The Nutrient Content of *Paspalum atratum* Grass Associated with *Macroptilium lathyroides* Legume Inoculated with Rhizobium through the Application of Molybdenum and Phosphorus Fertilizers

Anak Agung Ayu Sri Trisnadewi*, I Wayan Suarna, I Gede Mahardika, Ni Nyoman Suryani

Doctor Program, Faculty of Animal Science, Udayana University

*Corresponding author

Received: 15 Jul 2021; Received in revised form: 12 Aug 2021; Accepted: 18 Aug 2021; Available online: 23 Aug 2021

©2021 The Author(s). Published by Infogain Publication. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/).

Abstract—The experiment aimed to increase the nutrient content of *Paspalum atratum* grass associated with *Macroptilium lathyroides* legume inoculated with Rhizobium through the application of molybdenum (Mo) and phosphorus (P) fertilizer. The experiment used randomized block design (RBD) with factorial pattern. The first factor was Rhizobium inoculation consisted of Mo and P fertilizers without Rhizobium inoculation (R0) and with Rhizobium inoculation (R1). The second factor was planting pattern: grass monoculture (A1), legume monoculture (A2), one row of grass between one row of legumes (A3), and one row of grass between two rows of legumes (A4). There were eight treatments and each combination was repeated three times so that there were 24 experimental plots. The results showed that application of R1 increased the nutrient content of forage. A3 and A4 treatments were increased the nutrient content of forage compared with A1 dan A2. The conclusion of the research that the association of *Paspalum atratum* grass and *Macroptilium lathyroides* legume inoculated with Rhizobium and fertilized with Mo and P was able to increase nutrient content.

Keywords—grass-legume association, molybdenum, nutrient content, phosphorus, Rhizobium

1. INTRODUCTION

The availability of forage is still hampered due to the limited land for forage planting and the low of quantity and quality of forage. Efforts to improve the quality and quantity of forage feed can be carried out with various efforts, both extensively and intensively.

The association between grasses and suitable legumes will be able to meet the nitrogen deficiency in the grass so that can complement each other. Generally, grasses contain lower protein content than legumes. Legumes have the ability to fix nitrogen in the air due to the presence of nodules on the legume roots, then nitrogen will be returned to the soil and can be used by grass as a nutrient. Reksohadiprojo (1994) stated that mixed planting of grass and legumes is better than grass monoculture, besides protein, legumes also contain higher levels of phosphorus and calcium. According to Suarna et al. (2014) grass that is planted with legumes or the association of grass with legumes will provide good interactions with the physical, chemical and biological environment between the two plant species.

The availability of sufficient nutrients in the soil is important for growth and productivity of plant. Several nutrients play an important role in the symbiotic process of Rhizobium such as molybdenum (Mo) as part of the nitrogenase enzyme and every nitrogen-fixing bacteria requires Mo during the fixation process. *Rhizobium spp.* is a type of microorganism that lives in symbiosis with...
leguminous plants and functions to fix nitrogen biologically. Biological nitrogen fixation is a major source of nitronen (N) input in agricultural soils and rhizobia chemically converts nitrogen from the air to make it available to plants. The N fixation process is influenced by many factors including phosphorus (P) (Badar et al., 2015). Without proper P fertilization, rhizobia activity and nitrogen fixation are suppressed because P promotes early root formation, lateral root formation, and strong roots. It is very important for nodule formation and for binding to atmospheric nitrogen (Rahman et al., 2008). Togay et al. (2008) found that the application of phosphorus and molybdenuin caused an increase in all characters of the lentil legume (Lens culinaris Medic.). Soils that have low phosphorus content and very alkaline, fertilization with phosphorus 60 kg/ha and molybdenum 6 g/kg seeds resulted the highest in plant height, number of branches, pods, seeds, number of nodules, root and shoot dry weight and protein.

The research is about the use of Mo and P fertilizers in the association of *Paspalum atratum* with *Macroptilium lathyroides* inoculated with *Rhizobium* to increase forage productivity through the contribution of legumes.

II. MATERIAL AND METHODS

The study used fertilizer dose of 1.0 kg Mo ha⁻¹ and 20 kg P ha⁻¹, and *Rhizobium* inoculation. The experiment used randomized block design (RBD) with factorial pattern. The first factor was *Rhizobium* inoculation consisted of Mo and P fertilizers without *Rhizobium* inoculation (R₀) and with *Rhizobium* inoculation (R₁). The second factor was planting pattern: grass monoculture (A₁), legume monoculture (A₂), one row of grass between one row of legumes (A₃), and one row of grass between two rows of legumes (A₄). There were eight treatments and each combination was repeated three times so that there were 24 experimental plots. There were 8 treatment units: R₀A₁, R₀A₂, R₀A₃, R₀A₄, R₁A₁, R₁A₂, R₁A₃, and R₁A₄. Each treatment unit consisted of three replications, so there were 24 plots or experimental units.

The variables observed were dry matter, ash, organic matter, crude protein, crude fiber, crude fat, total digestible nutrient, nitrogen free extract, and gross energy content. Analysis of dry matter, ash, organic matter, crude protein, crude fiber, total digestible nutrients, nitrogen free extracts, and gross energy content using the method of Association of Official Analytical Chemists (1990).

The materials used in the study were poles of *Paspalum atratum* grass, seeds of legume *Macroptilium lathyroides*, triple super phosphate (TSP) fertilizer, ammonium molybdate fertilizer, and *Rhizobium* inoculants.

The data obtained were analyzed by means of variance and if the treatment showed a significant difference (P<0.05), and was continued with Duncan's multiple-distance test (Steel and Torrie, 1991).

II. MATERIAL AND METHODS

The study used fertilizer dose of 1.0 kg Mo ha⁻¹ and 20 kg P ha⁻¹, and *Rhizobium* inoculation. The experiment used randomized block design (RBD) with factorial pattern. The first factor was *Rhizobium* inoculation consisted of Mo and P fertilizers without *Rhizobium* inoculation (R₀) and with *Rhizobium* inoculation (R₁). The second factor was planting pattern: grass monoculture (A₁), legume monoculture (A₂), one row of grass between one row of legumes (A₃), and one row of grass between two rows of legumes (A₄). There were eight treatments and each combination was repeated three times so that there were 24 experimental plots. There were 8 treatment units: R₀A₁, R₀A₂, R₀A₃, R₀A₄, R₁A₁, R₁A₂, R₁A₃, and R₁A₄. Each treatment unit consisted of three replications, so there were 24 plots or experimental units.

The variables observed were dry matter, ash, organic matter, crude protein, crude fiber, crude fat, total digestible nutrient, nitrogen free extract, and gross energy content. Analysis of dry matter, ash, organic matter, crude protein, crude fiber, total digestible nutrients, nitrogen free extracts, and gross energy content using the method of Association of Official Analytical Chemists (1990).

The materials used in the study were poles of *Paspalum atratum* grass, seeds of legume *Macroptilium lathyroides*, triple super phosphate (TSP) fertilizer, ammonium molybdate fertilizer, and *Rhizobium* inoculants.

The data obtained were analyzed by means of variance and if the treatment showed a significant difference (P<0.05), and was continued with Duncan's multiple-distance test (Steel and Torrie, 1991).

III. RESULTS AND DISCUSSIONS

The combination of treatment with Mo and P fertilizers with *Rhizobium* (R₁) and without *Rhizobium* (R₀) inoculation had no significant effect (P>0.05) on the content of dry matter, ash, organic matter, crude protein, crude fiber, total digestible nutrients, and the gross energy of *Paspalum atratum* grass and *Macroptilium lathyroides* legume and their associations (Table 1). Leguminous plants have the ability to bind N₂ in the air when in symbiosis with *Rhizobium* bacteria. The role of Mo and P combined with *Rhizobium* in the nitrogen fixation process has not been optimal so that the nutrients needed by plants cannot be produced optimally so that there is no difference between the nutrient content of forages in treatment R₀ and R₁. Susilawati et al. (2014) found that giving molybdenuin had no significant effect on forage yields and the content of crude fiber components of *Panicum maximum* grass forage.

The content of nitrogen free extract (NFE) in treatment R₀ was significantly higher (P<0.05) compared to R₁ (Table 1). This is because the crude protein content in treatment R₀ which tends to be lower causes the NFE levels to increase and even shows a significant difference with the treatment R₁. NFE is a digestible carbohydrate. In accordance with Koten (2018) that the decreasing nitrogen content in plant tissue, it will further increase NFE levels of plant.

The dry matter content in treatments A₁ and A₂ was significantly (P<0.05) higher compared to treatments A₃ and A₄ (Table 1). The high dry matter content in treatments A₁ and A₂ indicated a lower water content, whereas the water content in treatments A₃ and A₄ was higher. There was an interaction between *Rhizobium* inoculants and planting patterns, indicating that the two treatments influenced each other.
Table 1. The Nutrient Content of *Paspalum atratum* Grass Associated with *Macroptilium lathyroides* Legume Inoculated with *Rhizobium* through the Application of Molybdenum and Phosphorus Fertilizer

| Variables                      | Rhizobium<sup>1)</sup> Inoculation | Planting Pattern<sup>2)</sup> | Average          |
|--------------------------------|------------------------------------|------------------------------|------------------|
|                                |                                    | A<sub>1</sub> | A<sub>2</sub> | A<sub>3</sub> | A<sub>4</sub> |                 |
| Dry matter                     |                                    |                      |                 |                 |                 |                 |
|                                | R<sub>0</sub>                      | 20.12 a A           | 17.32 b A      | 20.55 a A      | 18.11 b A      | 19.02 A         |
|                                | R<sub>1</sub>                      | 19.54 a A           | 18.74 a A      | 18.12 ab B     | 16.75 b A      | 18.28 A         |
|                                | Average                            | 19.83 a             | 18.03 b        | 19.33 a        | 17.43 b        |                 |
| Ash                            | R<sub>0</sub>                      | 12.70               | 8.61           | 10.01          | 10.27          | 10.40 A         |
|                                | R<sub>1</sub>                      | 12.95               | 8.51           | 10.61          | 10.88          | 10.74 A         |
|                                | Average                            | 12.82 a             | 8.56 c         | 10.31 bc       | 10.58 b        |                 |
| Organic matter                 | R<sub>0</sub>                      | 87.30               | 91.39          | 89.73          | 89.12          | 89.61 A         |
|                                | R<sub>1</sub>                      | 87.05               | 91.49          | 89.39          | 89.12          | 89.26 A         |
|                                | Average                            | 87.18 c             | 91.44 a        | 89.69 b        | 89.42 bc       |                 |
| Crude protein                  | R<sub>0</sub>                      | 7.73                | 22.79          | 15.81          | 18.67          | 16.25 A         |
|                                | R<sub>1</sub>                      | 8.67                | 22.52          | 16.54          | 18.40          | 16.53 A         |
|                                | Average                            | 8.20 d              | 22.65 a        | 16.18 c        | 18.53 b        | 23.14 A         |
| Crude fiber                    | R<sub>0</sub>                      | 27.28               | 20.38          | 23.31          | 21.59          | 24.72 A         |
|                                | R<sub>1</sub>                      | 26.91               | 22.39          | 24.08          | 25.47          |                 |
|                                | Average                            | 27.10 a             | 21.39 b        | 23.70 b        | 23.52 b        |                 |
| Ether extract                  | R<sub>0</sub>                      | 14.07               | 15.74          | 15.50          | 16.02          | 15.33 A         |
|                                | R<sub>1</sub>                      | 14.73               | 17.61          | 16.18          | 17.96          | 16.62 A         |
|                                | Average                            | 14.40 a             | 16.68 a        | 15.84 a        | 16.99 a        |                 |
| Total digestible nutrient (TDN)| R<sub>0</sub>                      | 32.53               | 49.59          | 42.88          | 47.27          | 43.07 A         |
|                                | R<sub>1</sub>                      | 34.09               | 47.26          | 41.93          | 40.87          | 41.04 A         |
|                                | Average                            | 33.31 b             | 48.42 a        | 42.40 a        | 44.07 a        |                 |
| Nitrogen free extract (NFE)    | R<sub>0</sub>                      | 28.58               | 20.40          | 23.42          | 21.58          | 23.49 A         |
|                                | R<sub>1</sub>                      | 26.52               | 16.78          | 21.79          | 15.53          | 20.16 B         |
|                                | Average                            | 27.55 a             | 18.59 c        | 22.61 b        | 18.56 c        |                 |
| Gross energy                   | R<sub>0</sub>                      | 3.51                | 3.97           | 3.37           | 4.00           | 3.71 A          |
|                                | R<sub>1</sub>                      | 2.97                | 4.01           | 3.61           | 3.81           | 3.60 A          |
|                                | Average                            | 3.24 b              | 3.99 a         | 3.49 b         | 3.90 a         |                 |

**Noted:**

1) R<sub>0</sub> = combination of Mo and P fertilizers without *Rhizobium* inoculation, R<sub>1</sub> = combination of Mo and P fertilizers with *Rhizobium* inoculation

2) A<sub>1</sub> = grass monoculture, A<sub>2</sub> = legume monoculture, A<sub>3</sub> = one row of grass between one row of legumes, A<sub>4</sub> = one row of grass between two rows of legumes

3) The average value of the treatment followed by the same lowercase letter in one row and the same capital letter in one column was not significantly different (P>0.05)
The highest ash content in treatment A1 and significantly different (P<0.05) was higher than treatment A2, A3, and A4, while the lowest ash content was in treatment A2 (Table 1). There is a tendency that the presence of grass in the association pattern results in higher ash content and can be seen from the cropping pattern in the A1 and A2 treatments. The ash content indicates the mineral content of the plant. On the other hand, the organic matter content of the A2 treatment showed the highest and the lowest values in the A1 treatment. Organic matter consists of protein, ether extract, and carbohydrates.

Crude protein content between all treatments showed significant differences (P<0.05) and the highest was in treatment A2 which was legume monoculture while the lowest was in treatment A1 (grass monoculture) (Table 1). The association of grasses and legumes in the A1 treatment showed a higher value than the A2 treatment. This shows that as a source of protein, legumes contain higher protein than grass. The increasing population of legumes in pattern planting with association between grass and legume, the crude protein content in treatment A4 is higher than A3. Skerman et al. (1988) stated that *Macroptilium lathyroides* has high nutritional value with crude protein content between 7.6 - 19.2%. The N content varies from 1% (after losing most of the leaves) to 4% in the vegetative period with digestibility between 40 - 70%.

Ether extract content in treatments A1, A2, A3, and A4 showed no significant difference (P>0.05) (Table 1). Ether extract content is influenced by plant age and plant growth phase. The experiment used *Paspalum atratum* and *Macroptilium lathyroides* with almost the same age and growth phase so that it had no effect on ether extract content. Farda et al. (2020) stated that fats contained in plants usually forming in unsaturated fats both linoleic acid and linolenic acid.

Crude fiber content in treatments A2, A3, and A4 showed no significant difference (P>0.05) and the three treatments showed significant differences (P<0.05) with treatment A1 (Table 1). Crude fiber content was closely related to the age of cutting and all treatments, both grass and legumes were cut at the same age. The lower crude fiber content in treatments A2, A3, and A4 was influenced by the presence of legumes in these treatments because legumes are forages with high protein content and lower fiber content than grass. Crude fiber content is affected by the age of the plant and also the part of the plant.

The content of total digestible nutrients (TDN) in treatments A2, A3, and A4 showed no significant difference (P>0.05) and the three treatments showed significant differences (P<0.05) with treatment A1 (Table 1). TDN content is closely related to crude fiber content because crude fiber is classified as a food substance that is difficult to digest so that the low crude fiber content in treatments A2, A3, and A4 causes the TDN content was increased while decreased in treatment A1.

The content of the nitrogen-free extract (NFE) in treatment A1 was the highest and significantly different from treatments A2, A3, and A4 and between treatments A2 and A4 was not significantly different (P>0.05) (Table 1). NFE is an digestible carbohydrate because it does not contain cell walls or crude fiber. NFE is related to the content of water, ash, crude protein, crude fiber, crude fat and crude fiber. The value of NFE will be higher if the content of water, ash, crude protein, crude fiber, and crude fat is lower, vice versa. The highest gross energy content was in treatment A2 and not significantly different with treatment A1 and the two treatments were significantly different (P<0.05) higher than treatments A1 and A3. This is because grass as source of energy while legumes as source of protein.

### IV. CONCLUSION

The application of Mo and P fertilizers to the association of *Paspalum atratum* grass and *Macroptilium lathyroides* legume inoculated with *Rhizobium* could increase the nutrient content of forage.

### REFERENCES

1. Association of Official Analytical Chemist (AOAC). (1990). Official Method of Analysis. 15th Ed. Association of Official Analytical Chemist, Inc. Virginia
2. Badar R., Z. Nisa, and S. Ibrahim. (2015). Supplementation of P with rhizobial inoculants to improve growth of Peanut plants. International Journal of Applied Research 2015; 1(4): 19-23. Diunduh 1 Mei 2017.
3. Farda, F. T., A. K. Wijaya, Liman, Muhtarudin, D. Putri, dan M. Hasanah. (2020). Pengaruh varietas dan jarak tanam yang berbeda terhadap kandungan nutrien hijauan jagung. Jurnal Ilmiah Peternakan Terpadu Vol. 8(2): 83 – 90. Diunduh 2 April 2021.
4. Reksohadiprodjo, S. (1994). Produksi Tanaman Hijauan Makanan Ternak Tropik. Edisi Ketiga. BPFE. Gajah Mada, Yogyakarta.
5. Koten, B. B., R. Naisoko, R. Wea, dan A. Semang. (2018). Produksi bahan organik, protein kasar dan bahan ekstrak tanpa nitrogen hijauan pastura alam yang diintroduksi jenis rumput dan legum yang berbeda. Partner, Tahun 23 Nomor 2, 773 – 781.
6. Skerman, P. J., Cameron, D. G. and Riveros, F. (1988). Tropical Forage Legumes. Food and Agriculture Organisation of the United Nations: Rome, Italy.
7. Steel, R. G. D. and J. H. Torrie. (1991). Principles and Procedures of Statistic. New York: McGraw-Hill Book Co. Inc.
[8] Suarna, W., N. N. Candraasih K., dan M. A. P. Duarsa. (2014). Model asosiasi tanaman pakan adaptif untuk perbaikan lahan pasca tambang di Kabupaten Karangasem. *Jurnal Bumi Lestari*, Volume 14 No. 1, Pebruari 2014. 9 – 14. Available at: https://ojs.unud.ac.id/index.php/blje/article/view/11211/797

[9] Susilawati, I., H. K. Mustafa, dan L. Khairani. (2014). Hasil dan kandungan komponen serat kasar hijauan rumput benggala dengan pemberian molibdenum dan jenis legum pada perataan campuran rumput dan legum. *Pastura* Vol. 2, No. 2. Juni. 74 – 78. ISSN 2549-8444. Available at: https://ojs.unud.ac.id/index.php/pastura/article/view/9024. Accessed: 17 Juli 2020

[10] Rahman, M. M., M. M. H. Bhuiyan, G. N. C. Sutradhar, M. M. Rahman, and A. K. Paul. (2008). Effect of Phosphorus, Molybdenum and *Rhizobium* inoculation on yield and yield attributes of mungbean. *Int. J. Sustain. Crop Prod*. 3(6):26-33. Accessed 1 Mei 2017.

[11] Togay, Y., N. Togay, and Y. Dogan. (2008). Research on the effect of phosphorus and molybdenum applications on the yield and yield parameters in lentil (*Lens culinaris* Medic.). *African Journal of Biotechnology*. Vol. 7 (9), pp. 1256-1260, 2 May, 2008. Available online at http://www.academicjournals.org/AJB. ISSN 1684–5315 © 2008 Academic Journals.