 2007). Conversely, the acid detergent fiber (ADF) percentage was significantly higher in the 25:75 mixture of Kluang Comel and Stylo 184, reaching 41.06%. ADF levels increased as the proportion of legumes in the mixture rose. ADF consists of highly indigestible fiber and is negatively associated with forage digestibility (Rayburn 1991; Ball et al., 2001), suggesting that a higher legume proportion leads to reduced digestibility of the forage. This reduced digestibility may result from the high cellulose content in legume leaves and the lignification of stems, both of which limit the benefits of legume inclusion in mixed pasture swards with higher legume proportions (López et al., 2005; Gomes et al., 2011; Ratnawaty and Chuzaemi 2018; Kaithwas et al., 2020).

The Stylo 184 monoculture sward demonstrated the highest Ca and P percentages overall. However, among the mixed pasture groups, the 25:75 Kluang Comel-Stylo 184 mixture recorded the highest mineral levels, outperforming the 50:50 and 75:25 grass-legume combinations (Table 2). This can be attributed to the greater proportion of Stylo 184 in the mixed pasture sward. Previous research has shown that various varieties of *S. guianensis* possess a remarkable ability to extract Ca efficiently even from soils with low levels of this essential nutrient (Edye, 1987; Gilbert et al., 1989; Stace, 2012). The legume's superior Ca uptake capabilities allow it to accumulate higher concentrations of this mineral in its tissues when grown in a mixed sward with the grass species such as *B. decumbens* (Wissal et al., 2020). In addition to enhancing mineral nutrition, the inclusion of legumes in pasture systems can also contribute significantly to improving soil fertility and reducing erosion (Guerra et al., 2019).

4. CONCLUSION

The present study evaluates the performance of a forage mixture comprising *Brachiaria decumbens* cv. Kluang Comel (Kluang Comel) and *Stylosanthes guianensis* CIAT 184 (Stylo 184), highlighting its potential for enhanced DM yield and CP content. Findings indicate that the optimal DM yield was achieved at a 50:50 planting ratio of grass to legume, suggesting this balance as the most favourable for productive output. Furthermore, as the proportion of legume increased, there were concurrent rises in CP, Ca, and P levels, underscoring the nutritional benefits associated with higher legume content in the mixture. However, while the inclusion of more legumes improves protein and mineral content, the associated rise in ADF may negatively impact digestibility, which warrants consideration in developing an optimal forage mixture. Consequently, a 50:50 ratio of Kluang Comel to Stylo 184 emerges as the most preferable configuration for a more sustainable forage production. Nevertheless, additional research is warranted to investigate the longevity and N fixation rate of the legume component to reinforce the robustness and practical implementation of these findings.

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