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Morphological Responses and Productivity of Indigofera (*Indigofera zollingeriana*) with Varied Fertilization in Limestone Post-mining Land

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Abstract

Indigofera is a protein-rich plant source for ruminants, with potential for introduction to marginal lands, such as limestone post-mining areas, through integration with reclamation activities. This study aims to determine the response of varying levels of organic fertilizer and NPK on the morphology and productivity of *Indigofera zollingeriana* introduced to limestone post-mining. The study employed a randomized block design with a 3 × 4 factorial pattern and four replications. The first factor was organic fertilizer at the rates of 0, 5, and 10 $tons.ha^{-1}$, and the second factor was NPK, 0, 50, 100, and 150 $kg.ha^{-1}$. Indigofera was planted in a 3 x 4 m^2 plot with a 1 m planting distance and harvested four times. The results showed that the combination of organic fertilizer with NPK affected ($p<0.05$) the morphology, biomass production, and nutrient profile. The combination of 10 $tons.ha^{-1}$ of organic fertilizer and 100 $kg.ha^{-1}$ NPK optimally produced plant with up to 217.64 cm height, 18.28 $tons.ha^{-1}$ fresh production per harvest, 4.96 $tons.ha^{-1}$ dry matter per harvest, 28.78% crude protein, and 72.47% total digestible nutrient. Biomass production decreased in the dry season (third harvest). Conclusively, the combination of 10 $tons.ha^{-1}$ organic fertilizer and 100 $kg.ha^{-1}$ NPK produced Indigofera plants with optimum plant height, biomass production, crude protein, and total digestible nutrients. Indigofera plants can be utilized as revegetation plants and as a source of green fodder on limestone post-mining land.

Keywords: fertilization, forage, *Indigofera zollingeriana*, limestone post-mining land, productivity

Introduction

Forage is the primary feed for ruminant livestock, requiring constant availability and reliable quality to support the development of the livestock industry. However, the decline in land use conversion (BPS, 2024) results in the area providing forage, decreasing forage availability. Therefore, efforts are needed to optimize the use value of marginal lands as planting media.

Limestone post-mining land is marginal land with potential to be developed as a green fodder area through reclamation integrated with the livestock sector. Mining activities alter the physical, biological, and chemical properties of the soil, which can damage the soil structure, decrease water retention, and lead to low fertility levels (Bandyopadhyay and Maiti, 2019; Carvalho et al., 2023). The chemical characteristics of limestone post-mining soil include pH 8.08, 0.10% nitrogen, 16.13 ppm phosphorus, and 58.90 $cmol^{(+)}.kg^{-1}$ calcium (Harwanto et al., 2023). The phosphorus content in alkaline soil is bound by calcium, which is difficult for plants to absorb. High calcium levels stimulate phosphate precipitation because phosphate is more reactive with with Ca^{2+} ion in forming $Ca_3(PO_4)_2$ and is difficult to dissolve (Msimbira and Smith, 2020). These conditions suggest that post-lime mining land requires ongoing restoration of soil fertility over time to support plant growth and development.

Improving post-mining land fertility is a crucial factor in supporting the success of ecological restoration and promoting the growth of vegetation. Adding

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organic matter (manure or compost) is the primary key to increase substrate fertility and improve the physical properties of the soil (Suwardi, 2019), such as structure, aggregate, density, and porosity, increase water retention capacity (Howe et al., 2024) and the survival of vegetation in the early stages of development (Joona et al., 2024). In addition, inorganic fertilizers can improve soil chemical properties (Pratiwi et al., 2021). Macro-minerals, such as N, P, and K, are essential nutrients that promote plant growth and development (Maccari et al., 2021). Mycorrhiza can also improve soil biological properties and help the absorption of nutrients for plant growth (Karti et al., 2012; Infitria et al., 2024).

The introduction of forage plants to post-mining land revegetation requires selecting the right plant species to recover the soil and then provide the necessary forage. The selection of plant species for this purpose should consider the physiological capabilities and productivity under certain conditions. *Indigofera zollingeriana*, one of the potential forages for ruminants, produces high crude protein biomass, exhibits a higher digestibility than other legumes (Suharlina et al., 2016), and produces DM forage up to 5.41 tons.ha^{-1} per harvest and 29.83% of crude protein (Abdullah, 2014). *Indigofera zollingeriana* is tolerant to drought (Abdullah, 2014) and acid soil (Herdiawan and Sutedi, 2015), and it promotes soil microfauna symbiosis, which positively contributes to the population of phosphate-solubilizing bacteria and soil fertility. Rhizobium bacterial symbiosis can fix nitrogen, and mycorrhiza can increase the absorption of nutrients and water (Hutapea et al., 2018; Infitria et al., 2024). It shows the potential of Indigofera plants to be introduced in a post-mining land with alkaline soil.

However, the development of Indigofera as ruminant feed is constrained by limited information on the quantity and quality of nutrients in limestone post-mining land. Preliminary research suggests the potential of Indigofera in dryland areas (Abdullah, 2014) and on acid soils (Herdiawan and Sutedi, 2015). However, forage productivity in the alkaline pH soil has yet to be discovered. This study examines the morphological response and productivity of Indigofera, introduced as a forage provider and reclamation plant, on limestone post-mining land.

Materials and Methods

The field experiment was conducted from January to October 2024 at the PT. Sinar Tambang Arthalestari in Ajibarang District, Banyumas Regency, Central Java (7°25’59.0”S 109°04’47.4”E, 211 m above sea level). The experimental location has an average temperature of 30.12°C, relative humidity of 79.43%, and an average rainfall of 155.52 mm per month (in a range of 0-344 mm per month between January and October). The highest rainfall intensity occurred during the wet season (223-344 mm per month) from January to April. In contrast, the lowest rainfall happened in the dry season (0-42 mm per month) between July and August (see Figure 1). The soil used in the experiment was limestone post-mining soil, which was slightly alkaline and less fertile (see Table 1).

Experimental Design

This study used a completely randomized block design with a 3 x 4 factorial pattern and four replications. The first factor was 0, 5, and 10 tons.ha^{-1} manure and the second factor was 0, 50, 100, and 150 kg.ha^{-1} NPK. Twelve treatment combinations were tested, including control (without

Table 1. Physical and chemical characteristics of limestone post-mining soil

Soil parameter	Extraction method	Value*	Status
pH	Electrometry	8.08	Slightly alkaline
C-Organic (%)	Walkley and Black	0.52	Very low
Total nitrogen (%)	Kjeldahl	0.24	Low
Phosphorus (P_2O_5, ppm)	Olsen	16.13	Low
Potassium (cmol$^{(+)}$.kg^{-1})	NH_4OAc 1 M, pH 7.00	0.52	Moderate
Cation exchange capacity (cmol$^{(+)}$.kg^{-1})	NH_4OAc 1 M, pH 7.00	42.20	Moderate
Electrical conductivity (µs/cm)	EC-Meter	220.00	Moderate
Texture:	Pipette method		Sandy clay
Sand (%)		45.21	
Silt (%)		24.19	
Clay (%)		30.60	

Note: *Soil properties result according to Harwanto et al. (2023).

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fertilizer), manure-only (5 and 10 tons.ha^{-1}), NPK-only (50, 100, and 150 kg.ha^{-1}), and combinations of manure and NPK. The chemical properties of cattle manure used in the experiment are shown in Table 2. Each treatment combination was supplemented with 10 g of mycorrhizae per plant, as described by Karti and Setiadi (2011).

Research Procedure

Four-month-old Indigofera seedlings were introduced to limestone post-mining land, planted in a 3 x 4 m^2 plot for each treatment combination with a 100 cm planting distance and a 100 cm distance between rows. Manure was applied two weeks before planting in the first and third maintenance periods. NPK fertilizers were applied two weeks after planting and after each harvest. The land was non-irrigated, as it relied entirely on rainfall. The environmental conditions during the maintenance period, including rainfall, temperature, and humidity, are shown in Figure 1. The plants were pruned at the first harvest on the 120th day (January-April), then harvested every 60 days during the second (May-June), third (July-August), and fourth (September-October) maintenance.

Data Collection

The morphological and production parameters of the plants were observed until the fourth harvest. The morphological variables were plant height (cm), measured from the base of the stem to the tip, and the height of the highest stem node. The stem diameter (mm) was measured with a caliper at a height of 15 cm from the base of the stem, the number of branches was measured from the branching of the main stem, and the number of twigs was calculated from the shoots that appeared on the branches.

Table 2. Chemical characteristics of cattle manure used in the experiment

Parameter	Extraction method*	Value**
pH	Electrometry	6.70
C-Organic (%)	Walkley and Black	24.12
Total Nitrogen (%)	Kjeldahl	1.80
Total Phosphorus (%)	Spectrophotometry	0.34
Potassium (%)	Atomic absorption spectroscopy	1.28
C/N ratio		13.40
Organic matter	Conversion	41.59

Notes: *Extraction method according to Eviati et al. (2023); **Manure chemical values according to Harwanto et al. (2025).

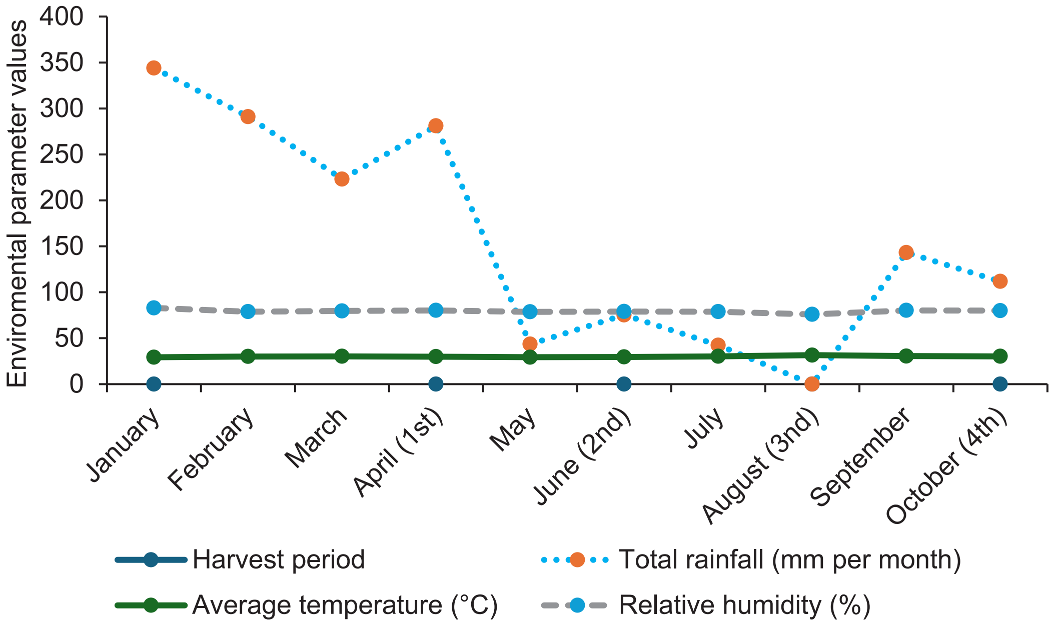

Figure 1. Environmental conditions at the experimental site from January to October 2024. The first harvest occurred during the wet season, and the third harvest occurred during the dry season

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Indigofera plants were pruned once they reached a height of 100 cm measured from the soil surface, and allowed to regrow until defoliation. The edible parts of the herbage were the base parts (leaves, petioles, and succulent branches) and the tips (all parts of the shoot tips) (Abdullah and Suharlina, 2010). The length of the shoot tip obtained in this research was 15-20 cm. Defoliation intensity was considered to optimize Indigofera production and stimulate regrowth. The observed parameters of the new biomass production (tons.ha^{-1}) were the fresh weight, biomass production (tons), and harvested area (ha).

The samples from the first and third harvests in each treatment were weighed as fresh weight, dried at 60°C for 48 hours, and analyzed to compare dry weight and nutrient quality using near-infrared reflectance spectroscopy, as described by Despal et al. (2021). The analyzed nutrient profile, based on Despal et al. (2021), consisted of crude protein (CP), crude fiber (CF), and ether extract (EE). Total digestible nutrients (TDN) were calculated based on Hartadi et al. (2005). The data were subjected to an Analysis of Variance Test, and any significant differences were analyzed using Duncan's Multiple Range Test.

Results and Discussion

Morphological Parameters of Indigofera

The analysis revealed an interaction ($p<0.05$) between manure and NPK fertilizer on the plant height and stem diameter of Indigofera in limestone post-mining land (see Table 3). Indigofera without fertilizer (control) showed the lowest growth of all treatments

Table 3. Plant height and stem diameter of Indigofera at each harvest

Treatments	Harvest			
	1st	2nd	3rd	4th
	January-April	May-June	July-August	September-October
Treatments	Plant height (cm)			
Control	126.40 ± 11.91^{a}	138.50 ± 15.54^{a}	138.63 ± 2.56^{a}	155.38 ± 10.51^{a}
5 t.ha^{-1} manure	127.73 ± 4.56^{a}	142.05 ± 5.32^{a}	142.75 ± 10.04^{a}	168.68 ± 5.01^{ab}
10 t.ha^{-1} manure	137.58 ± 7.56^{a}	148.60 ± 6.91^{ab}	142.08 ± 8.52^{a}	170.13 ± 3.92^{ab}
50 kg.ha^{-1} NPK	153.55 ± 4.02^{b}	165.60 ± 7.71^{bc}	155.50 ± 5.68^{b}	174.48 ± 4.07^{ab}
100 kg.ha^{-1} NPK	167.30 ± 8.27^{c}	178.80 ± 11.32^{cd}	161.25 ± 4.50^{bc}	176.43 ± 9.42^{ab}
150 kg.ha^{-1} NPK	179.68 ± 7.89^{c}	187.70 ± 8.11^{ef}	168.23 ± 3.66^{cd}	179.80 ± 10.08^{bc}
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	186.25 ± 14.93^{d}	181.27 ± 24.03^{de}	173.36 ± 11.44^{de}	191.10 ± 15.85^{cd}
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	183.65 ± 6.39^{d}	205.13 ± 40.21^{ef}	174.41 ± 11.17^{de}	199.73 ± 14.71^{cd}
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	209.15 ± 15.93^{e}	211.71 ± 33.85^{ef}	182.16 ± 5.71^{e}	205.12 ± 20.98^{d}
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	183.25 ± 6.88^{d}	195.80 ± 24.14^{ef}	176.53 ± 11.21^{de}	199.27 ± 10.37^{cd}
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	213.50 ± 8.24^{e}	217.64 ± 10.12^{f}	184.98 ± 10.33^{e}	209.46 ± 26.03^{d}
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	212.34 ± 7.12^{e}	215.54 ± 16.61^{f}	186.01 ± 6.37^{e}	210.32 ± 22.23^{d}
	Stem diameter (mm)			
Control	13.15 ± 1.71^{a}	15.23 ± 1.67^{a}	22.40 ± 1.64^{a}	27.25 ± 2.05^{a}
5 t.ha^{-1} manure	14.23 ± 1.25^{a}	16.05 ± 1.84^{ab}	24.83 ± 1.33^{ab}	29.20 ± 1.43^{ab}
10 t.ha^{-1} manure	14.95 ± 1.63^{a}	16.33 ± 1.12^{ab}	25.38 ± 1.73^{bc}	30.25 ± 1.79^{ab}
50 kg.ha^{-1} NPK	16.25 ± 2.20^{ab}	17.53 ± 2.29^{bc}	26.73 ± 5.66^{bc}	32.18 ± 3.23^{bc}
100 kg.ha^{-1} NPK	18.75 ± 2.75^{bc}	22.48 ± 2.14^{cd}	29.15 ± 2.40^{cd}	36.35 ± 4.65^{cd}
150 kg.ha^{-1} NPK	19.08 ± 1.85^{bc}	24.48 ± 1.05^{d}	32.03 ± 4.10^{d}	39.93 ± 4.66^{de}
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	20.43 ± 2.88^{d}	23.45 ± 6.36^{cd}	31.59 ± 1.96^{d}	39.31 ± 3.12^{de}
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	22.14 ± 3.23^{de}	25.69 ± 4.49^{d}	32.98 ± 2.21^{de}	42.81 ± 1.30^{e}
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	22.72 ± 3.35^{de}	29.82 ± 7.14^{d}	36.44 ± 2.41^{ef}	43.41 ± 3.08^{e}
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	23.82 ± 2.88^{ef}	26.67 ± 5.22^{d}	32.49 ± 1.50^{de}	43.47 ± 3.85^{e}
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	25.45 ± 3.15^{f}	28.89 ± 8.17^{d}	36.35 ± 1.94^{ef}	48.17 ± 4.31^{f}
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	24.96 ± 1.55^{f}	25.02 ± 3.76^{d}	37.23 ± 1.51^{f}	49.44 ± 2.16^{f}

Notes: Different superscripts within columns show significant differences ($p<0.05$). Control (without fertilizer).

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from the first to the fourth harvest, indicating that land with low nutrient content can lead to plant growth deficiency. In general, the combination of manure and NPK fertilizer produced plants with larger stem diameters than those of the control, manure-only, and NPK-only. The addition of 10 tons.ha^{-1} of manure-only, starting from the second harvest, resulted in higher growth and larger diameter than those of the control, but lower than those with 100 and 150 kg.ha^{-1} NPK-only. This result indicates that organic fertilizer, serving as a source of carbon and energy, combined with NPK as a macro-nutrient in the soil, promotes optimal plant growth and productivity.

These results are in line with those of Ortega et al. (2020) and Komolafe et al. (2022), who found that organic fertilizer improves the physical and chemical properties of the soil, provides essential nutrients (e.g., organic carbon, nitrogen, and phosphorus) for plants, increases soil microbial activity, and stimulates biochemical cycles for land recovery. Maccari et al. (2021) added that NPK fertilizer acts as a source of essential minerals (N, P, and K), which are critical to the process of photosynthesis and plant growth. Additionally, Indigofera is a legume capable of nitrogen fixation, which supports the availability of nitrogen in the soil and enhances growth (Hutapea et al., 2018).

The combination of 10 tons.ha^{-1} manure and 100 kg.ha^{-1} NPK fertilizer had a significant effect ($p<0.05$) (Table 3) on increasing plant height and stem diameter more efficiently than other treatments. From the first to fourth harvests, the plant heights were 213.50, 217.64, 184.98, and 210.46 cm, respectively, and the stem diameters were 25.45, 28.89, 36.35, and 48.17 mm, respectively. The increase in stem diameter (Table 3) was proportional to the number of branches and twigs (Table 4). The average plant height in this study was lower than that of Indigofera on peat land (236.4 cm) reported by Ali et al. (2014), but higher than that in latosol soil (177.15 cm) reported by Kumalasari et al. (2017). According to Hilty et al. (2021), plant growth is determined by fertilizer doses, planting media, and environmental factors.

The interaction ($p<0.05$) between manure and NPK was significant for the number of branches and twigs (see Table 4). The combined manure and NPK produced more branches and twigs than the control group, and Indigofera fertilized with NPK or manure separately from the first to fourth harvests. In Table 4, the combination of 10 tons.ha^{-1} of manure and 100 kg.ha^{-1} NPK fertilizer had a more significant effect ($p<0.05$) on the number of branches and twigs than the other treatments from the first to fourth harvests, namely 8.70, 12.08, 8.85, and 12.99 branches per individual plant and 51.29, 77.32, 37.79, and 104 twigs/individual plant, respectively.

The control group (non-fertilized Indigofera) had the fewest branches of all fertilized Indigofera. This condition shows that the availability of NPK minerals as macronutrients must be balanced with organic carbon to support optimal growth of the plant branches. The number of branches and twigs in the third harvest (Table 4) was lower than in the first, second, and fourth harvests. It is likely due to the maintenance period of the third harvest, which occurs during the dry season in July-August (see Figure 1), resulting in low water availability for plant shoot growth and metabolism. According to Abdullah (2014), the number of branches of Indigofera positively correlates with production, with a reported range of 8-30 branches and 2-6 twigs following the first pruning. The number of branches and twigs produced in the Indigofera plants in Table 4 was linearly related to the amount of fresh biomass production (Table 5).

Indigofera Biomass Production

The response of fertilizer application to biomass production of Indigofera legumes in limestone post-mining land is shown in Table 5. The results showed an interaction ($p<0.05$) between manure and NPK fertilizer on fresh biomass production and dry matter (DM) production. The combination of manure and NPK increased biomass production more efficiently compared to the control group and Indigofera fertilized with NPK or manure separately.

Non-fertilized Indigofera produced the least amount of fresh biomass compared to Indigofera fertilized from the first to fourth harvest (see Table 5). The combination of 10 tons.ha^{-1} manure and 100 kg.ha^{-1} NPK fertilizer had a significant effect ($p<0.05$) on increasing the production of fresh biomass and dry matter in Indigofera compared to other treatments. From the first to the fourth harvest, this combination of treatments produced fresh biomass of 18.28, 17.70, 5.83, and 17.67 tons.ha^{-1} per harvest, respectively, and dry matter of 4.24, 4.62, 1.97, and 4.96 tons.ha^{-1} per harvest. The fresh biomass in this study was lower than that reported by Abdullah (2014), specifically 6.40-7.40 tons DM.ha^{-1} per harvest, but higher than 1.56-4.08 tons of DM.ha^{-1} per harvest reported by Abdullah and Suharlina (2010). Cumulatively (Figure 2), the combination of 10 tons.ha^{-1} manure with 100 kg.ha^{-1} NPK significantly ($p<0.05$) produced 59.48 tons.ha^{-1} per year of fresh biomass and 15.79 tons DM.ha^{-1} per year (four harvests).

Fresh biomass and DM production in the third harvest (see Table 5) were lower than those in the

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first, second, and fourth harvests because the third harvest occurred in the dry season in July-August (see Figure 1), where water availability was scarce, so it disrupted plants' absorption and metabolism processes. This result indicates that Indigofera is a type of legume tree that exhibits excellent adaptation to a range of environments and alkaline soil pH in sub-optimal conditions by reducing its size to minimize evaporation and maintain its survival. Habermann et al. (2021) noted that plants can adapt to suboptimal environments through physiological changes, enhanced forage production, and improved forage quality. According to Msimbira and Smith (2020), plants reduce their size to minimize evaporation and maintain their survival through the cytoplasmic membrane proton transfer system and symbiosis with rhizobia-mycorrhiza, which helps maintain ion homeostasis and nutrient absorption.

Indigofera Nutritional Characteristics

The effect of fertilizer application on the crude protein (CP) content of Indigofera legume introduced into limestone post-mining land is shown in Table 6. The results showed that a combination of 10 $t.ha^{-1}$ manure + 100 $kg.ha^{-1}$ NPK (T2N2) or 10 $t.ha^{-1}$ manure + 150 $kg.ha^{-1}$ NPK (T2N3) significantly ($p<0.05$) produced higher CP compared to the control and Indigofera fertilized with manure or NPK separately. However, the combination T2N2 appeared more efficient than T2N3 because the latter produced 28.78% CP in the first harvest (rainy season) and 25.58% in the

Table 4. Number of branches and twigs of Indigofera at each harvest

Treatments	Harvest			
	1st	2nd	3rd	4th
	January-April	May-June	July-August	September-October
	Number of branches per plant			
Control	4.53 ± 1.02[a]	6.63 ± 1.14[a]	4.72 ± 0.98[a]	7.20 ± 0.77[a]
5 $t.ha^{-1}$ manure	5.25 ± 0.69[a]	7.33 ± 0.58[a]	5.38 ± 0.63[a]	8.75 ± 0.97[ab]
10 $t.ha^{-1}$ manure	5.38 ± 0.75[a]	7.50 ± 1.15[ab]	5.35 ± 0.31[a]	8.50 ± 0.86[ab]
50 $kg.ha^{-1}$ NPK	5.48 ± 1.02[a]	7.85 ± 0.92[ab]	5.58 ± 0.73[a]	9.65 ± 1.23[bc]
100 $kg.ha^{-1}$ NPK	7.10 ± 0.74[b]	8.70 ± 0.93[bc]	6.15 ± 0.99[ab]	9.80 ± 1.16[bc]
150 $kg.ha^{-1}$ NPK	7.68 ± 1.09[bc]	9.73 ± 0.50[cd]	7.53 ± 1.71[cd]	10.93 ± 0.98[cd]
5 $t.ha^{-1}$ manure + 50 $kg.ha^{-1}$ NPK	7.75 ± 0.62[bc]	10.25 ± 0.93[d]	7.03 ± 1.27[bc]	11.80 ± 1.42[de]
5 $t.ha^{-1}$ manure + 100 $kg.ha^{-1}$ NPK	8.30 ± 0.62[c]	10.68 ± 0.62[d]	7.25 ± 0.59[bc]	12.15 ± 1.17[ef]
5 $t.ha^{-1}$ manure + 150 $kg.ha^{-1}$ NPK	8.60 ± 0.53[c]	11.93 ± 0.68[e]	8.08 ± 1.15[cd]	12.31 ± 0.71[ef]
10 $t.ha^{-1}$ manure + 50 $kg.ha^{-1}$ NPK	7.67 ± 0.43[bc]	10.45 ± 0.60[d]	7.43 ± 0.45[cd]	12.04 ± 0.84[de]
10 $t.ha^{-1}$ manure + 100 $kg.ha^{-1}$ NPK	8.70 ± 0.43[c]	12.08 ± 0.83[e]	8.85 ± 0.41[d]	12.99 ± 1.12[ef]
10 $t.ha^{-1}$ manure + 150 $kg.ha^{-1}$ NPK	8.75 ± 0.42[c]	12.20 ± 0.90[e]	8.35 ± 0.83[cd]	13.85 ± 1.82[f]
	Number of twigs per plant			
Control	14.96 ± 2.15[a]	33.68 ± 2.87[a]	20.27 ± 4.50[a]	50.77 ± 7.82[a]
5 $t.ha^{-1}$ manure	17.98 ± 3.13[a]	36.47 ± 4.21[a]	26.79 ± 6.97[ab]	60.84 ± 9.18[ab]
10 $t.ha^{-1}$ manure	18.61 ± 2.14[a]	39.24 ± 4.06[ab]	27.29 ± 6.50[ab]	66.20 ± 4.71[bc]
50 $kg.ha^{-1}$ NPK	26.11 ± 3.51[b]	52.17 ± 4.43[bc]	28.06 ± 4.78[bc]	75.35 ± 13.34[cd]
100 $kg.ha^{-1}$ NPK	30.61 ± 4.15[bc]	54.62 ± 7.90[c]	29.60 ± 5.54[cd]	78.63 ± 9.55[de]
150 $kg.ha^{-1}$ NPK	32.11 ± 5.26[bc]	58.07 ± 6.30[c]	32.66 ± 6.82[cd]	88.73 ± 7.97[ef]
5 $t.ha^{-1}$ manure + 50 $kg.ha^{-1}$ NPK	36.07 ± 3.11[cd]	62.88 ± 11.02[cd]	30.46 ± 4.91[cd]	89.08 ± 9.11[ef]
5 $t.ha^{-1}$ manure + 100 $kg.ha^{-1}$ NPK	43.59 ± 9.23[ef]	64.88 ± 9.82[cd]	32.09 ± 6.42[cd]	93.30 ± 13.79[ef]
5 $t.ha^{-1}$ manure + 150 $kg.ha^{-1}$ NPK	45.20 ± 2.43[fg]	67.17 ± 10.63[cd]	40.50 ± 2.38[d]	95.24 ± 10.96[fg]
10 $t.ha^{-1}$ manure + 50 $kg.ha^{-1}$ NPK	39.42 ± 5.49[de]	65.63 ± 6.07[cd]	32.43 ± 1.89[cd]	94.23 ± 10.43[f]
10 $t.ha^{-1}$ manure + 100 $kg.ha^{-1}$ NPK	51.29 ± 7.48[g]	77.32 ± 16.45[d]	37.79 ± 5.93[cd]	104.14 ± 6.91[fg]
10 $t.ha^{-1}$ manure + 150 $kg.ha^{-1}$ NPK	50.90 ± 6.45[g]	76.34 ± 18.69[d]	38.95 ± 4.78[cd]	109.74 ± 8.14[g]

Notes: Different superscripts within one column show significant differences ($p<0.05$). Control (without fertilizer).

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Table 5. Fresh biomass and dry matter production of Indigofera at each harvest

Treatments	Harvest			
	1st	2nd	3rd	4th
	January-April	May-June	July-August	September-October
	Fresh biomass production (tons.ha^{-1})			
Control	2.23 ± 0.22^{a}	2.32 ± 0.26^{a}	0.84 ± 0.08^{a}	4.09 ± 0.49^{a}
5 t.ha^{-1} manure	2.75 ± 0.09^{a}	3.71 ± 0.31^{a}	1.37 ± 0.10^{ab}	5.66 ± 0.62^{b}
10 t.ha^{-1} manure	3.01 ± 0.48^{a}	3.94 ± 0.44^{ab}	1.57 ± 0.16^{b}	5.74 ± 0.61^{b}
50 kg.ha^{-1} NPK	4.97 ± 0.55^{b}	4.25 ± 0.46^{ab}	1.71 ± 0.12^{b}	7.58 ± 0.89^{c}
100 kg.ha^{-1} NPK	5.88 ± 0.62^{b}	5.92 ± 0.83^{bc}	2.73 ± 0.12^{c}	10.18 ± 0.80^{d}
150 kg.ha^{-1} NPK	7.29 ± 0.81^{c}	7.37 ± 0.56^{c}	3.25 ± 0.39^{cd}	11.93 ± 0.70^{e}
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	9.01 ± 0.74^{d}	10.10 ± 1.63^{d}	3.25 ± 0.38^{cd}	12.25 ± 1.09^{e}
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	10.60 ± 1.67^{e}	12.64 ± 1.60^{e}	3.83 ± 0.31^{d}	14.64 ± 1.04^{f}
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	15.09 ± 1.73^{f}	15.63 ± 1.94^{f}	5.29 ± 0.85^{e}	17.01 ± 0.70^{g}
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	9.99 ± 0.70^{de}	13.04 ± 1.89^{e}	3.85 ± 0.46^{d}	14.35 ± 1.25^{f}
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	18.28 ± 0.97^{g}	17.70 ± 2.24^{g}	5.83 ± 0.55^{e}	17.67 ± 1.84^{g}
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	18.61 ± 1.36^{g}	18.5 ± 1.30^{g}	6.80 ± 0.77^{f}	17.77 ± 1.46^{g}
	Dry matter production (tons.ha^{-1})			
Control	0.45 ± 0.04^{a}	0.64 ± 0.08^{a}	0.28 ± 0.03^{a}	0.99 ± 0.10^{a}
5 t.ha^{-1} manure	0.56 ± 0.04^{a}	0.97 ± 0.13^{ab}	0.44 ± 0.05^{ab}	1.35 ± 0.16^{a}
10 t.ha^{-1} manure	0.79 ± 0.13^{ab}	0.98 ± 0.13^{ab}	0.50 ± 0.03^{ab}	1.44 ± 0.18^{a}
50 kg.ha^{-1} NPK	0.94 ± 0.11^{b}	1.18 ± 0.12^{bc}	0.53 ± 0.05^{b}	1.94 ± 0.29^{b}
100 kg.ha^{-1} NPK	1.15 ± 0.06^{b}	1.49 ± 0.24^{cd}	0.94 ± 0.02^{c}	2.64 ± 0.28^{c}
150 kg.ha^{-1} NPK	1.54 ± 0.18^{c}	1.84 ± 0.22^{d}	1.04 ± 0.15^{cd}	3.00 ± 0.13^{cd}
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	1.85 ± 0.06^{c}	2.86 ± 0.43^{e}	1.12 ± 0.13^{de}	3.47 ± 0.23^{de}
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	2.37 ± 0.34^{d}	3.32 ± 0.51^{f}	1.25 ± 0.10^{de}	4.00 ± 0.39^{f}
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	3.20 ± 0.37^{e}	3.90 ± 0.21^{g}	1.91 ± 0.24^{f}	4.72 ± 0.25^{g}
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	2.24 ± 0.12^{d}	3.77 ± 0.49^{g}	1.31 ± 0.15^{e}	3.87 ± 0.38^{ef}
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	4.24 ± 0.45^{f}	4.62 ± 0.34^{h}	1.97 ± 0.27^{f}	4.96 ± 0.55^{g}
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	4.36 ± 0.40^{f}	4.49 ± 0.38^{h}	2.11 ± 0.30^{f}	5.08 ± 0.59^{g}

Notes: Different superscripts within one column show significant differences ($p<0.05$). Control (without fertilizer).

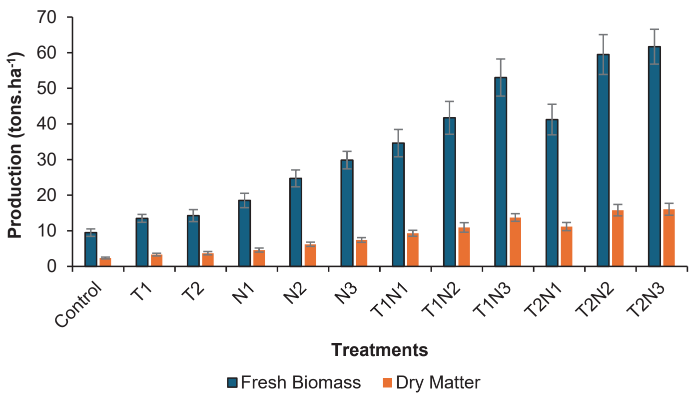

Figure 1. Total fresh biomass and dry matter production of Indigofera at four harvests (t.ha^{-1} per year) Control (without fertilizer); T1 and T2 (5 and 10 t.ha^{-1} manure-only); N1, N2 and N3 (50, 100 and 150 kg.ha^{-1} NPK-only); T1N1 (5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK); T1N2 (5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK), T1N3 (5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK); T2N1 (10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK); T2N2 (10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK); T2N3 (10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK).

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third harvest (dry season). It shows that low water availability (see Figure 1) and high environmental temperatures can affect the formation of Indigofera plant protein. Similarly, Ali et al. (2014) reported that low protein content is associated with plants' limited ability to supply nitrogen for protein synthesis due to the absence of fertilizer and unfavorable environmental conditions.

Crude protein is an important parameter for forage quality. Overall, the range of crude protein contents of Indigofera in this research was 25.6-28.83% in the first harvest and 22.48-25.58% in the third harvest. This range was consistent with that reported in a previous study, where the CP of Indigofera was 21.60%-31.20% (Royania et al., 2025). Based on the achievement of CP Indigofera, the results of this study are higher than those of *Centrocema pubescens* (16.85-21.63%) reported by Harwanto et al. (2025), and *Calliandra calothyrsus* (22.7%) reported by Lan et al. (2019).

On the other hand, adding fertilizer did not significantly affect the ether extract (EE) of the first harvest (Table 6). The EE produced in the first harvest (4.89-5.27%) was higher than that in the third harvest (2.77-3.42%), which was consistent with the decrease in crude protein. This condition was likely influenced by the season, water availability, and environmental temperature. According to Li and Liu (2024), the nutritive value (crude protein, ether extract, crude fiber, etc) of legumes is reported to be influenced by the type of legume, environment, land condition, and soil fertility.

The results in Table 7 show that the combination of 10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK produced lower crude fiber (CF) than other treatments in the first harvest (7.76%) and the third harvest (11.93%). Overall, the CF of the first harvest and the third harvest was 7.76%-10.10% vs. 7.76%-10.10%, which was comparable to the CF reported by Abdullah and Suharlina (2010), namely 10.97-15.02%. CF in the third harvest was higher than the first harvest because the former occurred in the dry season (Figure 1), where high environmental temperatures and low water availability caused plants to form more cell walls to protect and adapt to the environmental conditions. According to Houston et al. (2016), crude fiber is a cell wall component consisting of lignin, cellulose, and hemicellulose, which plays a role in protecting the contents of plant cells and reducing evaporation as an adaptation response to suboptimal environments.

Table 7 indicates that a decrease follows the increase in crude fiber (CF) in crude protein and ether extract (Table 6). These results are similar to those of Li and Liu (2024), who state that decreased availability of N and P in the soil will reduce the metabolism of plant protein formation and increase cell wall content. Ezquer et al. (2020) noted that plants adapt to drought through morphological and physiological changes, which occur via various signaling pathways that lead to osmotic adjustment. Cellulose microfibrils, consisting of ß-1,4-glucan chains, are major contributors to plant biomass formation and trigger cellulose accumulation as a defense against environmental fluctuations.

Table 6. Crude protein percentage and ether extract of Indigofera

Treatments	Crude protein (%)		Ether extract (%)	
	1st harvest January-April	3rd harvest July-August	1st harvest January-April	3rd harvest July-August
Control	25.68 ± 0.63^{a}	22.48 ± 1.17^{a}	5.04 ± 0.16	3.07 ± 0.35^{ab}
5 t.ha^{-1} manure	25.70 ± 0.53^{a}	23.14 ± 1.23^{ab}	4.98 ± 0.17	2.92 ± 0.11^{ab}
10 t.ha^{-1} manure	26.25 ± 1.50^{ab}	23.24 ± 1.13^{ab}	4.94 ± 0.29	3.08 ± 0.64^{ab}
50 kg.ha^{-1} NPK	26.22 ± 0.08^{ab}	23.29 ± 0.81^{ab}	5.10 ± 0.03	2.77 ± 0.30^{a}
100 kg.ha^{-1} NPK	27.07 ± 0.88^{bc}	23.46 ± 0.89^{bc}	5.20 ± 0.48	2.69 ± 0.11^{a}
150 kg.ha^{-1} NPK	27.50 ± 1.41^{cd}	23.49 ± 1.19^{bc}	5.32 ± 0.45	3.41 ± 0.27^{b}
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	27.49 ± 0.77^{cd}	24.48 ± 1.16^{cd}	4.96 ± 0.22	3.05 ± 0.23^{ab}
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	27.59 ± 1.41^{cd}	25.29 ± 1.36^{cd}	4.89 ± 0.13	2.90 ± 0.34^{ab}
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	28.86 ± 0.42^{d}	25.34 ± 0.91^{d}	5.28 ± 0.11	3.21 ± 0.39^{ab}
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	27.92 ± 1.20^{cd}	24.56 ± 0.65^{cd}	5.13 ± 0.27	3.07 ± 0.19^{ab}
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	28.78 ± 1.11^{d}	25.58 ± 1.58^{d}	5.27 ± 0.18	3.42 ± 0.32^{b}
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	28.83 ± 0.38^{d}	25.52 ± 1.01^{d}	5.07 ± 0.17	3.02 ± 0.23^{ab}

Notes: Different superscripts within one column show significant differences (p<0.05). Control= without fertilizer.

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Table 7. Crude fiber and total digestible nutrients of Indigofera

Treatments	Crude protein (%)		Ether extract (%)	
	1st harvest January-April	3rd harvest July-August	1st harvest January-April	3rd harvest July-August
Control	10.10 ± 0.26[d]	14.74 ± 0.23[d]	70.84 ± 0.81[a]	70.63 ± 0.59[a]
5 t.ha^{-1} manure	9.59 ± 0.74[d]	14.56 ± 0.29[d]	70.99 ± 0.72[a]	71.13 ± 1.39[ab]
10 t.ha^{-1} manure	9.67 ± 0.35[d]	14.13 ± 0.62[d]	71.01 ± 1.98[a]	71.11 ± 0.78[ab]
50 kg.ha^{-1} NPK	9.96 ± 0.50[d]	14.12 ± 0.88[d]	71.53 ± 0.30[ab]	71.33 ± 0.77[ab]
100 kg.ha^{-1} NPK	9.32 ± 0.83[cd]	13.40 ± 0.40[cd]	71.46 ± 2.41[ab]	71.51 ± 0.83[ab]
150 kg.ha^{-1} NPK	8.82 ± 0.61[bc]	13.71 ± 0.25[cd]	72.07 ± 0.76[ab]	71.37 ± 1.52[ab]
5 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	8.20 ± 0.10[ab]	12.61 ± 1.34[bc]	72.38 ± 0.59[ab]	71.94 ± 1.24[ab]
5 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	8.39 ± 0.29[ab]	12.31 ± 1.33[ab]	72.05 ± 0.83[ab]	72.08 ± 1.85[ab]
5 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	8.53 ± 0.42[b]	11.61 ± 0.58[a]	73.15 ± 1.13[b]	72.32 ± 0.91[ab]
10 t.ha^{-1} manure + 50 kg.ha^{-1} NPK	8.81 ± 0.72[bc]	12.39 ± 0.88[bc]	72.00 ± 1.98[ab]	71.57 ± 0.77[ab]
10 t.ha^{-1} manure + 100 kg.ha^{-1} NPK	8.64 ± 0.21[bc]	11.56 ± 0.65[a]	72.47 ± 1.75[ab]	72.02 ± 0.27[ab]
10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK	7.76 ± 0.17[a]	11.93 ± 0.47[a]	73.22 ± 0.35[c]	72.65 ± 1.71[b]

Notes: Different superscripts within one column show significant differences ($p<0.05$). Control (without fertilizer).

Table 7 shows that the combination of 10 t.ha^{-1} manure + 150 kg.ha^{-1} NPK significantly ($p<0.05$) produced a higher total digestible nutrient (TDN) than the other treatments. The average TDN in the first harvest was higher than that of the third harvest, while the average TDN in the third harvest was only higher ($p<0.05$) than that of the control. The TDN value was positively correlated with protein and fat content but inversely related to crude fiber content. In other words, an increase in crude fiber is associated with a decrease in the total digestible nutrient (TDN) value. According to Jayanegara et al. (2019), TDN is a value that describes the energy content that can be utilized from feed as a sufficient energy supply for livestock.

Conclusions

The combination of 10 t.ha^{-1} organic fertilizer and 100 kg.ha^{-1} NPK produced *Indigofera zollingeriana* with optimum plant height, biomass production, crude protein, and total digestible nutrients. Indigofera can be used as revegetation plants and providers of green fodder on limestone post-mining land.

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