EFFECT OF RESIDUAL ROCK PHOSPHATE AND BIOFERTILIZERS (*RHIZOBIUM* AND BIOPHOSPHATE) AND THEIR COMBINATIONS ON THE PRODUCTIVITY OF *Stylosanthes guianensis* CIAT 184

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ABSTRACT

The purpose of this research was to evaluate the agronomic effectiveness of rock phosphate, biological fertilizers (*Rhizobium* and bio-phosphate) and their combinations on dry matter (DM) production, nutrient content and *in vitro* dry matter digestibility (IVDMD) of *Stylosanthes guianensis* CIAT 184. This research was conducted at Forage and Pasture Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia from February – October 2010. A strip plot design which consisted of two fertilizer factors with three (3) replicates was used. The first factor was the horizontal factor and consisted of four levels of biological fertilizers (*Rhizobium* and bio-phosphate) namely: M_0 = control, M_1 = *Rhizobium* (0.5 g/plot), M_2 = bio-phosphate (0.6 g/plot) and M_3 = combinations of *Rhizobium* and bio-phosphate. The second factor was the vertical factor and consisted of three levels of rock phosphate, namely: P_0 = control, P_1 = 250 kg/ha (32.5 P kg/ha) and P_2 = 500 kg/ha (65 P kg/ha). Defoliation was carried out every three months for 9 months. Results of the study showed that there was no significant difference in DM production (kg/ha) amongst treatments. Although the current research showed no significance difference amongs the treatments, DM production increased in the 2nd harvest (12.3%) and 4th harvest (7.1%) between P_0 and P_1. Combinations treatment P_1M_2 (21073.63 kg/ha) had the highest average DM production amongs treatments. Statistical analysis followed by Duncan's new Multiple Range Test (DMRT), showed that rock phosphate can significantly ($P<0.05$) increase fiber production of *Stylosanthes guianensis*. It showed that treatment P_1 increased crude fiber production by 12.2% from 5864, 47 kg/ha to 6580,19 kg/ha. In addition, tests further revealed that there was a difference between M_1 and M_2, but had no effect on M_0. There was no significant effect on production (kg/ha) of crude protein, phosphorus, nitrogen free extract (NFE) and ash. In conclusion, treatments with rock phosphate and bio-fertilizers tend to increase productivity of *Stylosanthes guianensis* CIAT 184.

(Key words: *Stylosanthes guianensis* CIAT 184, Rock phosphate, Bio-phosphate, *Rhizobium*, and *In vitro* dry matter digestibility)

Introduction

The success of legumes is found in their ability to have a mutualistic symbioses with nitrogen (N)-fixing bacteria to directly capture atmospheric dinitrogen (N_2) to support plant growth (Hernandez *et al*., 2009). Nitrogen is commonly the most limiting plant nutrient in arable farming in the tropics and also the most expensive element as a mineral fertilizer, biological nitrogen fixation (BNF) therefore, holds great promise for smallholder farmers (Tripathi and Psychas, 1992).

However, although leguminous plants are expected to replenish soil nitrogen supplies, they are particularly hard hit by phosphorus (P) deficiency because low supply inhibits effective nodulation and retards the biological nitrogen fixation process (Whiteman, 1980).

The nitrogen fixing systems require more phosphorus (P) than non-N_2 fixing systems, because the process consumes large amounts of energy, and energy-generating metabolism strongly depends upon the availability of P (Schulze *et al.,* 2006).

Phosphorous is one of important mineral for the plant. Manufactured water soluble phosphorus (WSP) fertilizers such as superphosphates are commonly recommended for correcting P deficiencies. The application of these inorganic fertilizers, results in low efficiency of WSP fertilizers such as triple superphosphate (TSP) or diammonium phosphate (DAP) by crops (Chien *et al.*, 2010).

Another solution can be found in the direct application of rock phosphate which is an agronomic and economically sound alternative to the more expensive superphosphates in the tropics (Zapata and Roy, 2004). Phosphorus however, becomes unavailable to the plants after its application in soils; this is due to the formation of strong bonds between phosphorus with calcium and magnesium in alkaline pH and the same bonds with iron and aluminum in acidic soils (Mehrvars and Chaichi, 2008).

It is therefore, behind this background that this study aimed to investigate the use of bio-fertilizers (*Biophosphate* and *Rhizobium*), to increase the effectiveness of rock phosphate and thus the availability of P.

Stylosanthes guianensis CIAT 184 (stylo) is a legume that has been identified as a potential feed resource that could be used as a supplemental feed

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during periods of shortages. Another advantage of this legume is that as a N_2-fixing plant, it has a higher capacity to utilize P from rock phosphate than nitrate-fed plants (Perez *et al.,* 2007), because of their higher cation/anion uptake ratio and corresponding net release of H^+.

This research is a continuation of the research conducted by Hutasoit (2010) who aimed at investigating the effect of rock phosphate and biological fertilizers on the production of *Stylosanthes guianensis*. The purpose of this research is to measure the agronomic effectiveness of rock phosphate, *biofertilizers* (biophosphate and *Rhizobium*) and their combinations on: the dry matter yield, nutrient compostion content and *in vitro* dry matter digestibility of S*tylosanthes guianensis*. This research would provide valuable information on the agronomic effectiveness of rock phosphate and provide an environmentally and economically attractive means to increase production.

Materials and Methods

Description of the study area

This research was conducted at Pasture and Forage Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia, for 9 months (February – October 2010). Average rainfall, temperature and humidity during this study was 240.4 mm, 32.1°C (maximum temp.) and 82.4% respectively (Adisutjipto Meteorology Stasion, 2010).

Research materials

This research used *Stylosanthes guianensis* CIAT 184 seeds, rock phosphate (P_2O_5 13%), biophosphate (0.6 g/plot) and *Rhizobium* (0.5 g/plot) strain legume *Cowpea* (*Vigna sinensis*) with dosis of 1 kg/150 kg seeds.

Research method

This research used the *Strip Plot Design* that consisted of two factors (3 x 4 factors). The first one being the horizontal factor, namely rock phosphate which consisted of three stages, namely:
P0 = without *rock phosphate*
P1 = *rock phosphate* 250 kg/ha (32.5 P kg/ha)
P2 = *rock phosphate* 500 kg/ha (65 P kg/ha)

The second factor was the vertical factor namely, biological fertilizers *rhizobium* and *biofosfat* (M) which consist of four stages namely:
M0 = without *biofertilizers*
M1 = plus *rhizobium*
M2 = plus *biophosphate*
M3 = plus *rhizobium* dan *biophosphate*.

The current research did not apply any of the above-mentioned treatments during the course of the 9 month research period. Hence, was only applied once at the time of planting by Hutasoit (2010).

Treatment combinations

Plot No.	Treatment	Treament kombination
1	P_0M_0	No treatment – control
2	P_0M_1	Without *rock phosphate* + *Rhizobium*
3	P_0M_2	Without *rock phosphate* + biophosphate
4	P_0M_3	Without *rock phosphate* + *Rhizobium* + biophosphate
5	$P_1 M_0$	*rock phosphate* 250 kg/ha + no biofertilizers
6	$P_1 M_1$	*rock phosphate* 250 kg/ha + *Rhizobium*
7	$P_1 M_2$	*rock phosphate* 250 kg/ha + biophosphate
8	$P_1 M_3$	*rock phosphate* 250 kg/ha + *Rhizobium* + biophosphate
9	$P_2 M_0$	*rock phosphate* 500 kg/ha + no biofertilizers
10	$P_2 M_1$	*rock phosphate* 500 kg/ha + *Rhizobium*
11	$P_2 M_2$	*rock phosphate* 500 kg/ha + biophosphate
12	$P_2 M_3$	*rock phosphate* 500 kg/ha + *Rhizobium* + biophosphate

Variables measured

The following were variables observed: dry matter (DM) content, crude protein (CP), crude fiber (CF), content (AOAC, 2005), phosphorus concentration, soil analysis and *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry, 1963). Data of the proximate analysis and P concentration were used to calculate the production per hectare. Dry matter production was calculated by multiplying the dry matter content by the fresh weight, while the other variables were calculated based on the DM production.

Statistical analysis

Data were analyzed statistically using *SPSS for windows version 16* and followed by Duncan's new Multiple Range Test (DMRT) when there was a significant effect.

Results and Discussion

Soil analysis of research area

The total Ca also reduced from 0.54% to 0.02%, this coincides with the reduced pH level (from 6.9 to 6.7), because as Ca level drops, the more acidic soils become. In addition, as a legume, stylo has high demand for Ca (Zapata and Roy, 2004). The available cation exchange capacity (CEC) recorded was 22.37, this value lies within the normal range 15 – 40, and Kettering *et al.* (2007) reported that at these levels soils are more clay or more organic matter is present with high water holding capacity. Camberato (2001) further reported that pH 6.5 provides a near optimum CEC levels and P availability.

Dry matter (DM) content and production

Results showed that the 2nd harvest recorded an average DM content of 25.79% based on the interaction effect, and ranged between 24.90 (*rock phosphate* 250 kg/ha + *Rhizobium*) – 26.70% (*rock phosphate* 250 kg/ha + biophosphate); the 3rd harvest recorded an average DM of 30.19%, and ranged between 28.70–32.60% (without *rock phosphate* + *Rhizobium*) and finally the 4th harvest recorded an average DM (%) of 26.74% and ranged between 24.70 (*rock phosphate* 250 kg/ha + no biofertilizers) – 28.70% (*rock phosphate* 250 kg/ha + *Rhizobium*). The 1st harvest recorded (Hutasoit, 2010) an average of 23.54% and ranged between 22.20 (*rock phosphate* 250 kg/ha + *Rhizobium*) – 25.20% (*rock phosphate* 250 kg/ha + biophosphate). The 3rd harvest recorded the highest DM content, but had the lowest DM (kg/ha) production.

Results in Table 1 show that treatments had no significant effect on the DM production (kg/ha) of stylo.

Although no statistically significance difference, amongs treatments were found production increased in the 2nd harvest 12.3% and 4th harvest 7.1% between (P_0) and (P_1). Combinations treatment *rock phosphate* 250 kg/ha+ biophosphate (21073.63 kg/ha) had the highest average DM production amongst treatments.

Temperature probably had one of the greatest effects on the low DM average. If soil temperatures

Table 1. Effect of rock phosphate, bio-fertilizers and their interactions on DM production (kg/ha) of stylo

Treatment	Average DM production (kg/ha)			
	Harvest 2	Harvest 3	Harvest 4	Average
Rock phosphate				
P0	21624.06	15466.08	18437.34	18509.16
P1	24291.36	14978.51	19746.00	19671.96
P2	21097.70	14685.70	19626.65	18470.02
Bio-fertilizers				
M0	22146.76	15466.08	20167.95	19260.26
M1	20902.49	13256.86	19428.88	17862.74
M2	24173.44	15217.72	18995.48	19462.21
M3	22128.27	13679.24	18487.68	18098.40
Combination treatment of rock phosphate and bio-fertilizers				
P0M0	20668.77	12798.73	19231.81	17566.44
P0M1	23421.31	12530.47	18248.91	18066.90
P0M2	20428.34	14661.62	17252.68	17447.55
P0M3	21977.82	14212.04	19015.96	18401.94
P1M0	23774.10	17834.55	20293.99	20634.21
P1M1	23201.76	12724.86	19917.57	18614.73
P1M2	27343.98	15427.32	20449.60	21073.63
P1M3	22845.61	13927.32	18322.85	18365.26
P2M0	21997.41	15764.96	20978.04	19580.14
P2M1	16084.39	14515.25	20120.17	16906.60
P2M2	24747.99	15564.21	19284.17	19865.46
P2M3	21561.00	12898.36	18124.23	17527.86
Average	22337.71	14404.97	19270.00	18670.89

are high, dry matter can be lost through excessive respiration. Another reason may be due the excess defoliation in the first year (4 times), as according to Tripathi and Psychas (1992) defoliation decreases the photosynthetic ability of legumes and impairs N_2 fixation which can lead to nodule decay. Results also showed that increased levels of rock phosphate (P_2) 500 kg/ha (65 P kg/ha) did not increase yield, with treatment (P_2) 18470.02 kg/ha having a lower yield as compared to the control. Similarly in their study to test different levels of fertilizer (P) application ranging between 0, 20, 40 – 320 kg P/ha, Crespo and Curbelo (1990) found that increased levels more than 23.3 kg P/ha did not increase yields.

Increased production in the 2nd harvest may be because during the growing period dissolved P could have moved through the soil to the roots (Bushman *et al*., 2009) and was absorbed by stylo and hence increased growth. In addition, the characteristic of stylo's response to phosphatic fertilization can double its production t' Mannetje (1992). In addition, Mckenzie and Middleton (1997) stated that a good supply of P has been associated with increased root growth, which means the plants, can explore more soil for nutrients and moisture.

There was a trend between rainfall and DM production (kg/ha), this statement is supported by Bushman *et al.* (2009) who reported that the activity of microorganisms is highly influenced by soil temperature and soil moisture.

Crude protein (CP) content and production

The CP represents a composite analysis of 3 harvests (harvest 2 - 4) collected over a 9 month period. Treatment with rock phosphate had no effect on the CP content of stylo recording 17.67% (P_1) and 17.84% (P_2) as compared to the control 17.96 % (P_0). Treatment with bio-fertilizers also had no effect on CP content, with the control (P_0) recording the highest CP content at 18.08%.

The total average CP content based on the interaction effects recorded 17.82%, and varied between 17.23% (P_1M_3) – 18.60% (P_0M_0). D'Mello and Devendra (1995) reported CP figures ranged from 12.1 to 18.1% for the whole plant, this record

Table 2. Effect of rock phosphate, bio-fertilizers and their interactions on CP content of stylo

Treatment	Average CP content (%)			
	Rep 1	Rep 2	Rep 3	Average
Rock phosphate				
P0	17.51	18.08	18.29	17.96
P1	17.21	18.60	17.22	17.67
P2	17.43	18.23	17.86	17.84
Bio-fertilizers				
M0	18.09	18.18	17.97	18.08
M1	16.74	18.32	18.02	17.69
M2	17.87	18.38	17.46	17.90
M3	16.84	18.31	17.7	17.62
Combination treatment of rock phosphate and bio-fertilizers				
P0M0	19.63	17.05	19.11	18.60
P0M1	17.18	17.96	18.66	17.93
P0M2	17.24	18.29	17.52	17.68
P0M3	16.00	19.00	17.85	17.62
P1M0	17.63	19.77	17.34	18.25
P1M1	16.85	18.63	16.81	17.43
P1M2	17.12	18.77	17.49	17.79
P1M3	17.25	17.21	17.22	17.23
P2M0	17.00	17.73	17.45	17.39
P2M1	16.18	18.36	18.58	17.71
P2M2	19.25	18.09	17.38	18.24
P2M3	17.27	18.72	18.03	18.01
Average	17.38	18.3	17.79	17.82

is slightly lower than the CP recorded in this study, which had an average of 17.82% for the whole plant.

The total average production of CP recorded 3318.91 kg/ha and ranged between 3001.93 (P_2M_1) – 3745.41 kg/ha (P_1M_0) as shown in Table 2.

There was no significant effect amongst the different rock phosphate levels. Bio-fertilizers also had no significant effect on production. The biological fixation of nitrogen may have been affected at earlier stages of plant growth due to the harsh environmental conditions experienced at the initial stage of growth. Zahran (1999) reported that a decrease in the infectivity of cowpea rhizobia was found at 35°C. Serraj and Adu-Gyamti (2004) report that it is well documented that both low and high extremes temperature can prevent nodulation, or if nodulation occurs, can inhibit nitrogen fixation. Nodule functioning in common beans (*Phaseolus* spp.) is optimal between 25 and 30°C and is hampered by root temperatures between 30 and 33°C (Zahran, 1999). During the research, temperatures recorded an average of 32.1°C; this could have been one of the factors that affected the microbial activity and hampered nodulation.

Sensitivity of symbiotic nitrogen fixation to phosphorus deficiency could have affected the CP production; if bio-phosphate microbes were rendered dormant due to the above mentioned conditions, P-deficiency would drastically cause a reduction in nodulation (Hernandez *et al.,* 2009) and nitrogenase activity.

Crude fiber content and production

The total average CF (%) based on the interaction effects recorded 33.01% and ranged between 31.78 (P_0M_1) – 34.11% (P_1M_1). Treatment with P_1 (250 kg/ha) had the highest effect on CF content recorded 33.41%. Keoboualapheth and Mikled (2003) recorded CF of stylo was 30%, which is lower than results in current studies. Keopaseuth *et al.* (2004) recorded even lower CF at 17.3%. The apparent increasing may be due to the aging of stylo, as sub-samples were collected for a one year period. Maturity through its effects on plant composition is recognized as a major

Table 3. Effect of rock phosphate, bio-fertilizers and their interactions on CF contents of stylo

Treatment	Average CF content (%)			
	Rep 1	Rep 2	Rep 3	Average
Rock phosphate				
P0	33.66	32.05	32.49	32.73
P1	33.85	32.71	33.67	33.41
P2	34.87	30.61	33.22	32.90
Bio-fertilizers				
M0	34.45	31.68	33.43	33.19
M1	33.97	33.32	31.94	33.08
M2	33.87	31.51	33.57	32.98
M3	34.21	30.65	33.57	32.81
Combination treatment of rock phosphate and bio-fertilizers				
P0M0	33.31	34.4	32.40	33.37
P0M1	32.59	32.3	30.45	31.78
P0M2	35.05	32.45	33.20	33.57
P0M3	33.67	29.03	33.90	32.20
P1M0	36.36	31.70	34.05	34.04
P1M1	33.24	35.28	33.82	34.11
P1M2	33.00	29.98	33.62	32.20
P1M3	32.80	33.89	33.18	33.29
P2M0	33.67	28.93	33.83	32.14
P2M1	36.08	32.38	31.54	33.33
P2M2	33.56	32.10	33.89	33.18
P2M3	36.17	29.03	33.62	32.94
Average	34.13	31.79	33.13	33.01

determinant of forage quality (Onyeonagu *et al.*, 2012). Because, as plant matures the cell wall content increases and cell content decreases.

Table 3 shows that the total CF average production (kg/ha) recorded 6186.74 kg/ha and ranged between 5616.40 kg/ha (P_2M_1) – 7069.30 (P_1M_0). The highest CF was recorded in combination treatment (P_1M_0) 7069.30 kg/ha, similarly Hutasoit (2010) also recorded combination (P_1M_0) as the treatment with the highest CF content.

SPSS statistical analysis followed by DMRT showed that there was a significant effect ($P<0.05$) of rock phosphate application on CF production, and there was a significant difference between (P_0) and (P_1). It showed that treatment P_1 increased crude fiber production by 12.2% from 5864.47 kg/ha to 6580.19 kg/ha. The results also showed a significant difference between (M_1) and (M_2) on CF production (kg/ha).

Phosphorus content and production

Treatment P_2 had the highest effect on phosphorus concentration, and M_2 had highest concentration 0.42% amongst the bio-fertilizer treatments, as compared to the control (M_0) 0.38%. The total average P based on the interaction effects recorded 0.40% and ranged between 0.35% (P_1M_3) – 0.50% (P_2M_3). Compared to Hutasoit (2010) who recorded an average P of 0.13% and ranged between 0.12% (P_2M_2) – 0.16% (P_2M_0).

The highest P content 0.50% (P_2M_3) showed that combinations of rock phosphate levels at 500 kg/ha (65 P kg/ha) and bio-fertilizers combinations (bio-phosphate and *Rhizobium*) had an increasing effect on P content.

Crespo and Curbelo (1990) in their research to determine the response of *Stylosanthes guianensis* CIAT-184 to phosphoric fertilization, recorded critical minimum P concentration in the plantamounted to 0.16%, while P phytotoxicity symptoms were present when 320 kg P/ha was applied and a P content of 0.71% was attained. Furthermore, they reported that increased application of fertilizer increased P concentration in plant. Similarly, Underwood and Suttle (1999) reported that phosphate application increases herbage phosphorus concentrations.

The phosphorus production (kg/ha) of stylo as per treatment with rock phosphate, biological fertilizers and their interaction effect recorded an average P (kg/ha) 75.51 and ranged between 61.43 (P_1M_3) – 89.77% (P_2M_3). Statistical analysis

Table 4. Effect of rock phosphate, bio-fertilizers and their interactions on *in vitro* dry matter digestibility of stylo

Treatment	Average *in vitro* dry matter digestibility (%)			
	Rep 1	Rep 2	Rep 3	Average
Rock phosphate				
P0	50.11	49.19	47.81	49.04
P1	48.99	50.13	46.79	48.64
P2	51.05	48.17	50.11	49.78
Bio-fertilizers				
M0	51.14	49.08	49.17	49.80
M1	50.06	48.83	48.30	49.06
M2	49.30	48.79	47.72	48.60
M3	49.71	49.98	47.74	49.14
Combination treatment of rock phosphate and bio-fertilizer				
P0M0	49.99	51.38	48.21	49.86
P0M1	49.10	48.57	47.40	48.36
P0M2	53.68	49.26	45.90	49.61
P0M3	47.68	47.55	49.71	48.31
P1M0	50.01	48.52	47.41	48.65
P1M1	49.61	50.01	48.41	49.34
P1M2	46.24	47.38	48.36	47.33
P1M3	50.11	54.68	42.98	49.26
P2M0	53.42	47.35	51.90	50.89
P2M1	51.46	47.90	49.09	49.48
P2M2	47.97	49.74	48.90	48.87
P2M3	51.35	47.70	50.53	49.86
Average	50.05	49.17	48.23	49.15

showed that interaction treatment with rock phosphate and bio-fertilizers had no significant effect on P production (kg/ha) of stylo.

In vitro dry matter digestibility (IVDMD)

In this experiment, IVDMD averaged 49.15% and ranged between 47.33% (P_1M_2) to 50.89% (P_2M_0). Treatments with rock phosphate, bio-fertilizers and their interactions had no significant effect on *in vitro* digestibilities. Table 4 shows the digestibilities as affected by treatments.

Cook *et al.* (2005) reported IVDMD of stylo range between 52-60%. Valarini and Possenti (2006) had similar IVDMD of stylo at 57.0%. Phonepaseuth and Ledin (2003) recorded digestibility of stylo between 60-70%. They further reported that increased age and lignifications may reduce digestibility to below 40%.

The low digestibility was a direct effect of the increased fiber content. This increase in fiber content influenced the digestibility of stylo, because a fiber fraction of a feed is closely related to its digestibility (McDonald *et al.,* 1995).

Buxton and Redfearn (1997) reported that the major factor lowering digestibilites of forages as they mature is the higher fiber and lower cell-soluble concentrations of mature forages. In addition, lignin is also primarily responsible for limiting digestibility of fiber, as lignin interferes with microbial degradation of fiber polysaccharides by acting as a physical barrier. Legume stems have a ring of thick-walled, lignified cells resistant to digestion.

Conclusion and Recommendation

Conclusion

Results of the study showed that residual rock phosphate and bio-fertilizers application tended to increase re-growth productivity of *Stylosanthes guianensis* CIAT 184. The most effective interaction between treatments was (P_1) 250 kg/ha (32.5 P Kg/ha) and (M_2) (bio-phosphate). Harvest 3 showed that (P_1) and (P_2) had lower yields as compared to P_0. Rock phosphate significantly increased fiber production of stylo by 12.2%, while bio-fertilizers seemed not to influence the fiber production in statistical analysis. *In vitro* dry matter digestibility was not significantly affected by interaction treatment of rock phosphate, bio-phosphate and *Rhizobium.* Average P concentration increased from 0.13% (harvest 1) - 0.40% (in current research).

Recommendation

Further analysis on the chemical composition, mineralogy and its influence on the reactivity of rock phosphate can further be researched. Also, a detailed economic analysis including cost of production can be formulated to compare the cost of rock phosphate to that of water-soluble-phosphate fertilizer. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the Van Soest fiber fractionation system can be analyzed, as this estimates the structural carbohydrates and evaluates the usable area of the plant cell wall.

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