

## Herbage Yield and Quality of Two Vegetative Parts of *Indigofera* at Different Times of First Regrowth Defoliation

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### ABSTRACT

A field experiment using *Indigofera* sp. was conducted at the Farm Research Station of Faculty of Animal Science, Bogor Agricultural University, Darmaga Campus, during 2008-2009. The objectives of this study were to identify the effect of defoliation time on herbage dry-matter production, protein, fiber contents, and *in vitro* digestibility of different vegetative parts of *Indigofera* sp. Block randomized design comprising three levels of defoliation time at first regrowth after pruning (38, 68, and 88 days) with 3 replications were used in this experiment. Pruning was done 3 months after transplanted into the experimental plots. First defoliation was conducted after the plant had been pruned. Herbage was derived from different vegetative parts, i.e: leaves of branch base and all parts of shoot tips. The results revealed significant effect of defoliation time on dry matter (DM) production of both branch base and shoot tip herbages. Crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* organic matter digestibility (IVOMD) of branch base were influenced significantly by defoliation time, except crude protein (CP) and *in vitro* dry matter digestibility (IVDMD). Defoliation time significantly affected CP, CF, NDF, ADF, IVDMD and IVOMD of herbage derived from shoot tips.

*Key words: Indigofera, defoliation time, herbage quality, herbage yield*

### INTRODUCTION

Stability of ruminant productivity in Indonesia is determined by forage availability throughout the year. Almost 70% of forage fed to animals is derived from local grasses species (Abdullah, 2006). This circumstance leads to low productivity of dairy and beef cattle, particularly during dry seasons. The main reason is certainly due to low quality of local grasses herbage. Herbage originated from local grass species mostly contains about 5%-7% of crude protein (CP), meaning that the grasses contribute only about 30% to 40% of ideal protein on ruminant diet. Introduction of legumes species into ruminant ration is a popular feeding management to improve productivity of ruminant animals. A previous study on the use of fodder legumes into daily goat's ration improved performance of local goats. Addition 10% *Indigofera* to ruzi grass-based diet overcame CP

deficiency for goats and led to increase in total herbage intake (Tarigan, 2009). Use of legume fodder basically becomes a traditional feeding management, since indigenous knowledge has significantly contributed in improvement of nutrition intake for animals (Thapa *et al.*, 1997).

One of important forage legumes in Indonesia is *Indigofera* sp. because it is highly relished by livestock. It contributes up to 45% in Burka cross bred goat ration (Tarigan, 2009). The forage plant has high herbage productivity, quality and high edible herbage portion for animals. Its leave meal may contain CP 27,9%, crude fibre (CF) 15,25%, Ca 0,22%, P 0,18%, and contains xanthophyl and carotenoid (Akbarillah *et al.*, 2002). A previous study was conducted to identify the possible contribution of indigofera leaf-meal in quail ration (Soetianto *et al.*, 2005). However, the results revealed low dry matter consumption and daily weight gain of quail fed with *Indigofera*'s leave meal. A reason explaining this result may be due to high fiber content of the leaf mill. Information about dry matter production and quality of edible parts of *Indigofera*'s herbage is required to optimize its biomass utilization for feed, particularly for non ruminant feed. It is therefore, information regarding quality and dry matter production of different edible

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plant parts has to be precisely elucidated. By recognizing the nutritional potency of herbage originated from each edible part of the forage the use of *Indigofera's* herbage for poultry is expected to be more possible.

Besides edible parts of *Indigofera* herbage, agronomical treatment such as defoliation time is considerably important aspect that influences quality of herbage (Badi *et al.*, 2004). Its association with plant age affects accumulation of dry matter and leaf-stem ratio (Tarigan, 2009), which in turn influences chemical composition of the harvested herbage (Kabi & Bareeba, 2008). This study focused on identification of defoliation time effect on herbage production and quality of different edible parts of *Indigofera's* herbage. Study on quality of herbage originated from legume trees is an important aspect, because herbage quality denotes a potential of the forage to meet the nutritional requirements of a specific kind and class of animals (Allen & Segarra, 2001) and to produce a desired animal response. Agronomical studies focusing on investigation appropriate time of defoliation for resulting optimal nutritive value becomes fundamental to set up forage management in the field. Changing defoliation time on forage plant may cause change of herbage nutrition such as non structural carbohydrates, CP and dry mater digestibility (Burner & Belesky, 2000), due to dynamics of plant morphological development during growing periods.

The objectives of this study were to identify the effect of defoliation time on dry matter production, CP content, fiber components including CF content, neutral detergent fiber content, acid detergent fiber, and *in vitro* dry matter- and organic matter digestibility of different vegetative parts (branch base and shoot tips), and identify the correlation among parameters which were affected by defoliation time.

## MATERIALS AND METHODS

### Time and Location

The field experiment was conducted from December 2008 to September 2009 at the University Farm research station, Darmaga, Bogor Agricultural University, Indonesia. The average monthly rainfall, air temperature and relative humidity during the experimental period were 465 mm month<sup>-1</sup>, 26 °C and 84%, respectively. The highest rainfall intensity occurred on March-April 2009 at 657 mm (23 days), and it shifted to dry season during July-August 2009, with very rare rainy days (139 mm, 8-10 days).

### Nursery Preparation

*Indigofera* seeds were planted in nursery tray in a greenhouse. Three weeks after planting, young plants with 15-20 cm height were selected and transplanted to polybags which were filled 2 kg growing media (consisting of 1 kg latosols and one kg cattle manure). The plants were nursed for 2 months in the growing media. During nursery the plants were watered every morning at 8.00-9.00 o'clock with maximum 1.2 L or 60% water holding capacity. To avoid direct sun shine to the nursed plants,

shading with paranet (reducing 30% of sun shine) was conducted.

### Plots Preparation and Transplantation

The land was cleared and totally tilled by using hand tractor. The dimension of plots was 4x6 m for each plot and the distance among plots were 1.5 m. After 2 months- nursery period and the plant height reached about 48 cm, the plants were transplanted to experimental plots in the field. Each plots consisted of 25 plants with planting dimension 1x1.5 m, so that each plot area was 63 m<sup>2</sup>. The plots were irrigated regularly when the rainfall was absent to keep soil moisture.

### Fertilization

Chemical properties of soils were analyzed before planting to identify nutrient requirement and establish optimum growth of experimental plants. Results of soils analysis revealed low pH (5.4), low available P (bray-1) 5.6 ppm and low nitrogen content (NO<sub>3</sub>; lower than 10 ppm). Application of 2 ton/ha of CaCO<sub>3</sub> increased soil pH up to 6.2, which sufficient for growing *Indigofera*. Application of basal fertilizer containing manure (2.5 ton/ha), 60 kg P/ha of superphosphate, 60 kg N/ha (Urea) and 60 kg K/ha (KCl) at a depth of 2.5-5.0 cm directly around the plant was conducted to improve soil fertility and enable optimum growth of *Indigofera* (Teutsch *et al.*, 2000).

### Pruning and Defoliation

After 3 months growing on the plots (average plant height varied from 175-228 cm), the plants were then pruned at the height 100 cm from soil surface, and let the plants re-grow up to the time of defoliation. Thirty-eight days, 68 days and 88 days after pruning, the plants were defoliated at height level 100 cm above ground. The edible part of herbage that consisted of branch base part including leaves, petioles and succulent branch, and branch tips including all parts of shoot tips. The shoot tip length obtained in this study was 10-15 cm. Defoliation intensity was considered to optimize the production and utilization of *Indigofera* and stimulating regrowth.

### Sample Preparation and Chemical Analysis

Fresh herbage derived from cut branches was weighed, and 2 kg of it were sampled, air dried at 70 °C for 2 days. The dried samples were then ground to pass through a 1 mm sieve and representative subsamples were stored and prepared for laboratory analysis. Moisture content was determined by drying sample at 105 °C according to official standard methods (AOAC, 2005) for 3 hours. Nitrogen content was analyzed with Kjeldahl method and CF analysis was conducted according to AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were determined according to Van Soest (1964). One gram of sample (passed through one-mm mesh) was boiled in an acid detergent

solution (Cetyl-trimethyl-ammonium-bromide dissolved in 0.5 M H<sub>2</sub>SO<sub>4</sub>) for one hour. The substrate was then filtered through a crucible filter and dried in an oven at 105 °C. The difference in weight between the fresh sample and the filtrate was calculated as fiber, soluble in acid (ADF).

*In vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) analysis were conducted in rumen fluid of dairy cows. The IVDMD is a laboratory test used as a plant quality index for animal feed by animal nutritionists (Tilley & Terry, 1963). In this study, the method includes two consecutive digestion phases. During the first digestion phase, samples (*Indigofera's* herbage from both branch base and shoot tips) were incubated under anaerobic conditions with dairy-cattle rumen microorganisms for 48 hours at 39 °C. This was followed by a 24 hour acid-pepsin digestion phase at the same temperature, under aerobic conditions. Following this 72 hour incubation, residual herbage materials were collected and oven dried (105 °C for 12 hours). Calculations were made using the following equation: %IVDMD = (1 – Wi – Wb/Ws) × 100; where Wf = weight of dry plant residue, Wb = weight of dry residues from blank, and Ws = dry weight of original plant sample. Ash contents were determined by combustion (550 °C for 2 hours) and these data used to correct herbage sample weight for potential contamination with soil.

### Experimental Design

The completely-randomized design was chosen as experimental design. The treatment was three levels of defoliation times (38, 68, and 88 days) with 3 replications. Data were analyzed with analysis of variance. Each mean value at each incubation time was contrasted by using least significant differences (LSD) at level 5% (Steel & Torrie, 1995). The data of both branch base and shoot tips were separately analyzed and were not compared each others; the means within vegetative parts was compared.

## RESULTS AND DISCUSSIONS

### Dry Matter Production

Dry matter production of both vegetative parts of *Indigofera's* herbage was significantly (P<0.05) affected

by defoliation time (Table 1). Total dry matter production was comparable with Hasen *et al.* (2008) that revealed herbage production of *Indigofera* about 2.6 ton DM/ha/harvest. Within base branch, delaying defoliation time from 38 and 68 to 88 dap (day after pruning) resulted in increased dry matter production by 56.6% and 160.9%, respectively. Delaying defoliation time from 68 dap to 88 dap increased dry matter production by 114.3%. This is understandable; branch base accumulates older tissues during plant structure development. A dynamic accumulation of dry matter occurs, when the branch of plants get older as described by (Abdullah, 2008). This is indicated by the increase of herbage portion of branch base (Table 1) when the plant age increased.

Increased dry matter production by 53% was found in shoot tip, when defoliation time was delayed from 38 dap to 68 dap. However, dry matter production reduced 26.5% when the defoliation time delayed from 68 dap to 88 dap. This might be due to shift of shoot tips portion to branch base parts by delaying 20 days of defoliation time. This is indicated by reduction of shoot tip portion when the plant age increased, but increase in base parts by increasing defoliation time, as depicted in Table 1. Total dry matter production increased significantly (P<0.05) when the defoliation time delayed from 38 to 68 and 88 by 53.2% and 124%, respectively. A little increase in dry matter production occurred when defoliation time delayed from 68 dap to 88 dap. Herbage production is contributed by leaf and stem formation, which was affected by cell division and elongation. Cell division and elongation zones are sites of high metabolic activity (growth hormones) and dry matter accumulation, which associated with defoliation (Schaufele & Schnyder, 2000).

### Protein Content

Crude protein content is one important quality measure of forage. The results showed that *Indigofera's* vegetative parts contained high CP (20.47%-27.60%) (Table 2). This is comparable with Tjelele result (2006), who reported that CP content of *Indigofera arrecta* ranged between 24.61%-26.1%. High protein content of the herbage enables high protein digestibility, however degradation of protein in the rumen is possibly high as well (McDonald *et al.*, 2002) that may lead to reduce amino acid absorption in the intestines.

Table 1. Dry matter (DM) production (kg/ha/harvest) of *Indigofera* herbage harvested at different defoliation time of different vegetative parts

| Defoliation time (dap) | Base                          |                                       | Tip                           |                                       | Total DM production (kg/ha/harvest) |
|------------------------|-------------------------------|---------------------------------------|-------------------------------|---------------------------------------|-------------------------------------|
|                        | DM production (kg/ha/harvest) | Proportion to total DM production (%) | DM production (kg/ha/harvest) | Proportion to total DM production (%) |                                     |
| 38                     | 1,562±104 <sup>c</sup>        | 58.4 <sup>b</sup>                     | 1,111±120 <sup>c</sup>        | 41.6 <sup>a</sup>                     | 2,673±217 <sup>c</sup>              |
| 68                     | 2,291±360 <sup>b</sup>        | 55.9 <sup>b</sup>                     | 1,805±120 <sup>a</sup>        | 44.1 <sup>a</sup>                     | 4,096±434 <sup>b</sup>              |
| 88                     | 4,076±373 <sup>a</sup>        | 75.3 <sup>a</sup>                     | 1,333±289 <sup>b</sup>        | 24.7 <sup>b</sup>                     | 5,410±554 <sup>a</sup>              |

Different letters within column denote significant differences at level 5% LSD. Base means herbage obtained from branch base, and tip means herbage obtained from branch tip. DAP=day after pruning.

Table 2. Crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter- (DM) and organic matter (OM) digestibility of *Indigofera* herbage derived from branch base (%) at different defoliation time

| Defoliation time (dap) | CP         | CF                      | NDF                     | ADF                     | <i>In vitro</i> digestibility |                          |
|------------------------|------------|-------------------------|-------------------------|-------------------------|-------------------------------|--------------------------|
|                        |            |                         |                         |                         | DM                            | OM                       |
| 8                      | 21.39±0.23 | 17.73±0.48 <sup>b</sup> | 57.67±1.36 <sup>a</sup> | 32.18±2.77 <sup>b</sup> | 70.21±4.13                    | 68.95±3.87 <sup>ab</sup> |
| 68                     | 20.96±2.49 | 21.20±1.42 <sup>a</sup> | 49.41±2.03 <sup>b</sup> | 26.23±4.09 <sup>c</sup> | 69.17±6.82                    | 70.79±3.05 <sup>a</sup>  |
| 88                     | 20.47±1.55 | 21.40±0.50 <sup>a</sup> | 53.29±1.47 <sup>b</sup> | 37.82±1.91 <sup>a</sup> | 67.39 ±4.21                   | 65.77±4.63 <sup>b</sup>  |

Different letters within column denote significant differences at level 5% LSD. Base means herbage obtained from branch base, and tip means herbage obtained from branch tip. DAP=day after pruning.

There was no effect of defoliation time on CP content in herbage samples of branch base. However, defoliation time significantly affected CP content from the herbage samples of shoot tip. In the shoot tip herbage, delaying defoliation time from 38 dap to 68 dap reduced significantly (4.2%) CP content (Table 3), but shifting defoliation time from 68 dap to 88 dap again increased significantly (3.6%) CP content. The young vegetative parts contained more nitrogen than the older one. Mobilization of nitrogen from older tissue to younger tissue is a reason of high protein content of shoot tip (Whitehead, 2000). Branch base was relatively more stable in CP content than shoot tip. This indicating N-saturation occurred in older tissue after delivering the N to younger tissue. The dynamics of N was seen in shoot tips, indicated by change of CP content if the defoliation time changed. The changing of CP content was contributed by the change of DM production of shoot tips (Duru, 2003). Increasing DM production of shoot tip was followed by reduction of CP content of shoot tips as shown in Table 1.

**Crude Fiber Content**

The major components found in forage cell walls are categorized as CF (Moore & Jung, 2001). CF is structural carbohydrates which contain cellulose, hemicelluloses and lignin (Delmer, 1999). CF content of *Indigofera*'s herbage ranged from 10.97% to 21.40%. It showed that *Indigofera*'s herbage contains relatively low CF. CF content in herbage from branch base showed almost double of those of shoot tip (Table 2 and Table 3). CF content of branch base and shoot tip herbage was significantly (P<0.01) influenced by defoliation time. Delaying defo-

liation time from 38 dap to 68 dap or 88 dap increased significantly CF content 19% and 33% for branch base and shoot tip, respectively. The increase of CF value in branch base was less than those of CF value in shoot tip. This showed that development of structural carbohydrate in shoot tips was undergoing during studies.

Addition of new tissue in shoot tip led to dynamics of fiber formation in this vegetative part. Increasing plant age led to shift some parts of shoot tips to branch base parts, which showed dynamic change of fiber content of both branch base and shoot tip. In legumes plant, xylem tissue accounts for the majority of cell wall material at typical harvest maturity (Jung & Engels, 2002). After plant cells reach their mature physical dimension, additional development of the primary wall or deposition of secondary wall structure will take place. This may result in different cell wall concentrations and structures among mature tissues (Jung & Engels, 2002)

Based on the CF content of shoot tip, it can be recommended that it is suitable for non ruminant diet, since the fiber content was lower than 18% (13.53%). Fiber has good correlation with lignin (Moore & Jung, 2001) and NDF and ADF. In this study, significant correlation between CF and NDF (r=0.69) and ADF (r=0.73) was found from branch base samples. In shoot tip, correlation value between CF and NDF (r=0.81) and ADF (r=0.83) was higher than that in branch base. These parameters influence digestibility of herbage that will be discussed later.

**Neutral and Acid Detergent Fiber**

The most universal method used to analyze forage cell wall is detergent fiber procedure (Van Soest, 1964). Neutral detergent fiber is defined as the portion

Table 3. Crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter- (DM) and organic matter (OM) digestibility of *Indigofera* herbage derived from shoot tip (%) at different defoliation time

| Defoliation time (dap) | CP                      | CF                      | NDF                     | ADF                      | <i>In vitro</i> digestibility |                         |
|------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------------|-------------------------|
|                        |                         |                         |                         |                          | DM                            | OM                      |
| 38                     | 27.60±1.93 <sup>a</sup> | 10.97±0.43 <sup>b</sup> | 59.97±1.59 <sup>a</sup> | 27.15±2.03 <sup>b</sup>  | 81.80±3.16 <sup>a</sup>       | 80.47±2.91 <sup>a</sup> |
| 68                     | 23.40±0.48 <sup>b</sup> | 14.60±0.31 <sup>a</sup> | 56.10±1.39 <sup>b</sup> | 30.73±3.68 <sup>ab</sup> | 78.58±0.58 <sup>ab</sup>      | 77.63±0.80 <sup>b</sup> |
| 88                     | 27.03±1.28 <sup>a</sup> | 15.02±0.75 <sup>a</sup> | 51.16±1.03 <sup>c</sup> | 36.44±2.56 <sup>a</sup>  | 72.03±2.39 <sup>b</sup>       | 77.46±3.68 <sup>b</sup> |

Different letters within column denote significant differences at level 5% LSD. Base means herbage obtained from branch base, and tip means herbage obtained from branch tip. DAP=day after pruning.

of plant-derived feedstuffs of limited digestibility (Van Soest, 1964), which mostly equated as plant cell walls. Defoliation time significantly ( $P < 0.01$ ) influenced NDF and ADF of *Indigofera's* herbage. NDF of *Indigofera* was ranging at 49.4%-59.97%. This number was higher than those of *Indigofera arrecta* grown in spring (32.80%) found by Hassen *et al.* (2000). As shown in Table 2 and Table 3, NDF of branch base and shoot tips decreased by delaying defoliation time. On the other hand ADF increased significantly by delaying defoliation time. In shoot tips part NDF and ADF decreased to the lowest level (49% and 26%, respectively), when defoliation time delayed from 38 dap to 68 dap or 88 dap.

In branch base, NDF showed significant reduction by delaying defoliation time. The development of cell wall in plant age 38 dap tissue seemed to be active; hence, accumulation of NDF was quite higher than older tissue, but did not occur advanced lignifications yet. This was indicated by lower ADF content at 38 dap plant than those of 68 dap and 88 dap plants. Meanwhile, during growing, cell wall development slowly shifted from NDF to ADF at branch base. This was indicated by increase in ADF at older plants. The concentration of ADF is greater in stem material of forages than leaves (Belesky, 2006). In legumes, cell wall concentration of leaves changes with delaying defoliation time, whereas stem develop cambium, which add thick-walled xylem tissue (Jung & Engels, 2002). The xylem account for typical of dominant cell wall material in legumes if the plants is getting old due to late harvest time (Jung & Engels, 2002). According to the proportion of branch base and shoot tips data collected in this study, defoliation time led to changes of anatomy composition and plant morphology (Hassen *et al.*, 2006).

NDF and ADF are indicators to express organic content and dry matter and organic consumption of diets. In this study, negative correlation between NDF/ADF and IVDMD/IVOMD was found. In branch base, ADF significantly correlated ( $r = -0.58$ ) with IVOMD. However, no correlation between NDF and herbage digestibility was found in this study. Meanwhile, in shoot tip, NDF had significantly negative correlation with IVDMD ( $r = -0.71$ ) and IVOMD ( $r = -0.71$ ), but no correlation between ADF and herbage digestibility. This study revealed that NDF and ADF were important indicators for digestibility of shoot tip and branch base herbage, respectively.

#### *In vitro* Dry Matter and Organic Matter Digestibility

Dry matter and organic matter digestibility are main determinants of forage quality, influencing forage intake. Forage is a unique feedstuffs and present challenge to an animal's capacity to ingest and digest nutrient (Mertens, 2007). Digestibility of forage is determined by the composition of fiber content in plant tissue. The IVDMD and IVOMD digestibility value of *Indigofera's* were rather high for tropical legume like *Indigofera* (maximum 80%-81%) as found in this study. High value of IVDMD and IVOMD may be caused by high protein content (causing very active rumen microbes) and relatively low CF, NDF and ADF content. There was same trend of IVDMD and

IVOMD, which similarly revealed by Tarigan (2009).

In this study, delaying defoliation time reduced IVDMD and IVOMD of shoot tip, but reduced only IVOMD of branch base (Table 2 and Table 3). Delaying defoliation time from 38 dap to 68 dap or 88 dap led to reduce 7% and 3% of IVDMD and IVOMD, respectively in shoot tips. In branch base IVDMD was not significantly influenced by defoliation time. This seemed to be stable in older tissue, which is not correlated with the change of NDF and ADF. The reduction of digestibility might be due to increase of syringyl- to guaiacyl-type lignin (Jung & Engels, 2002) or increase of arabinoxylan cross-linking by ferulates (Hatfield, *et al.*, 2007) by maturing the plant tissues.

#### CONCLUSIONS

Defoliation time determined dry matter production, CP, CF, NDF, ADF and digestibility of *Indigofera's* herbage. The highest total dry matter production was found at 88 dap, however the highest dry matter production of shoot tip was found at 68 dap. Delaying defoliation time from 38 dap to 68 dap or 88 dap reduced CP, IVDMD and IVOMD in shoot tip, increased CF and ADF both in branch base and shoot tip, reduced NDF branch base and shoot tip, reduced IVOMD in branch base. NDF had significantly negative correlation with IVDMD and IVOMD in shoot tip, meanwhile in branch base ADF had significantly negative correlation with IVOMD.

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