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Increase nutritional content and *in vitro* digestibility of forage legumes by adding molybdenum with foliar spray methods

Iin Susilawati*, Nyimas Popi Indriani, Lizah Khairani and U. Hidayat Tanuwiria

Department of Animal Nutrition and Feed Technology,
Faculty of Animal Husbandry, Padjadjaran University-45363, Indonesia.
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ABSTRACT

Legumes as a source of protein is one of the most important nutrients for the ruminants. The experiment was carried out in the field of Forage Crop Laboratory of Padjadjaran University from october 2016 until January 2017, to study the effect of molybdenum fertilization on quality and *in vitro* digestibility on legumes. The design used was randomized block design with six treatment combinations consisting two levels of molybdenum (0 and 1.5 kg Mo ha^{-1}) with foliar spray methods and three species of legumes (*Pueraria phaseoloides*, *Calopogonium mucunoides* and *Centrosema pubescens*) replicated four times. The results showed that the type of legume and molybdenum fertilizer had significant effect on crude protein content, ash content and in vitro digestibility but no significant effect on crude fiber content of forage legume.

Key words: Foliar spray, Forage, In vitro digestibility, Legume, Molybdenum.

INTRODUCTION

The use of artificial nitrogen fertilizers has a negative impact on the sustainability of the environment, so in order to support the concept of sustainable agriculture, the effort to increase the supply of nitrogen nutrients through biological nitrogen fixation is one alternative (Simms and Taylor, 2002; Sutanto, 2002). Legumes, can absorb nitrogen from the air if there is symbiotic with rhizobium bacteria. One of the things that happens to rhizobium-legum symbiosis, legumes provide carbohydrates that rhizobium can use as a source of energy, rhizobium provides nitrogen that can be exploited by legumes to synthesize amino acids and proteins. The symbiosis causes the legumes to produce higher production and quality (Peoples and Craswell, 1992).

Molybdenum (Mo) is a important element to help in protein synthesis and fixation of atmospheric nitrogen in the root of legume by nodule bacteria (Singh *et al*, 2008). Molybdenum is a micro nutrient found in the soil and is necessary for the growth of almost all living organisms, both plants and animals. Molybdenum is used by certain plant enzymes to perform reduction and oxidation reactions. Molybdenum itself is biologically inactive but is found primarily as part of the cofactor molybdenum compound (Moco). Moco is bound to enzymes that require molybdenum (molybdoenzymes) found in plants, animals and prokaryotes (Kaiser *et al*., 2005). According to Togay *et al.,* (2008), molybdenum is a key ingredient in nitrogenase enzymes, and any bacterium that fixes nitrogen requires molybdenum during the fixation process. Molybdenum has a positive influence on the formation of nodules, nitrogenase activity, yields and legume crop quality. Molybdenum enhanced the accumulation of nutrients in plant tissues (Hristozkova *et al*., 2006) and it is important that the addition of Molybdenum could be reduced the Nitrogen environment. Foliar Mo application was more effective than soil application and seed treatment on chickpea (*Cicer arietinum*) growth and yield (Valenciano *et al*, 2011).

The Mo availability increases with increasing soil pH and acid soils with pH less than 5.2, the amount of available Mo to plants is extremely low, i.e., 0.10 to 0.25 mg kg^{-1} (Mengel and Kirkby, 2001). Since Mo is highly mobile in the xylem and phloem, and crops require low amounts, this micronutrient can be provided by seed treatments and/or foliar spray. Molybdenum foliar sprays are often more effective than soil applications, particularly for acid soils, and are most effective if applied at early stages of plant development (Valenciano *et al*., 2011). Effectiveness of foliar spray depends on the nutrient uptake rate by the leaves and its translocation into the plant (Boaretto *et al*., 2003 and Fabio and Zoz, 2015). The leaves rapidly absorb Mo applied by leaf spray. Leguminous showed a higher response to Mo compared to other crops (Velmurugan and Mahendran, 2015).

The availability of sustainable feed for both quality and quantity especially during the dry season is still a major problem in livestock productivity. Legumes forages as sources of ruminant feed have higher crude protein content compared to grass (Indriani *et al*., 2019).

*Corresponding author's e-mail: iin2susilawati@gmail.com

Forages of kudzu (*Pueraria phaseoloides*), calopo (*Calopogonium mucunoides*) and centro (*Centrosema pubescens*) as leguminous were evaluated as a potential ruminants feed. Their forages have high nutrient contents especially rich in crude protein content and was recommended for most ruminants. Therefore, to produce the forage that has high nutritional content, efforts are needed to increase the nutritional content of the calopo, centro and kudzu. Besides the nutritional content, the factor that must be considered in forage is digestibility of the forage.

MATERIALS AND METHODS

Field experiments were conducted at the field of Forage Crop Laboratory, Faculty of Animal Husbandry, Padjadjaran University, Sumedang, west Java, Indonesia. As planting was done in rainy season (October 2016 –January 2017). The field experiment was carried out in randomized block design with six treatments, consisting two levels of molybdenum (0 and 1.5 kg ha^{-1}) and three species of legumes (*Pueraria phaseoloides*, *Calopogonium mucunoides* and *Centrosema pubescens*) replicated four times. Total plots were 24 plots. Each experimental plot has a size of 3 x 4 m. Legume seeds were sowing at spacing 1 x 1 m. Three seeds were dibbled per hole and 10 days after emergence, thinned out to leave one healthy seedling per hole. Molybdenum foliar spraying was performed 20 days after legume plants emergence. Mo source was ammonium molybdate, $(NH_4)_6Mo_7O_{24}.2H_2O$.

The parameters were crude protein content (Kjeldhal Method), crude fiber content, ash content, IVDMD= In vitro dry matter digestibility, IVOMD= In vitro organic matter digestibilty, VFA= Volatile fatty acid content and NH_3 content. Kjeldahl method, ash content and crude fiber content using proximate analysis (AOAC, 2005). In vitro using Tilley and Terry method (1963).

The data obtained were analyzed by analysis of variance (F test). Differences between treatments were tested by Duncan Multiple Range Test.

Molybdenum was applied foliarly using a manual backpack sprayer at a dose of 1.5 kg ha^{-1} and a spray volume at 500 L ha^{-1} at 20 days after legumes plant emergence. The forage crop of legumes were harvested on 90 days after emergence. The sample was then weighed 500 grams fresh then cut into pieces approximately 3 cm long and dried to dry air for two days, then dried with oven at 70ºC until the samples attain a constant weight. After that, the sample was used for the purpose of measuring the chemical nutritional value and the in vitro digestibility of forage. The nitrogen content of forage legumes were analyzed by kjeldahl method and crude protein content was computed by multiplying nitrogen content with 6.25.

RESULTS AND DISCUSSION

The research data on legumes forage productivity through foliar spray molybdenum fertilization was presented in the following Table 1.

Crude protein content of forage legume: Data in Table 1 show that the crude protein content of legume forage with molybdenum fertilizer has a higher crude protein content than the crude protein content of legume forage without fertilization molybdenum (calopo: 18.52 vs. 14.93%), centro (27.7 vs. 24.86%) and Kudzu (22.56 vs 19.76%). This is because the fertilization of molybdenum affects nitrogen fixation by rhizobium bacteria at the legume root so that the nitrogen absorbed by the legume increases, whereas the increase in nitrogen uptake affects the crude protein content of forage legumes. This study was in line with Gad and Hala (2013) which states that the fertilization of molybdenum increases the nitrogen content in Cowpea plants. Molybdenum is part of a nitrogenase enzyme that catalyzes biological nitrogen fixation in rhizobium-legume symbiosis, so legume type crops require more molybdenum. Molybdenum deficiency in agricultural land limits nitrogen fixation biologically, therefore it is necessary to add molybdenum to agricultural land to increase biological nitrogen fixation (Jones, 1998). According to El-Bably (2002), the addition of 0.95 kg of ammonium molybdate ha^{-1} by foliar spray (spraying on leaves) can increase the production of fresh forage Clover (*Trifolium alexandrinum*), 16.48% and dry matter 18.63% on soils containing 0.2 mg kg^{-1} of molybdenum availability and soil pH 8.15. According to Togay *et al*., (2008) and Alloway (1995), the adding of molybdenum increases the activity of nitrogenase, because molybdenum is part of the nitrogenase molecule. Each molecule of nitrogenase contains 2 molybdenum atoms. Molybdenum is essential for the nitrogen fixation process, required for enzymatic reactions and aiding the reaction of nitrogen fixation. Any bacterium that fixes nitrogen requires molybdenum during the fixation process. Velmurugan and

Table 1: Ash content, crude protein content and crude fiber content of forage legumes as influenced by adding molybdenum fertilizer.

Parameter	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Ash (%)	10,29±0,58 ab	9,26±1,12 b	9,96±0,69 ab	10,57±0,31 a	7,78±0,57 c	10,20±0,77 ab
CP (%)	18,52±1,44 e	27,70±0,53 a	22,56±0,25 c	14,93±0,69 f	24,86±0,56 b	19,76±0,35 d
CF (%)	34,53±1,34 a	30,75±0,44 a	33,98±0,92 a	34,92±0,30 a	32,04±1,05 a	34,66±0,49 a

CP = Crude protein; CF = Crude Fiber; means ± std.; Letter of the same row with differet letter of its superscripts indicate significant different at probabilityof 0.05; T_1 = calopo + molibdenum; T_2 = centro + molibdenum; T_3 = Kudzu + molibdenum; T_4 = calopo without molibdenum; T_5 = centro without molibdenum; T_6 = kudzu without molibdenum.

Table 2: IVDMD, IVOMD, VFA and NH_3 of forage legumes as influenced by adding molybdenum fertilizer.

Parameter	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
IVDMD (%)	49,84±0,19 d	51,95±0,59 a	48,50±0,78 b	39,33±0,22 f	43,93±0,57 c	40,14±0,56 e
IVOMD (%)	40,75±14,60 a	49,76±0,23 a	46,26±1,00 ab	36,60±1,41 b	42,67±0,73 ab	38,64±0,55 b
VFA (mM)	115,63±2,87 e	179,50±1,96 a	154,13±4,46 b	97,48±2,15 f	144,25±4,77 c	125,38±3,15 d
NH_3 (mM)	3,71±0,14 cd	6,64±0,51 a	4,18±0,10 c	2,34±0,21 e	5,19±0,89 b	3,48±0,21 d

IVDMD= In vitro dry matter digestibility, IVOMD= In vitro organic matter digestibilty, NH_3= Ammonia, VFA= Volatile Fatty Acid; means ± std.; Letter of the same row with differet letter of its superscripts indicate significant different at probabilityof 0.05; T_1 = calopo + molibdenum; T_2 = centro + molibdenum; T_3 = Kudzu + molibdenum; T_4 = calopo without molibdenum; T_5 = centro without molibdenum; T_6 = kudzu without molibdenum.

Mahendran (2015) reported that Mo application enhanced the nutritional quality of green gram through effective nodulation, uptake and distribution of nutrients in the crop.

Crude fiber content: The results of variance showed that the effect of treatment on crude fiber content showed no significant difference. This was because forage legumes were cut at the same age that was 90 days, while the factors that greatly affect the content of crude fiber in plants was the age of cutting. The longer the age of cutting on the grass, the higher the production of dry matter and the increased crude fiber content (Reksohadiprodjo, 1994).

In Vitro Dry Matter Digestibility (IVDMD) and In Vitro Organic Matter Digestibilty (IVOMD): The result data in the Table 2 showed that IVDMD and IVOMD the forage legum material given the fertilization of molybdenum was higher than forage without the fertilization of molybdenum. This was due to an increase in the crude protein content of forage. Increased crude protein content in forage caused an increase that IVDMD and IVOMD. Besides that, ash content was also a part of dry matter, increased by Mo fertilization. Ash content reflects the mineral content. Gad and Hala (2013) reported that Molybdenum increased the content of N, P, K, Mn, Fe, Cu, Zn and Mo.

The Content of NH_3 and VFA (Volatile Fatty Acid): There is a close relationship between crude protein content, IVDMD and IVOMD with NH_3 and VFA contents. VFA is the final product of fermentation of carbohydrate by rumen microbes and is the main energy source for ruminants (Arora, 1995). Due to the increase in the crude protein content and digestibility, the NH_3 and VFA content increases as NH_3 and VFA are partly the result of protein digestion. According to Sutardi (1995) and Trisnadewi, *et al*. (2016), optimal VFA levels needed for the optimization of rumen microbial protein synthesis and degradation processes feed ranges between 8-160 mM. Levels of total VFA in this experiment results ranged from 115, 63 to 179, 50 mM. According to Nuswantara (2009) and Trisnadewi, *et al*. (2016), high concentrations of NH_3 and VFA in the rumen will cause higher microbial protein synthesis.

CONCLUSION

Fertilization of molybdenum in legume crops by foliar spray increased the crude protein content and mineral of legume forage but did not affect the content of crude fiber forage. Fertilization of molybdenum in the legumes increases the digestibility of dry matter, digestibility of organic matter, and increases the content of NH_3 and VFA.

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