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Determination of the Optimum Rate of N Fertilizers with Addition of Goat Manure for Production of Cowpea (*Vigna unguiculata* [L.] Walp)

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Abstract

Cowpea (*Vigna unguiculata* L. Walp) is one of the potential substitutes of soybean due to its similar nutritional content. This study aims to determine the optimum rate of N fertilizer for the production of cowpea, and to determine the effects of the interaction between N fertilizer rates and goat manure application on cowpea production. The experiment was organized in a split-plot with a complete randomized block design with three replications. The main plot was goat manure, (0 and 5 tons.ha^{-1}); the sub-plot was rates of nitrogen fertilizer (0, 50, 100, 150, and 200% of the recommended rate), or 0, 22.5, 45, 67.5, and 90 kg N.ha^{-1}. Application of N fertilizer reduced the 100-seed weight and slightly reduced cowpea yield and yield components. Application of goat manure increased seed dry weight per plant, number of pods per plant, dry pod weight per plant, seed dry weight per m^2, productivity, 100-seed weight, and the harvest index. There was no significant interaction between goat manure and different rates of nitrogen in affecting cowpea growth.

Keywords: inorganic, organic, legumes, yields and yield components

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is a species originating from West Africa (Kamai et al., 2014). Cowpea is a multi-functional crop as almost all parts of the plant can be used as source of foods for human as well as animal feeds (Kamai et al., 2014). Cowpea has high nutritional content; 100 g of cowpea tempeh contains 33 g of protein, 2 g of fat, 53 g of carbohydrates, 3 g of fibers, and 1 g of ashes (Haliza et al., 2007). Cowpea is one of the potential legumes that can replace soybean as raw materials to produce fermented soybean cake (Richana and Damardjati, 1999).

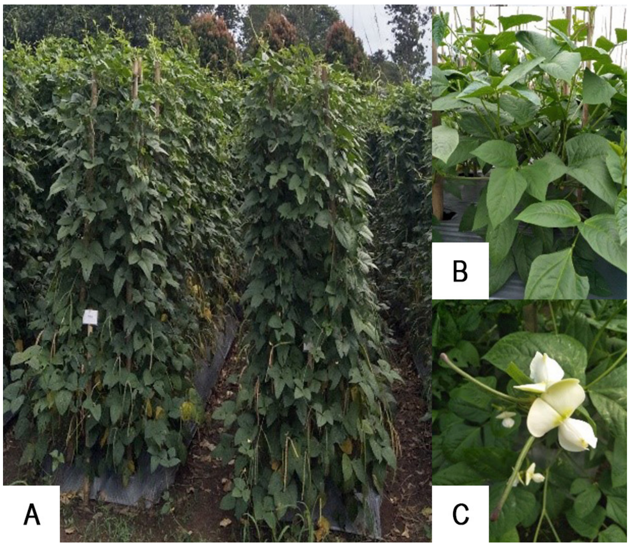

Figure 1. *Vigna unguiculata* plant (A), leaves (B) and flowers (C)

The average cowpea production in Indonesia is 1.5-2.0 ton.ha^{1} dry seeds, which is classified as very low according to Kasno et al. (1991). Optimizing fertilizer application is one of the options to increase production of cowpea, and information the correct fertilizer rates is important for adoption of improved technologies by cowpea growers.

N is one of the macro nutrients required by plants in large quantities, and its source can be in the form of either organic or inorganic fertilizers. Nitrogen deficiency symptoms first appear on older leaves, indicated by discoloration or yellowing. On the contrary, too much nitrogen will extend the vegetative growth period and reduce yields (Sugito, 2012).

The advantages of inorganic fertilizers include their high nutrient content in the forms that are immediately available for the crops. However, continuous use of inorganic fertilizers without being balanced by organic fertilizers can reduce soil fertility. The advantages of organic fertilizers include improvement of the physical, chemical and biological properties of the soil (Hartatik et al., 2015). Organic fertilizers have complete nutrient contents, but in relatively small amounts compared to those in chemical/inorganic fertilizers. The use of organic fertilizers combined with inorganic fertilizers can potentially increase production and reduce the requirements of the inorganic fertilizers. One of the organic fertilizers that can be used is goat manure; it is available in many Indonesian regions the compared to other animal manures.

Crop yields are highly influenced by the rates of fertilizer application. Dinariani et al. (2014) reported that sweet corn at density of 45.333 per ha applied with 10 tons.ha^{-1} goat manure produced the highest sugar content of 16.07 brix. In addition, application of goat manure at this rate increased the yield of fresh cob with husk by 19.46% compared to the untreated crops. Puspitasari and Elfarisna (2017) showed that the use of 100% inorganic fertilizer without the addition of liquid organic fertilizer promoted earlier flowering time. Application of liquid organic fertilizer at 150 ml + 50% inorganic fertilizer resulted in the tallest crops, the greatest number of pods and 100-seed weight, whereas application at 200 ml liquid organic fertilizer + 50% inorganic fertilizer resulted in crops with more branches, a higher percentage pod formation, and seed dry weight. The liquid organic fertilizer at 150 ml + 50% inorganic fertilizer was the best treatment for soybean (Puspitasari and Elfarisna, 2017). This study investigated the optimum dose of N fertilizer with the addition of goat manure for the production of cowpea.

Material and Methods

The study was conducted at the Leuwikopo Experimental field, Bogor Agricultural University, Darmaga at 6^{0}33'49.3"S, 106^{0}43'30.7" E in November 2017 until February 2018. Soil analysis was carried out at the Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor. Analysis of production variables included dry seed weight, number of pods, dry pod weight, weight of 100 seeds, harvest index, dry seed weight per m^2, and cowpea productivity at the Post Harvest Laboratory of the Department of Agronomy and Horticulture, Bogor Agricultural University. The materials used were cowpea seeds, Urea, KCl, SP 36, goat manure and Furadan (a.i. Carbofuran).

The experiment was organized in a split-plot with a complete randomized block design. Goat manures (0 and 5 ton.ha^{-1}) were assigned as the main plots, and rates of nitrogen fertilizer (0, 22.5, 45, 67.5, and 90 kg N.ha^{-1}) as the sub-plots. Rates of N were based on fertilizer recommendation for cowpea production, i.e. 45 kg N.ha^{-1} (Adisarwanto et al., 1998; Chozin et al., 2006; Setyowati and Sutoro, 2010; Sayekti et al., 2011). The plot size of 0.8 m x 4 m is considered as one experimental unit with 40 plants per plot. Distance between plots was 50 cm. All experimental plots were covered with plastic mulch. Goat manure was applied one week before planting; all inorganic fertilizers were applied one day before planting. The N fertilizer rates according to the treatment, K_2O, and SP-36 were applied at the same time. Planting distance was 40 cm x 40 cm; each planting hole was planted with three cowpea seeds, after two weeks left two seeds per hole. Furadan™ (a.i. carbofuran) insecticide was applied with the seeds at 10 kg.ha^{-1} at planting.

The cowpea crop growth rate was measured through destructive sampling of eight plants at 30, 42, 60, and 77 days after planting (DAP), or at the initial growth phase, flowering phase, the onset of pod formation, and seed filling, respectively. Harvesting was carried out at 70 days and 77 days after planting (DAP). Analysis of leaf content was conducted at the flowering phase (42 DAP) using the Kjeldahl method for N, and wet fogging method for determination of P and K levels. Nutrient uptake was calculated by multiplying the crop dry weight and leaf nutrient content. Measurement of nutrient uptake was carried out at 42 DAP.

The data were analyzed using the ANOVA; mean separations were performed with orthogonal polynomial contrast to evaluate the crop response patterns. All statistical analysis used SAS 9.4 software.

Result and Discussion

Soil nutrient levels at the beginning of the study were classified as moderate, and the soil was slightly acidic (Table 1).

Leaf Stomatal Density

Goat manure, N rates, and their interactions had no significance effect on stomatal density (Table 2). The lower density of stomata in the adaxial or the upper surface of the leaf was a form of adaptation of land plants in controlling the rate of transpiration due to direct exposure to solar radiation on the upper surface of the leaf (Zandroto, 2017). This was in line with the research of Zandroto (2017) in *Vigna unguiculata* that the average stomatal density on the upper surface of the leaves ranged from 128.64-136.28 mm^{-2} while in the lower surface of the leaves were 301.85-387.18 mm^{-2}.

Cowpea Growth Rate

Goat manure application did not have a significant

Table 1. The soil chemical properties prior to cowpea planting*

Soil parameteer	Extraction method	Value	Status
pH	H_2O	5.60	Slight acidic
C-Organic (%)	Walkley and Black	1.41	Low
Total N (%)	Kjeldahl	0.23	Medium
Available P (P_2O_5, ppm)	Olsen	102.65	Very high
K (cmol(+)/kg)	NH_4OAc 1M pH 7.00	0.61	High
CEC (cmol(+)/kg)	NH_4OAc 1M pH 7.00	18.36	Medium

Note: *Soil chemical properties criteria according to Sulaeman et al., (2005)

Table 2. Cowpea stomatal density with the application of goat manure and different rates of N fertilizer

Treatment	Stomatal density (per mm^2)	
	adaxial	abaxial
Goat manure (ton.ha^{-1})		
0	158.93	307.73
5	150.26	305.40
Rates of N fertilizer (kg.ha^{-1})		
0	148.62	268.33
22.5	173.00	324.33
45.0	148.50	341.33
67.5	162.00	257.50
90.0	141.00	341.30
Goat manure	ns	ns
N fertilizer rates	ns	ns
Goat manure x N fertilizer rates	ns	ns

Note: ns= not significant according to orthogonal polynomial contrast at 5%.

effect on plant growth rate at 30-42 DAP and 42-60 DAP, but had significant effects at 60-77 DAP (Table 3). Although it was not significant, there were indications that the increasing rates of N fertilizer slowed the cowpea growth.

Rates of fertilizer that exceeded the limit of the crop nutrient requirements could result in nutrient toxicity, stunted or/and abnormal growth (Hardjowigeno, 2010). In line with Hardjowigeno (2010), Budiyani et al. (2012) reported that increasing the nitrogen concentration from 150 μM and 200 μM promoted growth rate of a seaweed species *C. racemosa var. uvifera*, but plant growth started to decrease. Too high nitrogen concentration weakens the stem, resulting in the stem to break easily and stunted growth which eventually reduced the crop biomass.

The cowpea growth rate increased at pod formation stage (42-60 DAP) and decreased at seed filling stage (60-77 DAP) (Table 3).

Cowpeas started to flower at 32 DAP, and 50% of the population flowered at 40 DAP. The formation of cowpea pods occurred around four days after flowering. Seed filling lasted ten days after pod formation, and pods were fully filled then days later.

Cowpea growth rate increased particularly at the pod formation stage (42-60 DAP). The increase in cowpea growth was possibly to increase source capacity (foliage growth) to meet the requirement of the sink (pod formation). The growth rates decreased as the crops grew older at 60-77 DAP which was likely due to competition for assimilates between vegetative and reproductive organs. This was in line with reports by Zandroto (2017) that plant growth rates decreased with the increase in plant age until 44-84 DAP, and that continuous vegetative growth at flowering and pod formation could reduce crop yields.

Leaf Nutrient Content and Uptake

Application of goat manure did not have significant effects on the nutrient content of leaves and leaf nutrient uptake at the flowering phase (42 DAP) (Table 4). Application of manures increased leaf P content and P uptake, but decreased leaf N and K content.

Table 3. The cowpea growth rate with the application of goat manures and different rates of N fertilizer at six weeks after planting

Treatment	Plant growth rate (g per m^2 per day)		
	30-42 DAP	42-60 DAP	60-77 DAP
Goat manure (ton.ha^{-1})			
0	10.27	19.01	17.02
5	9.22	18.16	13.38
Rates of N fertilizer (kg.ha^{-1})			
0	11.01	16.35	14.47
22.5	9.68	20.46	13.79
45.0	9.45	21.58	15.60
67.5	9.76	21.44	18.97
90.0	8.83	13.10	13.16
Goat manure	ns	ns	*
N fertilizer rates	ns	ns	ns
Goat manure x N fertilizer rates	ns	ns	ns

Note: ns= not significant according to orthogonal polynomial contrast at 5%; *= significant at 5%.

N fertilizer did not significantly affect leaf nutrient content and K uptake, but the increasing rates of N fertilizer to 67.5 kg N^{-1} increased the N uptake, and reduced it at 90 kg N^{-1} (Figure 1). The leaf uptake of N and P increased with the increasing rates of N fertilizer in a quadratic response pattern (Figure 1).

Increasing the rates of N fertilizer application increased N content in leaves (Table 4). One of the nitrogen functions in plants is to promote the uptake of the other nutrients (Maschner, 2012), and in this study application of N fertilizer at different rates affected the P nutrient content of leaf. The highest leaf P content was achieved by administering N fertilizer at 22.5 kg N.ha^{-1}. This was probably because the higher the supply of N fertilizer, the higher the availability of N in the soil that could be absorbed by plants so that it might spur P uptake , hence increased the leaf P

Table 4. Cowpea leaf N, P, K content and uptake with application of goat manure and different rates of N fertilizer

Treatment	Leaf nutrient content (%)			Leaf nutrient uptake (g per plant)		
	N	P	K	N	P	K
Goat manure (ton.ha^{-1})						
0	5.29	0.42	3.25	2.37	0.18	1.44
5	5.19	0.43	3.05	2.29	0.19	1.35
Rates of N fertilizer (kg.ha^{-1})						
0	5.11	0.40	3.11	2.23	0.17	1.35
22.5	5.18	0.43	3.27	2.33	0.19	1.47
45.0	5.23	0.43	3.20	2.50	0.20	1.51
67.5	5.30	0.43	3.07	2.59	0.21	1.52
90.0	5.38	0.43	3.11	1.99	0.16	1.14
Goat manure	ns	ns	ns	ns	ns	ns
N fertilizer rates	ns	ns	ns	*	*	ns
Goat manure x N fertilizer rates	ns	ns	ns	ns	ns	ns
Response pattern^t	ns	ns	ns	Q**	Q**	ns

Note: ns= not significant according to orthogonal polynomial contrast at 5%; Q= quadratic; *= significant at 5%; **= significant at 1%.

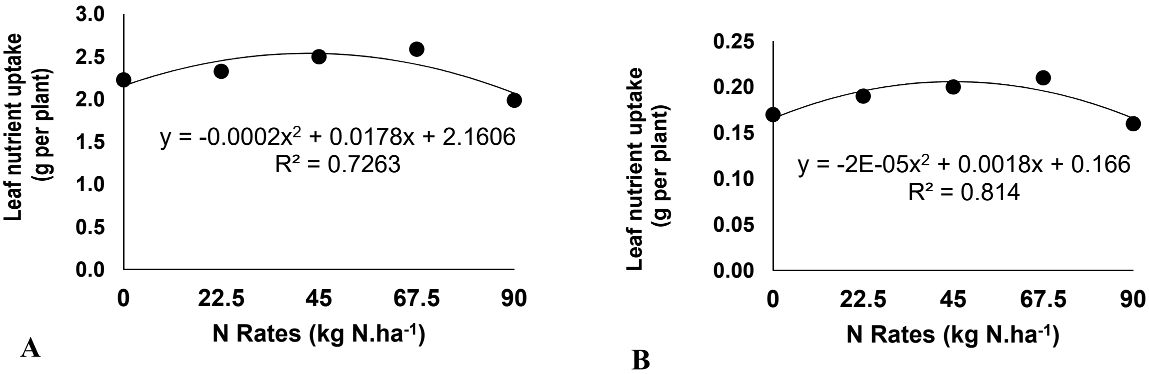

Figure 2. Effect of N fertilizer application on N (A) and P (B) uptake of cowpea leaves at 42 days after planting

levels (Lestari et al., 2015). This was confirmed Amin (2007) that N availability can stimulate P uptake, and vice versa. The N content in the leaves of legumes was usually 2 to 5% (Maschner, 2012). The results of the study showed that nutrient content of N on the leaves of cowpea was optimal, namely 5.11 to 5.38%.

The average content of P in leaves ranged from 0.40-0.43% (Table 4), so the P content of cowpea leaves seems to be sufficient. There is no information regarding the criteria for N, P and K nutrient sufficiency on cowpea leaves, so this criteria was assessed based on Rahmianna and Bel (2001) report on peanut which showed that the optimal P content of peanut leaves ranged from 0.3-0.5% of the total plant dry weight.

Application of higher rates of N fertilizer reduced leaf K uptake (Table 4), possibly due to the acidic soil at the study site. In addition, K content in the soil was already high. The average leaf K in this study ranged from 3.07-3.27%, which is sufficient according to Lestari et al. (2015) study on Bogor beans, which showed that the optimal leaf K ranged from 0.93-1.13%.

Leaf N and K uptake was influenced by N fertilization treatment in a quadratic pattern (Figure 1). According to Mulyadi (2012) application of NPK fertilizer, urea, and legume inoculant could increase N uptake in soybean, but the high N fertilizer application could decrease the total N of the shoots, this was due to nitrogen tethering activity by *Rhizobium* bacteria which decreased due to high nitrogen levels in the soil.

Cowpea Yield and Yield Components

Application of goat manure at 5 ton.ha^{-1} significantly increased dry seed weight, the number of pods, dry pod weight, 100-seed weight, and the harvest index by 71.6%, 51.1%, 71.8%, 9%, and 140 %, respectively, compared to control.

Rates of nitrogen fertilizer did not have significant effects on seed dry weight, number of pods, dry pod weight, and harvest index. However, increasing N rates significantly decreased 100-seed weight with a negative linear pattern. Similarly, increasing rates of N fertilizer reduced seed dry weight, number of pods, dry pod weight, 100-seed weight, and harvest index. There was no interaction between goat manures and N fertilizer rates in affecting cowpea yield components.

Application of 5 ton.ha^{-1} of goat manure significantly increased seed dry weight per m^2 and cowpea productivity by 71.6%, and 76.5% compared to control, respectively (Table 6). The rate of N fertilizer did not have significant effects on seed dry weight per m^2 and productivity. Application of N fertilizer tends to reduce seed dry weight per m^2 and productivity (Table 6). The interaction of the treatment level of manure and level of N fertilizer did not have a significant effect (Table 6).

The soil nutrient analysis before planting showed that the soil in the study area was relatively acidic (pH 5.60). C-Organic content was low (1.41%), N-total was classified as moderate (0.23%), P was very high (102.65 ppm), and K was high (0.61 cmol (+) per kg). Based on these results the soil had sufficient N nutrients for the growth of cowpea. However, the application of manure could increase the yield and yield components of cowpea. The goat manure in this had a water content of 55.68%, N of 0.61%, P_2O_5 of 0.38%, and K_2O of 3.68%. Five ton per ha of goat manures potentially supply 30.5 kg of N, 69 kg of P_2O_5, and 184 kg K_2O per ha. The application of manure could also improve the physical, chemical and biological properties of the soil, including C/N ratio (Putra et al., 2015).

The nitrogen requirement for crop growth can be obtained from the soil and from the air through the symbiosis with N fixating bacteria *Rhizobium*. The high rates of N fertilizer application had possibly inhibited the *Rhizobium* growth and their symbiosis with the cowpea roots. According to Maschner (2012) nitrogen in the soil was generally in the form of nitrate. Excessive N would reduce the N fixation process

Table 5. Seed dry weight, number of pods, dry pod weight, 100 seed weight, and cowpea harvest index in the treatment levels of manure and levels of N fertilizer

Treatment	Seed dry weight per plant (g)	Number of pods per plant	Pod dry weight per plant (g)	100 seed weight (g)	Harvest index
Goat manure (ton.ha^{-1})					
0	4.47	4.50	6.20	12.16	0.05
5	7.67	6.80	10.65	13.26	0.12
Rates of N fertilizer (kg.ha^{-1})					
0	8.10	7.07	10.95	13.19	0.12
22.5	6.94	5.92	9.70	13.31	0.09
45.0	4.70	4.90	6.77	12.41	0.07
67.5	5.47	5.47	7.46	12.72	0.07
90.0	5.15	4.89	7.25	11.92	0.09
Goat manures	**	**	**	**	**
N fertilizer rates	ns	ns	ns	*	ns
Goat manures x N fertilizer rates	ns	ns	ns	ns	ns
Response pattern^t	ns	ns	ns	L**	ns

Note: ns= not significant according to orthogonal polynomial contrast at 5%; L: linear;
*= significant at 5%; **= significant at 1%.

Table 6. Seed dry weight and cowpea productivity with goat manure application and different rates of N fertilizer

Treatment	Seed dry weight per m^2 (g)	Productivity (ton.ha^{-1})
Goat manure (ton.ha^{-1})		
0	35.78	0.17
5	61.40	0.30
N fertilizer rates (kg.ha^{-1})		
0	64.82	0.32
22.5	55.51	0.27
45.0	37.57	0.18
67.5	43.79	0.21
90.0	41.25	0.20
Goat manures	**	**
N fertilizer rates	ns	ns
Manures x N fertilizer rates	ns	ns

Note: ns= not significant according to orthogonal polynomial contrast at 5%; **= significant at 1%.

by *Rhizobium*. Thus, overdose of N fertilizer can decrease yield, as reported by Ferguson et al. (2018) Low levels of exogenously applied nitrogen (<2 mM) enhance nodulation, likely by promoting plant health without being sufficient to make nitrogen fixation redundant, whereas nodule numbers gradually decrease with the application of increasing nitrogen concentrations.

N fertilization rates in this study had reduced cowpea yield and yield components (Table 5 and Table 6). There were several possibilities to explain these findings; the first possibility was that the seeds used were low yielding, the average production of cowpea planted by farmers in Indonesia is 1.5-2.0 tons ha^1 dry seeds (Kasno et al., 1991). The second possibility was that cowpea need low levels of nitrogen. As reported by Rahman et al. (2018) the results suggest that small-scale farmers could apply starter N fertilizer at either 15 kg.ha^{-1} N for grain only or 30 kg.ha^{-1} N for both grain and fodder yields improvement of cowpea in West Africa and similar ecologies.

The potential yields of cowpea according to Kasno et al. (1991) are 1.5-2.0 tons.ha$^{-1;}$ therefore the cowpea production in this study (0.32 tons.ha^{-1} with goat manure application and nitrogen fertilizer) was relatively low (Table 6). Low cowpea production in this study could be due to the low light sunlight intensity and too high humidity due to extended heavy rains during the course of the experiment. According to Gardner et al. (1991) yield components are highly influenced by management or cultivation technology, genotypes, and the environment, and the environmental factors affects the ability of the crops to reach their genetic potentials.

This study had demonstrated that the soil nitrogen level with the application of goat manure at 5 ton per ha was 0.23 %, and at this level application of N fertilizer up to 90 kg.ha^{-1} did not increase cowpea yield.

Conclusion

Goat manures application at 5 tons.ha^{-1} increased seed dry weight per plant, number of pods per plant, dry pod weight per plant, seed dry weight per m^2, productivity, 100-seed-weight, and harvest index of cowpea crops. Application of N fertilizer in addition to goat manures did not increase cowpea yield. Interactions between goat manure and rates of N fertilizer were not significant in all measured variables.

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