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Capacity of *Gliricidia sepium* and *Indigofera zollingeriana* plants which were given *Rhizobium* inoculants as a source of feed for Bali caws on post-mining lands

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Abstract
The aim of this research was to analyze the effect of differences in *rhizobium* inoculant levels on the quality and digestibility of forage *G. sepium* and *I. zollingeriana* planted on post-rock mining land. The research was conducted on post-rock mining land in Sebudi Village, Selat District, Karangasem Regency. The experimental design used was a nested randomized block design (RBD) with two factors, the first factor was plant type, namely *G. sepium* and *I. zollingeriana*, the second factor was the dose of *rhizobium* inoculant (without inoculant, 1 g/plant, 2 g/plant and 4 g/ The data obtained were analyzed by means of variance and if there were significant differences (p<0.05) between treatments, then continued with Duncan's multiple distance test. The results of the study were that administration of various doses of rhizobium inoculant had no significant effect (p>0.05) on forage quality except for the crude protein variable, R3 treatment had a significant effect (p<0.05) on the crude protein of *G. sepium* and *I. zollingeriana* plants. Dosage and type of plant had a significant effect (p>0.05) on the level of digestibility vitro. It was concluded that rhizobium inoculant was able to improve the quality and in vitro digestibility of *G. sepium* and *I. zollingeriana* forages grown on post-mining land.

Keywords: Post-mining land, *Gliricidia sepium*, *Indigofera zollingeriana*, organic fertilizer, *rhizobium*

Introduction
The increase in population, increasing welfare and education of the Indonesian people, has resulted in demand for livestock products increasing. This is because people are increasingly aware and concerned about meeting their protein needs. To fulfill protein needs, beef is one of the largest supply source products. According to data from the Director General of Nak and Keswan, (2021) national meat needs will be met from beef and buffalo in 2021 amounting to 696,965 tonnes, but 36% of this will come from foreign imports. Increasing ruminant livestock production is influenced by three factors, namely land, feed and livestock. Feed plays a role of up to 80% of livestock production costs. The availability of forage is still hampered due to limited land for planting forage and the low quantity and quality of forage.

As land conversion increases due to the increase in population, the availability of land that can be used for extensive development of forage for livestock is decreasing, because it has been used for the development of food agriculture and other infrastructure. The use of ex-mining land as an expansion area for forage plantations has been growing since the last few years, however the use of ex-mining land as a livestock area has not been utilized optimally because ex-mining land is sub-optimal land so it requires good and appropriate handling to increase post-mining land fertility mine.

Fertilization and plant revegetation are ways to improve the physical and chemical properties of ex-mining soil. *G. sepium* and *I. zollingeriana* are tree legume plants that are able to adapt to all types of soil, are drought tolerant and always produce forage in the dry season if defoliated regularly. *G. sepium* and *I. zollingeriana* have several advantages, namely high forage production, climate resistance, easy to plant and have quite high food content. Increasing the production of *G. sepium* and *I. zollingeriana* can be done by applying organic fertilizer and adding rhizobium inoculum.

Rhizobium sp bacteria, this group of bacteria, are capable of infecting plant roots and forming root nodules. Providing solid organic fertilizer will increase rhizobium activity so that it can infect the roots of legume plants and can increase the nitrogen needed by *G. sepium* and *I. zollingeriana plants.*
Based on this background, the findings from the research carried out were the effect of giving organic fertilizer and *rhizobium* inoculant on the quality and in vitro digestibility of *G. sepium* and *I. zollingeriana* forages.

Materials and Methods

Ethical Approval

The research was carried out on post-mining land in Sebudi Village, Selat District, Karangasem Regency for 4 months and at the Animal Nutrition and Forage Laboratory, Faculty of Animal Husbandry, Udayana University, Denpasar.

Experimental Design

There are 8 treatment units, namely: GR_0, GR_1, GR_2, GR_3, IR_0, IR_1, IR_2, and IR_3. Each treatment unit consisted of three replications, so there were 3 groups and each group consisted of 8 treatments and the size of each plot was 2 m × 2 m. The spacing between rows is 50 cm. The distance between plots is 50 cm. *Rhizobium* inoculant is given at the time of planting.
The research used a nested randomized block design (RAK) with two factors. The first factor is the type of plant which consists of: G= *G. Sepium* and I = *I. zollingeriana*. The second factor of *rhizobium* inoculation dose consists of: R_0 = No *rhizobium* inoculation, R_1 = 1 g *rhizobium* inoculation/plant, R_2 = 2 g *rhizobium* inoculation/plant, R_3 = 4 g *rhizobium* inoculation/plant.
Variables observed in *G. sepium* and *I. zollingeriana* plants included forage quality variables (dry matter, ash, organic matter, crude protein, crude fiber, crude fat, total gestible nutrient (TDN), extract material without nitrogen (BETN), energy gross (GE)) and in vitro digestibility (dry matter and organic matter). The data obtained is analyzed if there is a real influence, it will be followed by Duncan's test.

Results and Discussion

The administration of *rhizobium* inoculant did not have a significant effect ($p>0.05$) on the content of dry matter, ash, organic matter, crude fiber, crude fat, total digestible nutrients, extract materials without nitrogen (BETN), and gross energy. The crude protein of the R_3 treatment was 12.64% ($p<0.05$) significantly higher than that of the R_0 treatment. There was a tendency for the content of dry matter, ash, organic matter, crude fat, total digestible nutrient, extract material without nitrogen (BETN), and gross energy to be higher compared to the R_0, R_1 and R_2 treatments, while the crude fiber content of the R_0 treatment was higher than the R_0 treatment other.

Table 1: Effect of different *Rhizobium* inoculant doses on forage quality of *G. sepium* and *I. zollingeriana*

Variable			Inoculan *Rhizobium*			Average
	Ttpe Plant 2	R0	R1	R2	R3	
Dry matter	I	26,7	24,3	25,0	27,9	26.0A[3)]
	G	26,0	21,0	22,5	36,1	26.4A
	Average	26,4a	22,7a	23,8a	32,0a	
Ash	I	12,3	12,1	11,5	11,6	11,9A
	G	13,8	8,5	8,5	8,7	9,9A
	Average	13,1a	10,3a	10,0a	10,2a	
Organic matter	I	87,7	87,9	88,5	88,4	88,1A
	G	86,2	91,5	91,5	92,0	90,3A
	Average	86,9a	89,7a	90,0a	90,2a	
Crude protein	I	28,4	30,3	31,1	31,8	30,4A
	G	23,3	25,6	27,1	27,4	25,9A
	Average	25,9b	27,9a	29,1a	29,6a	
Crude fiber	I	19,8	17,1	16,3	15,9	17,3A
	G	15,5	17,4	16,6	15,2	16,2A
	Average	17,6a	17,2a	16,4a	15,5a	
Crude fat	I	5,0	4,9	4,7	5,1	4,9A
	G	6,1	6,0	5,5	6,3	6,0A
	Rata-rata	5,6a	5,5a	5,1a	5,7a	
Total digestible nutrient (TDN)	I	38,9	44,8	46,3	47,6	44,4A
	G	49,6	45,3	46,4	50,5	48,0A
	Average	44,3a	45,0a	46,4a	49,1a	
Total digestible nutrient (TDN)	I	23,4	25,6	29,8	31,0	27.,5B
	G	30,2	30,7	30,1	31,5	30,6A
	Average	26,8a	28,2a	29,9a	31,2a	
Gross Energy (GE)	I	4,0	4,0	4,0	4,5	4,1A
	G	3,8	3,9	3,9	4,3	4,0A
	Average	3,9a	3,9a	4,0a	4,4a	

Note.
1. R_0 = No *rhizobium* inoculation, R_1 = 1 g *rhizobium* inoculation/plant, R_2 = 2 g *rhizobium* inoculation/plant, R_3 = 4 g *rhizobium* inoculation/plant.
2. I = *I zollingeriana,* G = *G. Sepium*
3. The average treatment value followed by the same lowercase letter in one row and the same capital letter in one column is not significantly different (p>0.05)

The provision of *rhizobium* inoculant had a significant effect on the crude protein content of plants and was highest in the R_3 treatment, namely 29.6%. The results of this research are higher than the results of research by Suharlina (2020) which was carried out on post-coal mining land, namely the crude protein value of *I. zollingeriana* was 28.66%.

The highest dry matter content was in treatment R3 (32.0%), namely the administration of rhizobium inoculant at a dose of 4 g/plant, respectively 17.58%, 22.7% and 25.6% higher than in treatments R_0, R_1 and R_2. The results of this study are lower than the results of research by Suharlina *et al*. (2019) [6] where *Indigofera zollingeriana* fertilized with Saritana cypramine produced dry matter ranging from 68.21-73.15%.

The ash content in this study ranged from 10.2-13.1%. The results of this study were higher than the results of research by Suharlina (2020) [7] where the ash content of *I. zollingeriana* planted on post-coal mining land ranged from 8.09-8.96%. The results of this study were also higher than the results of research by Purwantari and Sajimin (2017) [4], the ash content of *I. zollingeriana* forage fertilized with *rhizobium* inoculant was 8.23-9.64%. The ash content indicates the mineral content of the plant. The decrease in ash content indicates that there was an increase in organic matter content in the forage of *G. sepium* and *I. zollingeriana* in this study.

The highest crude fiber content in treatment R0 (17.6%) was higher than in treatments R_1, R_2 and R_3, namely 2.38%, 6.84% and 12.02%, but statistically it was not significantly different (p>0.05). The higher the dose of fertilizer given, the lower the crude fiber content and the higher the protein content produced. In accordance with research by Purwantari and Sajimin (2017) [4], administering IG strain *rhizobium* inoculant to *I. zolliringiana* forage reduced the crude fiber content of the forage. In line with research by Suharlina (2020) [7], the crude fiber content of *I. zolliringiana* planted on post-coal mining land treated with AMF fertilizer ranges from 13.9-16.69%. Likewise, the results of measurements by Abdullah and Suharlina (2010) [1], namely that the crude fiber content of *I. zolliringiana* ranges from 10.97-15.02% with a plant defoliation age of 38-88 days. The results of this study are also in line with the results of research by Purwantari and Sajimin (2017) [4], the crude fiber content of *I. zollingeriana* forage fertilized with *rhizobium* inoculant was 17.33-21.56%. There was no interaction between plant types and the administration of various doses of *rhizobium* inoculant because the factor that influenced crude fiber was plant age. The older the plant, the higher the crude fiber content of the plant, which is indicated by the harder and stronger the plant cell walls are to support the plant. The crude fiber content of *G. sepium* was 6.4% lower than that of *I. zollingeriana* and was not statistically significantly different (p>0.05). This is because the *I. zollingeriana* plant is taller than the *G. sepium* plant. This is supported by the statement of Wahyuni and Kamaliyah (2012) [9] that a high proportion of stems influences crude fiber content, increasing plant cell wall components will increase crude fiber content. The highest crude fat content was in treatment R_3 (5.7%).

The crude fat content in this study was 5.1% - 5.7%. The crude fat content in this study was higher compared to the results of research by Suharlina (2020) [7] which stated that the crude fat content of indigofera forage planted on post-mining land given AMF fertilizer was around 1, 64-2.74%. The crude fat value of *I. zollingeriana* forage is also higher than Abdullah's (2010) [1] results of 2.63%. The results of this study are also higher than the results of research by Purwantari and Sajimin (2017) [4], the crude fat content of *I. zollingeriana* forage fertilized with *rhizobium* inoculant ranged from 2.76-2.80%. The high crude fat content of *I. zollingeriana* and *G. sepium* obtained in this study is still considered safe for ruminants. Crude fat content of feed below 6% does not have a negative effect on microbial population and activity in the rumen of beef cattle (Suharti *et al*., 2015). The crude fat content of *G. sepium* was 17.3% higher than that of *I. zollingeriana* and was statistically not significantly different (p>0.05). There was no interaction between plant types and the administration of various doses of *rhizobium* inoculant. This occurs due to factors that influence crude fat, namely differences in plant fat content which vary depending on the type of plant, intensity and duration of sunlight and nutrients (Lakitan, 2004) [3].

Table 2: Effect of different *Rhizobium* Inoculant doses on *in vitro* digestibility of *G. sepium* and *I. zollingeriana* forages

Variable	Ttpe Plant 2	Inoculan *Rhizobium* 1				Variable
		R0	R1	R2	R0	
Dry matter digestibility	I	56,5	65,4	71,1	67,2	65,1A[3)]
	G	52,1	51,9	53,1	57,2	53,6B
	Rata-rata	54,3b	58,6a	62,1a	62,2a	
Digestibility of organic matter	I	67,0	78,2	78,0	75,2	74.6A
	G	57,1	60,0	60,8	64,6	60.6B
	Rata-rata	62,0b	69,1a	69,4a	69,9a	

Note.

1. R_0 = No *rhizobium* inoculation, R_1 = 1 g *rhizobium* inoculation/plant, R_2 = 2 g *rhizobium* inoculation/plant, R_3 = 4 g *rhizobium* inoculation/plant.
2. I = *I zollingeriana*, G = *G. Sepium*
3. The average treatment value followed by the same lowercase letter in one row and the same capital letter in one column is not significantly different (p>0.05)

The highest total digestible nutrient (TDN) was in the R_3 treatment (49.1%), there was an increase in the TDN value when the *rhizobium* inoculant was increased. There was no interaction between plant types and the administration of various doses of *rhizobium* inoculant. The TDN content of *G. sepium* was 7.4% higher than that of *I. zolliringeriana* but the difference was not statistically significant (p>0.05).

Extract material without nitrogen (BETN) from treatment R_3 (31.2%) showed the highest yield compared to other treatments R_0, R_1 and R_2. The BETN value of this research is lower than the results of Suharlina's (2020) [7] research of 47-49%. There was no interaction between plant types and the administration of various doses of *rhizobium* inoculant. The BETN value of feed ingredients depends on the composition

value of other nutrients such as ash, crude protein, crude fiber and crude fat. This is because BETN is obtained from the reduction of dry matter with organic (crude protein, crude fiber and crude fat) and inorganic (ash) components.

The highest gross energy content was in treatment R3 (4.4 kcal g^{-1}). The results of this study are higher than the results of research by Purwantari and Sajimin (2017) [4], the energy content of I. *zollingeriana* forage fertilized with *rhizobium* inoculant ranged from 4.19-4.25 kcal g^{-1}.

The administration of *rhizobium* inoculant increased the *in vitro* digestibility of *G. sepium* and *I. zollingeriana* forages. Statistically, it shows a significant difference ($P<0.05$) compared to R0. These results are lower than the results of research by Purwantari and Sajimin (2017) [4]. The dry matter digestibility of *I. zollingeriana* forage inoculated with IG strain *rhizobium* ranged from 64.47-65.12%. The results of this research are also lower than the results of research by Abdullah (2010) [1] where *I. zollingeriana* planted in dry land had a dry matter digestibility of 67.50%. The addition of *rhizobium* can increase the KCBK value, this is due to the increase in organic matter or the nutritional content of the forage which also increases. The KCBO value in this study ranged from 69.1-69.9%, which is equivalent to the research results of Suharlina *et al.* (2019) [6] amounted to 65.33-70.64% in *Indigofera* forage fertilized using food flavoring industry waste. The results of this research are higher than the results of research by Abdullah (2010) [1] where the organic matter digestibility of *I. zollingeriana* planted in dry land was 60.32%. The digestibility of organic matter describes the availability of feed nutrients. The in vitro digestibility of *I. zolliringiana* plants was significantly higher than that of *G. sepium* plants. The very low tannin content in *I. zolliringiana* plants ranges from 0.6-1.4 ppm, far below the level that can cause anti-nutritional properties. This low tannin content also has a positive impact on its palatability (which livestock like).

Providing *rhizobium* inoculant has an effect on the conservation of forage areas for *G. sepium* and *I. zolliringiana*. The root length variable statistically showed a significant difference ($p<0.05$) with the R_0 treatment. The longest root length in treatment R3 (67.3 cm). There was no interaction between plant types and the administration of various doses of *rhizobium* inoculant. The root length of *G. sepium* was 47.1% lower than that of *I. zollingeriana* and statistically showed a very significant difference ($p<0.05$) (Table 5.6). *Rhizobia bacteria* are a group of microorganisms that are able to infect the roots of legume plants and cause root nodules (nodules). The *rhizobium* in the root nodules is able to fix nitrogen from the atmosphere and convert it into NH_3, which is then converted into amino acids which can be directly absorbed by plants to support the photosynthesis process for plant development, including the development of stems, leaves and roots. This condition is very beneficial from a conservation perspective because the roots of *G. sepium* and *I. zollingeriana* will be able to inhibit soil surface erosion and prevent landslides. The highest number of litters in treatment R_2 (31.2) statistically showed no significant difference ($p>0.05$). Vegetation and layers of litter protect the soil surface from direct hits from raindrops which can destroy soil aggregates, resulting in soil compaction. The destruction of soil particles will cause blockage of macro soil pores, thereby inhibiting groundwater infiltration, as a result surface runoff will increase. The role of the litter layer in protecting the soil surface is greatly influenced by its resistance to weathering; High quality litter (containing nutrients, especially high N) will rot easily so that the function of covering the soil surface does not last long. The presence of litter on the soil surface accompanied by changes in soil porosity due to the development of the root system allows the capacity and rate of infiltration to increase (Swank and Miner, 1968).

Conclusion

Based on the research results, it can be concluded that giving 4 g of *rhizobium* inoculant/plant had the highest influence on the quality and digestibility of *G. sepium* and *I. zollingeriana* forages planted on post-rock mining land in Sebudi Village, Karangasem Regency.

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Authors' Contributions

All authors (Ni Nyoman Suryani, I Wayan Suarna Dan Anak Agung Ayu Sri Trisnadewi) contributed equally to the research and writing of this article.

Conflict of Interest

There is no conflict of interest related to this research.

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