ISSN 1411 – 0067 Jurnal Ilmu-Ilmu Pertanian Indonesia. Volume 9, No. 1, 2007, Hlm. 1 - 5

DRY MATTER PRODUCTION AND DIGESTIBILITY IMPROVEMENT OF *Centrosema pubescens* AND *Pueraria phaseoloides* WITH ROCK PHOSPHATE FERTILIZATION AND VAM INOCULATION

PENINGKATAN PRODUKSI DAN KECERNAAN BAHAN KERING Centrosema pubescens DAN Pueraria phaseoloides OLEH PEMUPUKAN BATUAN FOSFAT DAN INOKULASI MVA

Dwi Retno Lukiwati
Faculty of Animal Agriculture Diponegoro University
Campus UNDIP Tembalang, Semarang-Indonesia
dwiretno_lukiwati@yahoo.com

ABSTRACT

Centrosema pubescens (centro) and *Pueraria phaseoloides* (puero) are important forage legumes as protein and mineral sources for ruminant livestock in the tropics. However, most of the land that used for forage production is characterized by a low phosphorus content. As the high cost of superphosphate is a major limiting factor, a combination of rock phosphate (RP) fertilization and vesicular-arbuscular mycorrhizae (VAM) fungi inoculation maybe a promising technique. A field experiment was conducted in Bogor during 7 months, on a latosolic soil (low pH and low available Bray II extractable P) to evaluate the effects of RP fertilization and VAM inoculation and their interaction on dry matter (DM) production and in vitro dry matter digestibility. A completely randomized block design with 3 replicates was used**.** The main experiment consisted therein the combination of three factors as follows 1) legume species (centro, puero), 2) VAM inoculation (with, and without VAM inoculation), and 3) rock phosphate fertilization (0, 44, 87, 131, and 175 kg P ha^{-1}). The period of defoliation was used as sub factor (defoliation I, II, and III). Results showed that DM production and DM digestibility of puero was significantly higher ($P<0.05$) compared to centro after defoliation. Dry matter digestibility of VAM inoculated puero was significantly higher ($P<0.05$) compared to uninoculated one. Rock phosphate fertilization significantly increased ($P<0.05$) DM production of VAM inoculated legume. Dry matter production was not significantly different ($P>0.05$) with or without VAM inoculation. When inoculated, rock phosphate fertilization increased DM production of legume. Success of VAM inoculation in the field affected by effectiveness of indigenous-VAM fungi or depending upon VAM inoculum potential.

Key words : *Centrosema pubescens*, *Pueraria phaseoloides*, rock phosphate, mycorrhizae, dry matter, digestibility

ABSTRAK

Centrosema pubescens (centro) dan *Pueraria phaseoloides* (puero) adalah legum penting sebagai sumber protein dan mineral hijauan untuk ternak ruminansia. Namun demikian, sebagian besar lahan untuk produksi hijauan pakan dicirikan oleh kekurangan unsur hara fosfor. Mahalnya harga superfosfat (SP) merupakan faktor pembatas utama, dan kombinasi pemupukan batuan fosfat (BP) dengan inokulasi cendawan mikoriza vesikular-arbuskular (MVA) sebagai solusi untuk mengatasi masalah tersebut. Percobaan lapang telah dilaksanakan di Bogor selama 7 bulan pada tanah latosol (pH masam dan rendah ketersediaan P) untuk mengevaluasi pengaruh pemupukan BP dan inokulasi MVA serta interaksinya terhadap produksi bahan kering (BK)dan kecernaan BK secara *in vitro*. Digunakan rancangan acak kelompok dengan 3 kali ulangan. Percobaan utama meliputi kombinasi 3 faktor sebagai berikut 1) spesies legum (centro, puero), 2) inokulasi MVA (inokulasi dan tanpa inokulasi MVA), 3) pemupukan BP (0, 44, 87, 131, dan 175 kg P ha^{-1}). Periode defoliasi sebagai sub faktor (defoliasi I, II, dan III). Hasil penelitian menunjukkan bahwa produksi BK dan kecernaan BK puero nyata lebih tinggi ($P<0.05$) dibanding

centro setelah mengalami defoliasi. Kecernaan bahan kering puero nyata lebih tinggi (P<0.05) dengan inokulasi MVA dibanding tanpa inokulasi. Pemupukan BP nyata meningkatkan (P<0.05) produksi BK hijauan legum dengan inokulasi MVA. Tanpa inokulasi MVA, pemupukan BP menghasilkan produksi BK hijauan legum tidak berbeda nyata (P>0.05). Apabila di inokulasi MVA, pemupukan BP dapat meningkatkan produksi BK legum. Keberhasilan inokulasi MVA di lapang ditentukan oleh efektivitas cendawan MVA *indigenous* atau tergantung pada potensi inokulum MVA.

Kata kunci: *Centrosema pubescens*, *Pueraria phaseoloides*, batuan fosfat, mikoriza, bahan kering, kecernaan

INTRODUCTION

Centro (*Centrosema pubescens*) and puero (*Pueraria phaseoloides*) are important forage legumes as protein and mineral sources for ruminant livestock in the tropics. Centro and puero have also been used as cover crops in forest plantations or in agroforestry systems. However, most land used for forage production in Indonesia is characterized by a low phosphorus (P) content and a low soil pH (latosolic soil). The application of P fertilizer during the periods of active growth increases forage legumes production and quality (Coates *et al.*,1990),

Superphosphate (SP) fertilizer has been widely used to improve agricultural production. However, its high cost makes the use of rock phosphate (RP) attractive. Rock phosphate is a slow release source of phosphorus, thus the inoculation with vesicular-arbuscular mycorrhizae (VAM) fungus is a promising technique to increase P bio-availability (Lukiwati and Simanungkalit, 2001). According to Jones (1990), the response obtained to applications of P fertilizers is a function of many factors i.e. the initial availability of soil P, the form of fertilizer applied, and the presence or absence of effective mycorrhizae in the soil.

Most research on VAM inoculation has been done on forest-trees and agricultural crops, but rarely on forage crops. The VAM fungus of *Glomus mosseae*, for instance, is the most common species associated with agricultural crops or forests (Yinglong *et al.*, 1998). Centro and puero are suitable host plants for VAM fungi culture (Lukiwati and Supriyanto, 1995). Two species of VAM (*Glomus fasciculatum*, *Entrophosphora columbiana*) proved to increase dry matter production and nutrient uptake of *Pueraria phaseoloides* similarly (Lukiwati and Simanungkalit, 2004). The results showed that SP can be replaced by RP when combined with VAM fungi inoculation (Lukiwati and Simanungkalit, 2001). Success of VAM inoculation in the field affected by effectiveness of indigenous-VAM fungi or depending upon VAM inoculum potential (Mitiku-Habte and Fox, 1993).

The symbiosis between VAM fungi and legumes has been less studied in unsterilized soils than in sterilized soils. The objective of this work was to investigate under field conditions the effect of RP fertilization, VAM fungi inoculation, and their combination on dry matter production and digestibility of centro and puero in a latosolic soil low in available P.

METHODOLOGY

A completely randomized field experiment with three blocks was conducted in Bogor for 7 months on an acid (pH (H_2O) 5.1 to 5.3) latosolic soil low in available P (Bray II extractable between 4.0 and 5.7 g kg^{-1}). The experiment was conducted on 4x5 m plots.

The design consisted in three factors as follows 1) legume species (centro, puero), 2) VAM inoculation (with, and without VAM inoculation), and 3) five levels of rock phosphate fertilization (0, 44, 87, 131, and 175 kg Pha^{-1}). The periods of defoliation were used as sub factors. The defoliation of the plants was done three times. Standard fertilizers, i.e. 50 kg N ha^{-1} as urea and 83 kg K ha^{-1} as KCl, were applied to each plot. Legume seed of centro and puero were dibbled into small holes made with a wooden stick at the rate of two seeds per hole, spaced 100x50 cm. Each plot was inoculated with 100 gram of VAM inoculum/hole at sowing. The

inoculum of VAM fungi contained approximately 820 spores per 100 g. The parameters measured were dry matter (DM) production at the three times of defoliation, and in vitro DM digestibility on the second and third defoliation only, because of the limited biomass at the time of the first defoliation.

The first defoliation was conducted three months after sowing and the following defoliations were conducted every two months. The plants were cut close to the soil surface to determine dry matter production. Dry matter production of each replicate was calculated from $1m^2$ sub plots. To measure DM production the defoliated forage legumes was chopped, sub-sampled, and oven-dried to constant weight at 70 ^{0}C for 48 hours (Islam *et al.*, 1992). The samples of the second and the third defoliation were finely ground and analyzed to determine in vitro digestibility by the Terry and Tilley method (1963).

The analysis of variance on DM production and DM digestibility was done using the general linear model procedure of SAS. The significant differences among the treatments were tested using Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Table 1. Dry matter production (kg ha^{-1})of forage legumes with rock phosphate fertilization and mycorrhiza inoculation

P levels (kg P ha^{-1})	Uninoculated	Inoculated
0	92.2 ab	67.0 c*
44	92.7 ab	80.7 bc
87	90.1 ab	88.4 ab
131	88.7 ab	93.9 ab
175	97.4 ab	106.8 a

* Means followed by the same letters are not significantly different at DMRT 5%

RESULTS AND DISCUSSION

Results

The effect of RP fertilization was not significant (P>0.05) on the DM production of uninoculated legume (Table 1). Rock phosphate fertilization increased DM production on inoculated legume. The DM production of inoculated legume was not significantly higher (P>0.05) than of the uninoculated one, at the same level of RP except on the unfertilized treatment.

Dry matter production of centro and puero increased after the first and after the second defoliation (Table 2). Dry matter production was higher for puero than for centro at the second period of defoliation (120.8 kg ha^{-1} against 90.6 kg ha^{-1}).

For the same level of RP, dry matter production of the second and third defoliation was significantly higher (P<0.05) compared to the first defoliation. Dry matter production of the third defoliation was significantly higher (P<0.05) compared to the second defoliation for 0, 131, and 175 kg Pha^{-1}. Rock phosphate fertilization did not significantly increase (P>0.05) DM production at the first defoliation. However, RP fertilization increased DM production on the second and the third defoliation (Table 2).

Dry matter digestibility of puero was significantly higher (P<0.05) compared to centro, with or without VAM inoculation on the second and third defoliation (Table 3). Dry matter digestibility of centro and puero inoculated with VAM was not significantly different (P>0.05) compared to uninoculated one on the second defoliation. However, DM digestibility of centro was significantly lower (P<0.05) compared to uninoculated one on the third defoliation. Contrasting this, DM digestibility of puero inoculated by VAM was significantly higher (P<0.05) compared to uninoculated one on the same defoliation.

Table 2. Dry matter production (kg ha^{-1}) of forage legumes on three periods of defoliation

Treatment	Period of defoliation		
	I	II	III
Species of legume:			
Puero	40.7 c	120.8 a	121.1 a*
Centro	50.3 c	90.6 b	115.1 a
P levels (kg ha^{-1}) :			
0	36.6 e	89.5 d	109.8 bc
44	47.9 e	110.3 bc	102.0 c
87	45.9 e	116.6 bc	110.4 bc
131	48.0 e	99.1 cd	126.7 ab
175	46.3 e	118.2 bc	141.9 a

* Means followed by the same letters are not significantly different at DMRT 5%

Table 3. Dry matter digestibility (%) of forage legumes on the second and third period of defoliation with mycorrhiza inoculation

Species of legume	Uninoculated		Inoculated	
	Period of defoliation			
	I	II	I	II
Puero	47.3 cd	49.4 b	46.1 cd	50.8 a*
Centro	43.4 e	47.3 c	43.5 e	45.3 d

* Means followed by the same letters are not significantly different at DMRT 5%

Discussion

Dry matter production of VAM inoculated legumes was increased by rock phosphate fertilizer. However, both VAM inoculated and uninoculated legumes gave a similar DM production at the same level of rock phosphate (Table 1). Previous field experiments have shown that the response to VAM inoculation in the field greatly varies, and sometimes inoculation did not increase the production (Gui and Yin, 1991). This is because most of agricultural soils already contain indigenous populations of VAM fungi. Mycorrhizal inoculation would be successful in the field only if the native population was low and low effectiveness of indigenous-VAM fungi or depending upon VAM inoculum potential (Mitiku-Habte and Fox, 1993). Field experiment was carried out on the unsterilized soils. A year before, cassava had been harvested from the field, and since then the field was underfallow. Cassava is a VAM-obligate type. Cassava rhizosphere could increase the effectiveness of indigenous-VAM fungi (Potty, 1988). Spore isolation in the beginning of the field experiment showed that, there was an indigenous population of VAM fungi with a density up to 496 spores per 100 g. During an experiment aiming at isolating spores from the soil, some spores infected by pathogen fungi were found. These pathogens could have decreased the effectiveness of VAM inoculum. Mycorrhizal spores could have been infected by pathogen fungi during the storage of the soil inoculum (Bagyaraj, 1988).

Rock phosphate fertilization did not significantly increase DM production of forage legumes at the first defoliation (Table 2). That was because the plants were still at the initial growth, while rock phosphate belongs to the group of slow release source of phosphorus (Jones, 1990).

The DM production of puero was higher compared to centro after first and second defoliation (Table 2). Performance of plant growth and root geometry (number of roots and distribution in the soil) of each plant species are different, as well as their response to the treatments (Kerridge and Ratcliff, 1982). Dry matter production stimulated after the first and the second defoliation promoted new stolon growth. Defoliation promoted vegetative regrowth of legume as shown in the DM production. At the earliest period, the growth of puero was slower compared to centro, therefore, DM production of puero was lower compared to centro. However, the regrowth of puero was faster than centro, therefore, the DM production of puero was higher than centro after each defoliation which in turn decreased the copper (Cu) content of puero (data not showed). This is so because of Cu as co-factor phenoloxidase enzyme which influenced the lignification process (Dell *et al.*, 1995). Increase in phenoloxidase activity tend to increase lignification process. As a result, invitro DM digestibility of centro was lower than puero on the second and the third defoliation with or without VAM inoculation (Table 3).

CONCLUSION

Dry matter production on the second defoliation and DM digestibility of puero was higher than centro. Rock phosphate fertilization could increased the DM production of VAM inoculated legumes. Success of VAM inoculation in the field affected by effectiveness of indigenous-VAM fungi or depending upon VAM inoculum potential.

ACKNOWLEDGEMENT

Thanks to Directorate General of Higher Education, Ministry of Education and Culture of Republic Indonesia (:Grant Competition Research Project) for the financial support.

REFERENCES

Bagyaraj, D.J. 1988. Technology for inoculum production of VAM fungi. *In* Mahadevan, A., Raman, N., and Natarajan, K., eds., 1988. Proc. of the First Asian Conference on Mycorrhizae. Madras, 29-31 January, p.326-328.

Coates, D.B., Kerridge, P.C., Miller, C.P., and Winter, W.H. 1990. Phosphorus and beef production in northern Australia. 7. The effect of phosphorus on the composition, yield and quality of legume-based pasture and their relation to animal production. Tropical Grasslands. 24:209-220.

Dell, B., Malajczuk, N., and Grove, T.S. 1995. Nutrient Disorders in Plantation *Eucalyptus*. ACIAR Monograph

Gui,L.X., and Yin, H.W. 1991. Occurrence and efficiency of VA mycorrhizae in Fluvo-Aquic soils in North China. BIOTROP Special Publication. 42:111-121

Islam, A.K.M.S., Kerven, G., and Oweczkin, J. 1992. Methods of Plant Analysis. ACIAR 904. IBSRAM QC.

Jones, R.J. 1990. Phosphorus and beef production in northern Australia. 1. Phosphorus and pasture productivity. Tropical Grasslands. 24:131-139

Kerridge, P.C., and Ratcliff, D. 1982. Comparative growth of four tropical pasture legumes and guinea grass with different phosphorus sources. Tropical Grasslands. 16:33-40.

Lukiwati, D.R. and Simanungkalit, R.D.M. 2001. Improvement of maize productivity with combination of phosphorus fertilizer from difference sources and vesicular- arbuscular mycorrhizae inoculation. *p*.329-333. *In* Rajan, S.S.S, and Chien, S.H.,(eds). 2001. Proc.of International Meeting "Direct Application of Phosphate Rock and Related Appropriate Technology-Latest Developments and Practical Experiences". Kuala Lumpur, 16-20 July.

Lukiwati, D.R., and Simanungkalit, R.D.M. 2004. Production and nutritive value of *Pueraria phaseoloides* with vesicular-arbuscular mycorrhizae inoculation and phosphorus fertilization, abstr.*p*.90, Abstract of the 4^{h} International Symposium of the Working Group MO "Environmental Significance of Mineral-Organic Component-Microorganism Interactions in Terrestrial Systems". Wuhan-China, 20-23 September.

Lukiwati, D.R. and Supriyanto. 1995. Performance of three VAM species from India for inoculum production in centro and puero. *p*.257-265. *In* Prana, S.S., ed. 1995. International Workshop on Biotechnology and Development of Species for Industrial Timber Estates. Proc. LIPI Bogor. 27-29 June.

Mitiku-Habte and Fox, R.L. 1993. Effectiveness of VAM fungi in non sterile soils before and after optimization of P in soil solution. Plant and Soil. 151(2):219-226.

Potty, V.P. 1988. Response of cassava (*Manihot esculenta*) to VAM inoculation in acid lateritic soil. *p*.246-249. *In* Mahadevan, A., Raman, N, and Natarajan, K. 1988. Proc. of the First ACOM. Madras, 29-31 January.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. Second Edition. McGraw-Hill Book Company Inc, New York.

Terry, R.A.and Tilley, J.M.A. 1963. A two state technique for the in vitro digestion of forage crops. Journal British Grassland. F.O.C. 18:104-111

Yinglong, C., Mingqin, G., Fengzhen, W., Yu, C., Meiqing, Z., Dell, B., and Malajczuk, N. 1998. Diversity putative ectomycorrhizal fungi and arbuscular mycorrhizal fungi in *Eucalyptus* plantations in Southern China. *p* 21-28. *In* Mingqin,G., Daping, X., Chonglu, Z., Yinglong, C., Dell, B., and Brundrett, M.,eds.,1998. Proc. of Guangzhou ACIAR International Workshop. Guangzhou, 31 August-6 September,