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Submission date: 15-Apr-2023 11:36AM (UTC+0700)

Submission ID: 2065073248

File name: JURNAL_JPN_2019.pdf (486.4K)

Word count: 2776

Character count: 14833



Research Article

Utilization of Biourine and Arbuscular Mycorrhizal Fungi as Biotechnological Agents for Improving Land Productivity of Ex-Coal Mines and Testing Their Effects on the Content of Forage Fiber Fractions [*Hymenachne amplexicaulis* (Rudge) Nees]

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Abstract

Background and Objective: Liquid biourine organic fertilizer can affect the physical, chemical and biological fertility of soil. Mycorrhizal biofertilizers can help plants to supply and absorb nutrients, especially phosphorus, which is low in availability in acidic/critical soils. The aim of this study was to characterize, test and prove that the administration of biourine liquid organic fertilizer and Arbuscular mycorrhizal Fungi biofertilizer (AMF) as a biotechnology agent, was able to improve the productivity of ex-coal mine lands and also affect the content of forage fiber feed fractions. **Materials and Methods:** An experiment using a completely randomized design with 4 types of treatment and 5 repetitions was conducted. The treatments consisted of: (1) Biourine concentration of 0%+AMF 20 g pot⁻¹, (2) Biourine concentration of 15%+AMF 20 g pot⁻¹, (3) Biourine concentration of 30%+AMF 20 g pot⁻¹ and (4) Biourine concentration of 45%+AMF 20 g pot⁻¹. Variables observed were Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), hemicellulose, cellulose and lignin. **Results:** The results of various analyses showed that the treatment had a highly significant effect ($p < 0.01$) on all variables observed. The treatment with 45% biourine+AMF 20 g pot⁻¹ resulted in lower ADF, NDF and lignin contents and produced higher cellulose and hemicellulose content than the treatments with 30% biourine+AMF 20 g pot⁻¹, 15% biourine+AMF 20 g pot⁻¹ and 0% biourine+AMF 20 g pot⁻¹. **Conclusion:** Biourine liquid organic fertilizer and Arbuscular mycorrhizal Fungi biofertilizer (AMF) are biotechnology agents that improve the productivity of ex-coal mine land and also affect the content of forage fiber feed fractions.

Key words: ADF, arbuscular mycorrhizal fungi, biourine, fiber fraction, hymenachne amplexicaulis (Rudge) nees, lignin, NDF

Received:

Accepted:

Published:

Citation: Hardi Syafria, Novirman Jamarun and Roni Pazla, 2019. Utilization of biourine and arbuscular mycorrhizal fungi as biotechnological agents for improving land productivity of ex-coal mines and testing their effects on the content of forage fiber fractions (*Hymenachne amplexicaulis* (Rudge) nees). Pak. J. Nutr., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

To expand the diversity of forage fodder, local forages need to be developed. This is because some types of local forage show advantages over introduced forage fodder, one of which is the forage fiber fractions coexist. The grass *Hymenachne amplexicaulis* (Rudge) Ness is a natural resource that has high biological value, has the potential to serve as a forage source for ruminants and is based on local resources¹⁻³.

There are constraints to increase production and forage quality related to land resources in the tropics, including nutrient deficiencies, acidity, toxicity and soil water content. The land for forage planting is also decreasing since fertile land, in general, is used for food crops, plantations and various non-agricultural purposes⁴. One example is the increasing extent of ex-coal mining land located in Jambi Province in response to higher mining activities. This activity increases regional income and foreign exchange but also has a damaging impact on the environment. Hundreds and even thousands of hectares of land have become damaged and are now unproductive, resulting from damage to the physical structure and degradation of soil nutrients; this makes it difficult for plants to grow. One solution to solve the problem is to use the land again, with a small application of biourine liquid organic fertilizer and mycorrhizal biofertilizer technology. The use of biourine and mycorrhiza as biotechnology agents to increase the productivity of ex-mining land is one alternative that needs to be evaluated. This is in opposition to the use of chemical fertilizers, which, despite increasing production and quality but their continuous use will damage environmental sustainability.

Forage for tropical regions has a high fiber fraction, which is one of the factors decreasing the ability of livestock to consume forage; it affects digestibility as well as feed particle flow rates for forage. NDF represents the content of cell walls consisting of lignin, cellulose and hemicellulose. Cellulose is a major component of plant cell walls and is the main product of photosynthesis and it causes the ossification or strength of a plant. Hemicellulose is a chemical compound which is in a group with cellulose but has a lower molecular weight⁵.

This study aimed to find, obtain and prove the use of arbuscular mycorrhizal fungi and biourine in increasing the productivity of ex-coal mining land and their effects on the content of forage fiber fractions. This can be a solution in improving the productivity of the land, so that it can be used as land development/cultivation of fodder forage.

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MATERIALS AND METHODS

Time and place: The study was conducted in the Kotabaru Subdistrict in Jambi City for 5 months. An analysis of forage fiber fractions (ADF, NDF, Hemicellulose, Cellulose and Lignin) was conducted in the Ruminant Nutrition laboratory, Faculty of Animal Husbandry and Alas University.

Materials and equipment: As a planting medium, 5 kg of ex-mining coal was placed in each pot. Planted forage was grass, with planting material in the form of cuttings; each consisted of 3 cuttings and arbuscular mycorrhizal fungi (multiple spores were used with the trademark Cemiko I consisting of *Glomus* sp, *Acaulospora* sp. and *Scutellospora* sp). The organic fertilizer used was biourine and the basic fertilizer used was TSP fertilizer (45% P₂O₅), KCl (60% K₂O), Urea (46% N) and agricultural lime (CaCO₃). The equipment used was soil processing equipment, lawn mowers, ruler, sprinklers, plastic bags, scales and laboratory equipment for dry matter analysis and nutritional value.

Research methods: This experiment used a Completely Randomized Design (CRD), with four types of treatment and five replications. The four types of treatment were: (1) Biourine concentration of 0% + AMF 20 g pot⁻¹, (2) Biourine concentration of 15% + AMF 20 g pot⁻¹, (3) Biourine concentration of 30% + AMF 20 g pot⁻¹ and (4) Biourine concentration of 45% + AMF 20 g pot⁻¹.

Variables observed: The variables observed were the content of forage fiber fraction, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Cellulose, Hemicellulose and Lignin.

Research Implementation: Before the grass was planted, soil was taken for composite planting media from a depth of 0-20 cm. The soil was dried, aerated and cleaned of plant roots and other materials that were not needed. The administration of arbuscular mycorrhizal fungi as a treatment was based on the results of Syafria's³ study of 20 g pot⁻¹. Whereas for biourine treatment, the fermented biourine was used for a 6 h treatment. As a basic fertilizer, TSP fertilizer (45% P₂O₅) with a dose of 150 kg P₂O₅ ha⁻¹ ~0.85 g TSP pot⁻¹, KCl fertilizer (60% K₂O) with a dose of 100 kg K₂O ha⁻¹ ~0.43 g KCl pot⁻¹, Urea fertilizer (46% N) with a dose of 200 kg N ha⁻¹ ~1.14 g Urea pot⁻¹ and lime CaCO₃ with a dose of 2 t ha⁻¹ ~5.11 g pot⁻¹ were used. Two weeks before planting, a polybag was prepared and filled with soil from an ex-mining coal site to 5 kg pot⁻¹. The TSP, KCl, Urea and CaCO₃ fertilizers was added at the same time by mixing with the soil in the pot

and then stirring until homogeneous. Land that had been given basic fertilizer was left for one week until planting. The administration of mycorrhizae was done when the grass was planted by inserting the inoculum into each planting hole. Administration of biourine was conducted after the grass began to grow (approximately 2 weeks after planting). This was done to determine the content of forage fiber fractions using the Van Soest method, Georing and Van Soest⁶ and Van Soest and Robertson⁷.

Data processing: Data processing was performed statistically using a completely randomized design. The data were analyzed using one-way analysis of variance (ANOVA). The results of the analysis of variance showed a significant effect and was followed by Duncan Multiple-Range Test (DMRT) with 5% level of significance.

RESULTS AND DISCUSSION

The results of the analysis of variance showed that the treatment had a significant effect ($p < 0.01$) on the content of the fiber fraction (ADF, NDF, Hemicellulose, Cellulose and Lignin) in the forage. The mean value of the content of forage fiber fractions in various treatments is shown in Table 1.

NDF and ADF: The lowest NDF and ADF content was obtained in the 45% biourine+FMA 20 g pot⁻¹ treatment and it was significantly different from the 30% biourine +FMA 20 g pot⁻¹, 15% biourine+FMA 20 g pot⁻¹ and 0% treatment of biourine+FMA 20 g pot⁻¹. The higher concentration of biourine in this treatment provided different NDF and ADF results. The difference in NDF and ADF content in each of these treatments was caused by the response of plant growth in each treatment, especially in nutrient absorption, even though the dose of FMA was the same. Decreased content of NDF is also closely related to forage lignin content. Haris⁸ explained that the decrease in the content of NDF was caused by an increase in lignin in plants. According to Sutardi⁹, lignin is a component of crude fiber which is found partly in the cell wall from plant stems and is not easily digested. The decrease in NDF and ADF content can also occur during the

fermentation process because of the presence of microbes that are able to digest the components of the cell wall. According to Van Soest¹⁰ there is a relationship between NDF, ADF and lignin regarding the benefits of forage. If the green lignin content is high, the digestibility coefficient of the food is low. On the other hand, the nitrogen content in biourine can also increase the portion of protoplasm compared to cell walls, increase the water content of protoplasm and reduce calcium. Therefore, the increase in cell size and the addition of cell wall thickness causes the leaves and stems of the plant to become more difficult to digest¹¹.

Cellulose and hemicellulose: The difference in cellulose and hemicellulose content in each treatment is also due to differences in the ability of plants to absorb nutrients and water for plant cell formation. Cellulose and hemicellulose are the main components of the plant cell wall. The ability of plants to absorb nutrients and water increases with increasing doses of biourine liquid organic fertilizer. This is because biourine is a source of nutrition and energy for mycorrhizal growth and development. However, the decrease and increase in the content of cellulose and hemicellulose can also occur during the fermentation process, due to the presence of fiber-digesting enzymes. Hemicellulose has a lower degree of polymerization, is easier to digest than cellulose and does not form long fibers. According to Kusnandar⁵ hemicellulose can be hydrolyzed with the enzyme hemicellulase (xylanase). Hemicellulose binds to sheets of fiber; cellulose forms microfibrils which increase the stability of the cell wall. Hemicellulose is also crosslinked with lignin to form complex tissues and provide a strong structure. Tillman *et al.*¹² stated that hemicellulose was found together with cellulose in the bark and wood structure of all plant parts and plant seeds. Cellulose content in the plant cell wall is approximately 35-50% and hemicellulose is approximately 17.64-28.04%¹³.

Lignin: The lowest lignin content was observed in the treatment of 45% biourine+FMA 20 g pot⁻¹ and was not significantly different from the treatment of 30% biourine+FMA 20 g pot⁻¹ but it was significantly different from the treatment of 15% biourine+FMA 20 g pot⁻¹ and 0%

Table 1: Average values of ADF, NDF, cellulose, hemicellulose and lignin (%) in various treatments

Treatments	NDF	ADF	Cellulose	Hemicellulose	Lignin
0% biourine+FMA 20 g pot ⁻¹	59.20 ^a	34.90 ^a	35.30 ^a	24.30 ^a	6.79 ^a
15% biourine+FMA 20 g pot ⁻¹	56.55 ^b	31.35 ^b	37.50 ^b	24.20 ^a	5.61 ^b
30% biourine+FMA 20 g pot ⁻¹	53.20 ^c	29.80 ^c	38.70 ^c	23.40 ^b	5.32 ^c
45% biourine+FMA 20 g pot ⁻¹	52.80 ^d	27.25 ^d	39.10 ^c	25.55 ^c	5.20 ^c

Means are listed in the same column with different letters (a, b, c, d) indicating significance ($p < 0.05$)

biourine+FMA 20 g pot⁻¹. The higher the concentration of biourine, the more lignin content appeared to decrease. The difference in lignin content is due to differences in cell structure and walls. Plants that are treated with biourine+FMA are generally more fertile and have more leaves; stems were softer/more digestible than plants with mycorrhizae only. Another factor that causes this difference is the difference of protein compounds, because it can cause an increase in the C:N ratio and this excess carbohydrate causes the cell membrane to thicken. This will lead to increased lignin in the tissue¹¹. According to Sutardi⁹, lignin is a part or component of fiber that is found partly in the cell wall of plants.

CONCLUSION

Concentration of 45% biourine+FMA 20 g pot⁻¹ resulted in lower NDF, ADF and lignin content and higher cellulose and hemicellulose content than the treatments with 30% biourine+FMA 20 g pot⁻¹, 15% biourine+FMA 20 g pot⁻¹ and 0% biourine+FMA 20 g pot⁻¹.

SIGNIFICANCE STATEMENTS

Biourine liquid organic fertilizer and Arbuscular mycorrhizal Fungi biofertilizer (AMF) are biotechnology agents that improve the productivity of ex-coal mine land and also affect the content of forage fiber feed fractions. This will allow researchers to realize that the combination of biourine and FMA is important for supporting cellulose and hemicellulose. Thus, this research could lead to a new theory on the importance of biourine and AMF.

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ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Directorate of Research and Community Service of the Directorate General of Strengthening Research and Development of the Ministry of Research, Technology and Higher Education for the approval and assistance of research funds in the "Post-Doctoral Research" scheme for fiscal/budget in 2018.

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