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Effect of Bali Cattle Urine on Legume Cover Crop Puero (*Pueraria javanica*) Productivity on an East Borneo Oil Palm Plantation

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Abstract: This study examined the productivity of the legume cover crop puero (*Pueraria javanica*) used as Bali cattle feed on oil palm plantations in the East Kutai Regency in East Kalimantan Indonesia during November 2014-March 2015. Puero plants were grown in a 30 x 30 cm area in 24 plots with 45 plants per plot. Palm oil mill waste and Bali cattle feces were used as organic fertilizer. The plants were either left untreated (U-) or treated with Bali cattle urine (U+). Changes following urine treatment were measured as germination percentage, dry weight yields, leaves and stem weight, leaves and stem ratio, number and weight of root nodules, nutrient content (e.g., organic matter, crude protein, ash, crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF)), tannin and phenol, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). Statistical analysis of comparisons between the two groups were performed using a t-test. The results showed that the germination percentage was under 50% and did not differ significantly between U- and U+. In contrast, treatment of puero plants with urine significantly ($p<0.05$) affected dry weight production, yield per hectare, nitrogen content and stem production. These results indicate that the addition of urine to fertilizer produced higher (11.8%) puero yields and increased nitrogen content (24.6%). The TDN and digestibility (dry matter and organic matter) of puero would be sufficient for use of this plant in cattle feed.

Key words: *Pueraria javanica* productivity, fertilizer from oil palm plantation, Bali cattle urine

INTRODUCTION

Legume cover crops have notable protein content and thus have untapped potential as a feed source on palm oil plantations. Legumes are often grown as a cover crop between rows of oil palms (*Elaeis guineensis*) to increase soil fertility, maintain soil moisture and suppress weed growth (Lehmann *et al*., 2000). Typical legume varieties include Calopo (*Calopogonium mucunoides*), Centro (*Centrosema pubescens*), Puero (*Pueraria javanica*) and Mucuna (Mucuna bracteata) (Sunarko, 2009). The annual production of puero is 8-15 tons DM/ha/year, while 6 tons of callopo and centro are produced each year and 22 tons of mukuna, which has a crude protein content of >20% before flowering and 16% after flowering, are produced annually (Legel, 1990).

Puero has palatable, protein-rich leaves and does not contain harmful toxins. Cultivation of puero is required to enhance availability during the dry season, as well as to improve the quality, digestibility and palatability as cattle feed. Moreover, cultivation of legume forages promotes production of tannins that can inhibit protein degradation in cattle rumens.

Legume plants can grow symbiotically with *Rhizobium* Sp bacteria that interacts with plant roots. These bacteria cause the formation of nodules that can fix nitrogen from the air (Fuskhah, 2011). This nitrogen fixation activity occurs not at the end of the vegetative phase and the reproductive phase, but rather shortly after rhizosphere infection and colonization of legume roots (Beck *et al*., 1991). Nodules will appear after the legume plant reaches 28 days of age (Rao, 1996), so additional N sources are needed to promote growth. Urine is one such N source that can also provide potassium and growth hormones (Kamara, 2011). Urine contains substances arising from nitrogen metabolism (e.g., urea, uric acid and keratin). In livestock, urine composition can be influenced by feed, activity, external temperature, water consumption and season.

Maintenance of sufficient puero productivity on palm oil plantations is critical in order for this plant to be used as a feed crop. Urine is known to enhance puero productivity because of its N and K content, as well as the presence of plant growth hormones such as auxin-a, auxin-b and the auxin Indole Acetic Acid (IAA). Auxin encourages plant rooting and can be derived from a variety of substances contained in feed forage proteins. Auxin can also stimulate and encourage cell division in plant cambium vessels that support increases in stem diameter. Meanwhile, previous studies showed that IAA

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is transported through the cells of the cambium (Little and Savidge, 1987). Since auxin does not decompose in the body, it is thus a constituent of kidney filtrates and subsequently urine (Yunita, 2011).

MATERIALS AND METHODS

This study was conducted on a palm tree plantation in the Bengalon District, East Kutai Regency, in East Borneo during the period between November 2014 and March 2015. This region receives a monthly rainfall between 142 and 430 mm (Fig. 1) and 14-19 rainy days per month. Air temperature ranged from 22.6-35.2°C and the relative humidity was between 72 and 88% (Balikpapan Station of Climatology, 2015).

Materials: The research land for cultivating *Pueraria javanica* (puero) legume covered an area of 15 x 15 m that was divided into 24 3 x 2 m plots with 45 puero plants per plot. Soil samples were taken as deep as 20 cm in some places and the laboratory analysis of sample soils, organic fertilizer and Bali cattle urine is shown in Table 1. Polybags (10 cm) contained soil, sand and organic fertilizer (1:1:1).

The total area was seeded with 150 g (10 kg/ha) puero (Anonymous, 2009), with organic fertilizers added at 0.5 kg/plant (4,350 kg/ha). The fertilizer contained the following raw materials: fresh Bali cattle dung (60%), empty fruit bunches from oil palms (24%), ash boiler from a palm oil mill (6%), dolomyte (4%) and decomposer microbes (2%) (Mudhita and Saprudin, 2014). Other fertilizers included 270 L Bali cattle urine (5,000 l/ha) (Kamara, 2011), 21.6 kg (200 kg/ha) rock phosphate (Anonymous, 2009), 3.75 g (25 g/1 kg of seeds peuro) and Rhizobium Legin LCC (leguminose inoculant cover crop) (Anonymous, 2014).

Methodology: Puero plants in this study were divided into two groups with 12 plots left untreated (U-) and 12 plots treated with Bali cow urine (U+).

Puero legume seeds (150 g) were soaked for 6 h in a mixture of hot water and cold water (1: 2) for 1 h to soften the outer shell of the seed (Anonymous, 2014). Seeds were planted in polybags (5 seeds/bag) in the afternoon to a depth of 2 cm. After 21 days, puero legumes were transferred to plots with one plant per hole and with 30 x 30 cm spacing. Fertilizers and phosphate rock were placed in planting holes (Anonymous, 2009). Fertilization was achieved using 0.5 kg/plant organic fertilizer applied over the course of 30 days (0.15 kg seven days before planting, 0.15 when plants were 10 days old and 0.2 kg when plants were 30 days old) and treatment with cow urine (U+). Plants were treated with urine diluted with water (1:10) that was sprayed around the plant on day 15 and day 30. The plants were harvested at 3 months of age by taking cuttings 10 cm from the ground.

Parameters measured included: (1) production aspects: nodule (number, weight), legume length, ratio of leaves: stem, leaves:stem weight, dry weight yield (DM), organic matter (OM); (2) nutrient aspects: proximate analysis (AOAC, 2005), analysis of fiber NDF and ADF (Van Soest, 1982); (3) anti-nutrient aspects: tannins and phenols and (4) digestibility aspects: *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) (Tiley and Terry, 1963). Legume productivity data were analyzed with a t test.

RESULTS AND DISCUSSION

Production of *Pueraria javanica*: The production of dry matter and organic matter in *Pueraria javanica* legumes is shown in Table 2.

Dry matter production per plant and per m^2 was affected by treatment with cow urine ($p<0.05$). Compared to untreated plants (U-), the addition of urine (U+) resulted in 11.8% higher yields and nitrogen increased by 24.6%. This increase is consistent with earlier findings on coffee plantations in Nigeria by Nkwiine *et al.* (1999) that showed that puero production without additional fertilizer was 8.4 tonnes/ha with nitrogen levels of 336.6 kg/ha and increased to 11 tonnes/ha and 489.9 kg/ha nitrogen after the addition of organic cow manure as fertilizer. Puero legumes can take N from the air in the presence of symbiotic Rhizobium bacteria. However, since Rhizobium nodules capable of fixing nitrogen take some time to appear, N supplementation is needed to start early growth. Urine from Bali cattle can provide such a N source (Table 1). Dry plant weight can provide a measure of the quality of plant growth and crop yield and is associated with photosynthesis as well as nutrient assimilation and translocation and plant organ maintenance (Yunita, 2011). Treatment of puero plants with urine likely produced significant growth effects due to the hormone indole acetic acid (IAA), which is the main auxin in present in these plants. Moreover, auxin is known to promote bends in hair roots that are a prerequisite for Rhizobium infection (Gardner *et al.*, 1991; Kamara, 2011). Auxin also affects stem length, growth, differentiation and root branching, as well as fruit development, apical dominance, phototropism and geotropism (Gardner *et al.*, 1991).

Both treated and untreated plants showed similar amounts of organic matter. However, organic matter content was affected by the amount of ash in treated plants (Table 4). Fresh weight and dry weight of plants is associated with the number of leaves, shoot length and fresh weight of shoots. Since the dry weight of shoots is affected by leaf photosynthesis and nutrient absorption by the roots, this parameter can be used as a measure of plant growth and yield, which is also affected by photosynthesis and decreased nutrient assimilation and translocation to plant organs. Exogenous hormones can be translocated throughout

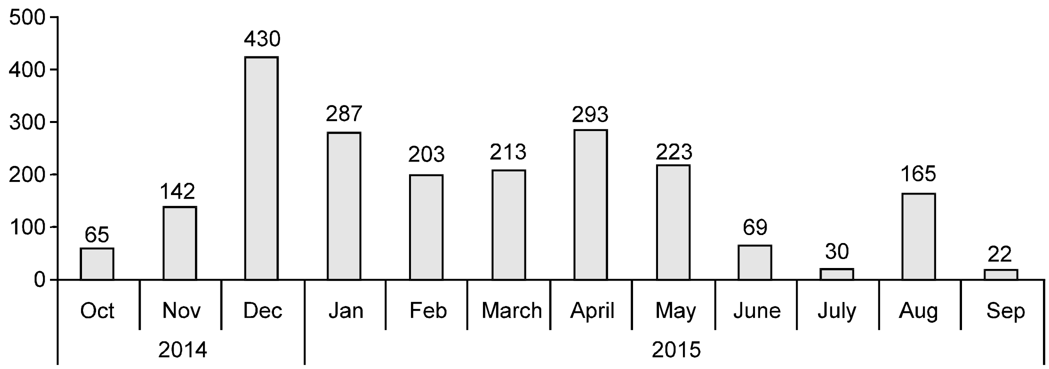

Fig. 1: Rainfall (mm) in Bengalon East Kutai Regency District in East Borneo, Indonesia

the plant to stimulate cells at the root tip that promote root cleavage and lengthening. Increased numbers and length of roots enhances water absorption and nutrient transport that in turn increases fresh weight and dry weight of roots. More rapid budding also accompanies increased rates of plant growth that also increases plant height and the number of leaves. All together these increases contribute to increased fresh and dry weights. Leaf production was not affected by urine treatments, although the stem weight was ($p<0.05$) (Fig. 2). The stems in particular would be expected to be affected by cattle urine, which can penetrate the soil and provide additional nutrients to promote puero growth. Beyond the additional nutrients for puero growth, there are sufficient levels of auxin in cow urine that interact with other growth regulators such as cytokinin. These factors could work together to promote cell division and increase the activity of enzymes that stimulate protein synthesis (Himanen *et al*., 2011). Untreated plants had lower stem weights, likely because the low content and activity of endogenous hormones lessens their effectiveness.

Germination parameters, including plant length, number and weight of nodules are presented in Table 3.

Neither growth rate nor length of puero plants was affected by urine treatment. The average length daily gains were 3.2 cm and 3.3 in the absence of presence of urine, respectively. Shoot length growth is also influenced by auxin and cytokinin, which can stimulate cell division through increased protein synthesis rates, whereas auxin spurs stem elongation by stimulating specific plasma membrane proton pumps. The increased proton pumping activity in turn activates other enzymes that promote hydrogen chain cross linking of cellulose molecules that comprise plant cell walls as well as increased water uptake through osmosis. After lengthening, the cells continued to grow with the return material to synthesize cell wall and cytoplasm (Yunita, 2011).

The number and weight of rhizobacteria nodules was not significantly different between urine-treated and untreated plants. Nodule weight reflects the effectiveness of root nodules of legumes in that as the nodule weight increases the nitrogen fixaction capacity also increases (Somaatmadja *et al*., 1988). Maximal nitrogen fixation activities can in turn provide sufficient N to meet the growth needs of legumes, which would, in

Table 1: Chemical analysis of soil chemistry, organic fertilizer and Bali cow urine

Parameter	Soil	Organic Fertilizer	Bali cow urine
pH (1:2.5) (H_2O)	4.40	7.47	7.32
C organic (%)	9.36	17.61	0.70
Organic matter (%)	16.14	30.66	1.21
Total N (%)	0.46	1.51	0.03
P Available (ppm)	2.16	0.47	0.02
K Available (me/100 g)	0.25	1.04	0.3
C/N ratio	20.35	11.66	23.33
Texture class	Red soil		

Source: Laboratory of Soil Faculty of Agriculture Gadjah Mada University (2014)

Table 2: Effect of Bali cattle urine treatment on *Pueraria javanica* production

	---------- Bali cattle urine ----------	
Parameters	U-	U+
Dry matter production/plant (g)	93.22±8.60[a]	104.13±12.59[b]
Dry matter production/m^2 (g)	838.98±67.49[a]	937.17±167[b]
Organic matter production/plant (g)	87.18±2.39	88.59±2.24
Yield per ha (t/ha DM)	8.4[a]	9.4[b]
Nitrogen content (kg/ha)	277.5[a]	344.9[b]
Leaf production/plant (g)	38.07±1.35	40.49±8.57
Stem production/plant (g)	55.15±8.61[a]	63.64±12.59[b]
Ratio stem and leaves	0.69±0.13	0.73±0.13

Different superscripts in the same row indicate significant differences ($p<0.05$)

Table 3: Germination, plant length, number and weight of nodules for *Pueraria javanica* plants treated with Bali cattle urine

	---------------Bali cattle urine --------------	
Parameters	U-	U+
Germination (%)	40.5±8.63	42.75±8.99
Plant length (cm)	293.62±41.85	301.09±43.91
Number of Nodules	38±20.22	39±24.92
Nodule weight (g)	2.87±1.65	2.98±1.22

Fig. 2: (a) Puero without Bali cattle urine treatment (U-) and (b) Puero with Bali cattle urine treatment (U+)

Table 4: *Pueraria javanican* nutrient content following Bali cattle urine treatment (% Dry matter)

	------- Bali cattle urine -------	
Parameter	U-	U+
Dry matter	26.95	25.82
Crude protein	20.65	22.93
Crude fiber	24.23[a]	17.88[b]
Ether extract	2.70	2.58
Ash	11.72	12.26
Non nitrogen extract (NNE)	40.70	44.35
Total digestible nutrient (TDN)	64.68	68.45
Neutral detergent fiber (NDF)	45.70	43.54
Acid detergent fiber (ADF)	29.45	29.11
Anti-nutrient (%)		
Tannin	3.64	3.87
Phenols	1.50	1.58

Different superscripts in the same row indicate significant differences ($p<0.05$)

Table 5: Dry matter and organic mater digestibility (*in vitro*) *Pueraria javanica* with added bali cattle urine

	--- Bali cattle urine ---	
Parameter	U-	U+
In vitro dry matter digestibility (%)	51.11	52.71
In vitro organic matter digestibility (%)	55.05	56.47

the context of this study, increase production of puero legumes. Most nodules on the puero plants are located at the base of the main root, but a small portion can be found at the root of the vine stems in the ground. In cross-section, the nodules have a pink color, which indicates the presence of leghemoglobin. This molecule is a hallmark of nodule effectiveness (Buchanan and Gibbans, 1974) and is directly related to nitrogen fixation activity (Rao, 1996).

Nutrient value of *Pueraria javanica*: The values for nutrients and anti-nutrients in puero legumes are shown in Table 4.

Bali cattle urine did not affect dry matter, ether extract, ash, crude protein, or NNE and TDN content, except that the crude fiber content in urine-treated plants was lower than untreated plants ($p<0.05$), as was the NDF level, although ADF was similar for both groups. Plants with higher contents of crude fiber and NDF are known to be of lower quality as crop plants (Tillman *et al.*, 1998). Moreover, the proportion of stems that formed more than one leaf can be increased by application of organic fertilizers containing phosphorus (P), which is also critical for stimulating root growth, especially in roots and stems of young plants and for promoting nutrient assimilation and respiration. According to Legel (1990) the crude protein content of puero before flowering is about 22%, while the values for puero grown on East and Central Borneo palm oil plantations are 18.25 and 14.24%, respectively (Mudhita, 2011).

TDN in both treated and untreated plants was around 65%; although TDN in treated plants was higher, the difference was not significant. This TDN content indicates that puero contains sufficient energy to meet the needs of cattle. Meanwhile, the TDN of puero legumes grown as a cover plant in Central Borneo palm plantations was 57%. The fiber content (NDF) of 43-45% indicates that puero legumes do not contain a lot of fiber, particularly as compared to the 71% NDF in *Mucuna bracteata* legumes (Sirait *et al.*, 2009). The anti-nutrient content of tannin and phenols, which should be limited in cattle feed, was at most 9% in this study and was typically lower.

***In vitro* dry matter and organic mater digestibility:** *In vitro* Digestibility is shown in Table 5.

Although the dry matter and organic matter digestibility in treated puero plants was higher than that of untreated plants, the differences were not significant. Fluid used in digestion is derived from the rumen in Bali cattle. Dry matter digestibility and organic matter are strongly influenced by the content of crude fiber, crude protein, rumen fluid and an aerobic conditions (Sutardi *et al.*, 1983).

Dry matter digestibility can affect crude protein levels that are available for use by rumen microbes, which in turn affect the number of proteolytic bacteria and increases deamination to improve organic matter digestibility (Sutardi, 1980). Given that puero plants are known to have high crude protein contents (Table 4), the high organic matter digestibility would be expected.

Conclusion: The legume plant *Pueraria javanica* showed good growth and production with a relatively high nutrient value following treatment with Bali cattle urine, although nutrient values did not significantly differ between treated and untreated plants. The highest production of dry matter per m^2 was 937 and 838 g, with and without urine, respectively, which represents an increase of 11.8%. Meanwhile, the nitrogen content of urine-treated plants increased by 24.6% relative to untreated plants. The TDN and digestibility (dry matter and organic matter) are also of levels that are suitable for cattle feed.

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