

IN VITRO EVALUATION OF CONCENTRATE FEED CONTAINING *Indigofera zollingeriana* IN GOAT

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi fermentabilitas dan pencernaan nutrisi pakan konsentrat berbasis *Indigofera zollingeriana* secara *in vitro*. Penelitian ini menggunakan rancangan acak kelompok pada 3 jenis pakan konsentrat dalam 4 kelompok cairan rumen yang berbeda. Pakan konsentrat yang digunakan yaitu R1, R2 dan R3 masing-masing mengandung 0, 20 dan 40% *I. zollingeriana*. Peubah yang diamati antara lain pencernaan nutrisi (bahan kering, bahan organik dan protein), produksi gas total, VFA parsial dan NH₃. Data dianalisis menggunakan analisis ragam (ANOVA). Hasil penelitian menunjukkan bahwa nilai pencernaan bahan kering (KCBK), bahan organik (KCBO) dan protein (KCPK) memperlihatkan pola yang sama yaitu R2 lebih tinggi dari R1 (P<0.05), sedangkan R3 memiliki nilai pencernaan yang sama dengan R2 dan R1. Tidak terdapat perbedaan produksi gas total pada ketiga jenis ransum konsentrat. Konsentrasi VFA parsial masing-masing ransum konsentrat tidak berbeda nyata. Perlakuan R2 memiliki konsentrasi NH₃ paling rendah (P<0.05) dibandingkan R1 dan R3. Dapat disimpulkan bahwa penambahan *I. zollingeriana* dapat digunakan sebagai penyusun pakan konsentrat maksimal 20%.

Kata kunci: fermentabilitas, Indigofera zollingeriana, in vitro, pakan konsentrat

ABSTRACT

This study was conducted to evaluate the fermentability and nutrient digestibility of concentrate feed containing *Indigofera zollingeriana* using *in vitro* technique. Randomized complete block design was used on 3 types of concentrate feed and 4 groups (replicates) of goat's rumen liquid. The concentrate feeds were R1, R2 and R3 containing *I. zollingeriana* at 0 (control), 20 and 40%, respectively. Variables observed were total gas production, *in vitro* dry matter digestibility (IVDMD), organic matter (IVDOM), and crude protein (IVDCP), volatile fatty acid (VFA) profiles and NH₃ concentration. Data were analyzed using analysis of variance (ANOVA). Results showed that there was no significant effect of R3 on IVDMD, IVDOM and IVDCP as compared to R1 and R2. However, there was significant difference (P<0.05) of the parameters between R1 and R2 (P<0.05). There was no significant effect (P<0.05) of different *Indigofera* levels on total gas production. The partial VFA of each concentrate feeds was not significantly different, but the percentage of acetic acids of concentrates containing *I. zollingeriana* were higher than those of control (P<0.05). The concentration of rumen NH₃ of R2 was lower than R1 and R3 (P<0.05) even though the IVCPD of R2 was higher than R1. It could be concluded that the inclusion of *I. zollingeriana* as concentrate feed ingredient at 20% maximum level.

Keywords: fermentability, Indigofera zollingeriana, in vitro, concentrate feed

INTRODUCTION

Ruminant have an important contribution in the provision of foods, especially milk and meat. Ruminants account for almost all of the milk and about one-third of the meat production worldwide (FAO, 2004). Even though milk and its product are dominated by dairy cow, goat milk is believed to have several advantages over cow's milk in several countries including Indonesia. The dairy goat farm in Indonesia is dominated by small-holder farmers. Poor quality feed such as tropical grasses given by small-holder farmers lead to low milk production. Feeding tropical grasses as the main source of feeds is not sufficient to meet the nutritional requirement of high-producing dairy goats (Fujisaka *et al.*, 2000), due to the tropical grasses are high of fiber content, and relatively low ranging of protein contents about 4-9 %DM, while the protein requirement of dairy goat feed reaches up to 18 % DM.

Productivity of goats in tropical areas could be improved by introducing forage legumes, such as *Leucaena leucocephala*, *Gliricidia sepium* or *Flemingia macrophylla* that rich in protein (Ondiek *et al.*, 2000; Mui *et al.*, 2002; Fagundes *et al.*, 2014), especially during the dry season when forages quality becomes poor. Another potential legume that can be used as feed for dairy goats is a forage namely *Indigofera zollingeriana*. The plant has a rapid regrowth in the defoliation interval of 60 days with a production of 51 tons/ha/year (Abdullah, 2010). *I. zollingeriana* is very adaptive to low soil fertility, easy on maintenance and low prize, and high seed production potential throughout the season (Abdullah and Suharlina, 2010). The branch base and shoot tip of *I. zollingeriana* that harvested at 68 days defoliation time containing of 20.96% and 23.40%DM crude protein (CP), 21.20% and 14.60%DM crude fiber (CF), 49.41% and 56.10% DM neutral detergent fiber (NDF), 26.23% and 30.73%DM acid detergent fiber (ADF) and the *in vitro* digestibility of 69.17% and 78.58%DM dry matter (IVDMD) and 70.79% and 77.63%DM organic matter (IVOMD), respectively (Abdullah and Suharlina, 2010). Suharlina and Abdullah (2010) reported that *I. zollingeriana* forage (leaves and twigs) treated by liquid organic fertilizer 0-40% containing 24.42-31.05%DM of CP; 50.51-51.77%DM of NDF; 48.19-49.15%DM of ADF; 68.21-73.73.15%DM, respectively. The use of fresh *I. zollingeriana* for local goats increased daily weight gain and feed efficiency up

to 45% and 30%, respectively (Tarigan and Ginting, 2011). Further, an experiment using pure pellet of *I. zollingeriana* leaves increased goat milk production, feed efficiency and nutrient efficiency by approximately 26%, 15-23% and 5-9%, respectively (Abdullah *et al.*, 2012).

This study was carried out to evaluate the fermentability and nutrient digestibility of concentrate feed containing *I. zollingeriana* in goat using *in vitro* technique.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Dairy Nutrition, Faculty of Animal Science, Bogor Agricultural University. Leaves and twigs of *I. zollingeriana* were collected from University Farm Research Station, Darmaga, Bogor Agricultural University, Indonesia. The samples were obtained from three plots with the dimension of 4×6 m each. Each plot consisted of *I. zollingeriana* plants with the planting dimension of 1×1.5 m. After 68 days of planting period as recommended from our previous study (Abdullah and Suharlina, 2010) the plants were harvested and leaf and twig samples were collected. Leaves and twigs of *I. zollingeriana* were air-dried and then oven-dried at 60°C for 24 h. *I. zollingeriana* forage was formulated in feed as R1, R2 and R3 with varying levels, i.e. 0, 20 and 40% (DM basis), respectively (Table 1). The feed materials were ground to pass a 1 mm sieve size and mixed homogeneously. Dry matter (DM), ash, crude protein (CP), extract ether and crude fiber (CF) composition of each concentrate feed were determined by proximate analysis (AOAC, 1995; Table 2).

Randomized complete block design was employed on 3 types of feeds and 4 groups of rumen liquid using *in vitro* technique. The *in vitro* method was based on Tilley and Terry (1963), the rumen fluid was collected from slaughter goats shortly after goat slaughtered at morning time. Rumen fluid was filtered through 100 µm nylon sieve size. An amount 0.5 g DM samples were filled up into fermentor tube and added by 40 ml McDougall solutions and 10 ml rumen fluid. The fermentor tubes were put into the shaker bath with 39°C temperature and flowed with CO₂ for 30 seconds, the pH was checked (6.5 up to 6.9), and then covered by ventilated rubber cap. The incubation was conducted during 48 hours. After 48 hours the rubber cap of tubes were open up and

Table 1. Formulation of Experimental Concentrate Feed

Ingredients	R1	R2	R3
	----- % DM -----		
Tapioca pulp	24	13	12
Corn	9	9	9
Coconut cake	35	39	20
<i>Indigofera zollingeriana</i>	0	20	40
Rice bran	28	15	15
Molases	1	1	1
Premix	1	1	1
CaCO ₃	1	1	1
DCP	0.5	0.5	0.5
NaCl	0.5	0.5	0.5

R1, concentrate feed without *I. zollingeriana* (control); R2, concentrate feed containing 20% DM *I. zollingeriana*; R3, concentrate feed containing 40% DM *I. zollingeriana*.

Table 2. Nutrient Contents of Experimental Concentrate Feed (%DM)

	Ash	EE	CP	CF	NFE	Ca	P
R1	11.4	8.82	14.0	10.5	55.3	1.47	0.27
R2	11.5	8.92	16.0	10.6	53.0	0.79	0.27
R3	12.0	9.33	17.2	11.1	50.4	2.14	0.31

R1, concentrate feed without *I. zollingeriana* (control); R2, concentrate feed containing 20% DM *I. zollingeriana*; R3, concentrate feed containing 40% DM *I. zollingeriana*.

added by 4 ml HgCl₂ with 5% concentration to stop the microbial activity, and subsequently centrifuged at 4000 rpm of speed for 15 minutes. The residual substrates are separated into sludge at the bottom and the clear supernatant is at the top. The residues were added by 50 ml solution of pepsin-HCl 0.2% and then incubated for 48 hours without a rubber cap. After 48 hours the tubes were centrifuged at 4000 rpm of speed for 15 minutes. The residues were filtered through Whatman paper No. 41 using vacuum pump, and the distillate residues were put in to a porcelain

cup. The crude protein of residue obtained by Kjeldal metod (AOAC, 1995), while the dry matter obtained by drying in oven at 105°C temperature for 8 hours. Subsequently, the residues were burnt to ashes using using oven at 450-600°C temperature for 6 hours to determin the organic matter. The residual substrates of the incubation without sample of feed materials were used as a blank. The *in vitro* digestibility nutrients calculated by these formulas:

$$IVDMD(\%) = \frac{DM \text{ sample (g)} - DM \text{ residue (g)} - DM \text{ Blank (g)}}{DM \text{ sample (g)}} \times 100\%$$

$$IVOMD(\%) = \frac{OM \text{ sample (g)} - OM \text{ residue (g)} - OM \text{ Blank (g)}}{OM \text{ sample (g)}} \times 100\%$$

$$IVCPD(\%) = \frac{CP \text{ sample (g)} - CP \text{ residue (g)} - OM \text{ Blank (g)}}{CP \text{ sample (g)}} \times 100\%$$

Where

IVDMD : *in vitro* dry matter digestibility

IVOMD : *in vitro* organic matter digestibility

IVCPD : *in vitro* crude protein digestibility

DM : Dry matter

OM : Organic matter

CP : Crude protein

The gas production method was based on Blümmel *et al.*, (1997). Rumen fluid was collected from slaughter goats shortly after goat slaughtered at morning time. Rumen fluid was filtered through 100 µm nylon sieve size and added by buffer being reduced. Rumen-buffer was saturated with CO₂ for 10 minutes before being inserted into the syringe glass to ensure anaerobic conditions in the reaction. An amount of 380 mg DM samples were inserted into the syringe and closed with a piston that has been lubricated by vaseline. As much as 30 ml of buffered-rumen fluid inserted into each syringe through the inlet channel, and then the syringe immediately put in a water bath at 39°C temperature. Gas production was observed at 0, 3, 6, 9, 12 and 24 after incubation.

Volatile fatty acids (VFA) were determined by the supernatant which incubation for 4 hours using gas chromatography, while NH₃ concentration was based on Conway diffusion method. The concentration of NH₃ was measured using a micro diffusion Conway method (Department of Dairy Science, 1966). A Conway plate and its cap were given by vaseline the edges of the cups, and then about 1 ml of the supernatant was put on one side of the bulkhead plate and on the other position was put 1 ml

solution of saturated Na₂CO₃. The plates were placed at an angle towards the bulkhead so that they were not mixed before the plate was sealed. At the center of the plate was put 1 ml of boric acid indicator solution, and then the plate was sealed. The plates were shake until Na₂CO₃ and supernatant mixed. Ammonia that released from the reaction will be captured by boric acid shown by a color change. After 24 hours, boric acid was titrated by 0.0059 N H₂SO₄ solution until the color changes return to the original color. The concentration of NH₃ measured by followed this formula:

$$\text{NH}_3 \text{ (mM)} = \frac{\text{ml H}_2\text{SO}_4 \times \text{N H}_2\text{SO}_4 \times 1000}{\text{gram sample} \times \text{DM sample}}$$

The data were analyzed using analysis of variance (ANOVA). If there were significant differences among treatments at P<0.05, the analysis was continued with Least Significance Difference (LSD) test.

RESULTS AND DISCUSSION

Higher inclusion levels of *Indigofera* increased CP contents of the experimental concentrates feed (Table 2). This was due to the high CP content of the plant (29.2% DM). High CP of *Indigofera* had been also reported by some authors and generally ranged from 23–27% DM (Kaitho *et al.*, 1998; Tscherning *et al.*, 2006; Bhatta *et al.*, 2012; Bhatta *et al.*, 2013). *In vitro*

dry matter (IVDMD) and organic matter digestibility (IVOMD) values of the experimental concentrates were 65.5–71.5% and 63.0–68.1%, respectively, while the *in vitro* crude protein digestibility (IVCPD) values were 79.1–83.8% (Figure 1). The IVDMD, IVOMD and IVCPD values had similar trends in which R2 were higher than R1 (P<0.01), while R3 had similar value with R1 and R2 feeds. The *in vitro* digestibility of *I. zollingeriana* had been reported by Suharlina and Abdullah (2010) ranged about 68.21-73.73.15%DM of IVDMD; 65.33-70.64%DM of IVOMD and 87.50-90.64%DM of IVCPD (in vitro crude protein digestibility), respectively. It was shown that the inclusion level of *I. zollingeriana* in feed by 20% improved the nutrient digestibility of R2 since the plant is characterized by its high protein and low fiber contents thus increase its digestibility (Suharlina *et al.*, 2016). The nutrient digestibility value of R3 was not different with that of R1 despite it contains *I. zollingeriana* higher than R1. Apparently higher fiber and lower NFE contents found in R3 than that found in R1 are among the plausible factors behind such response.

The digestibility of dry matter and organic matter are a primary determinant of the quality of feed and forage. Nutrient digestibility is determined by the composition of the fiber in the diet. Control concentrate feed (R1) contained high level of fiber and low level of protein. These

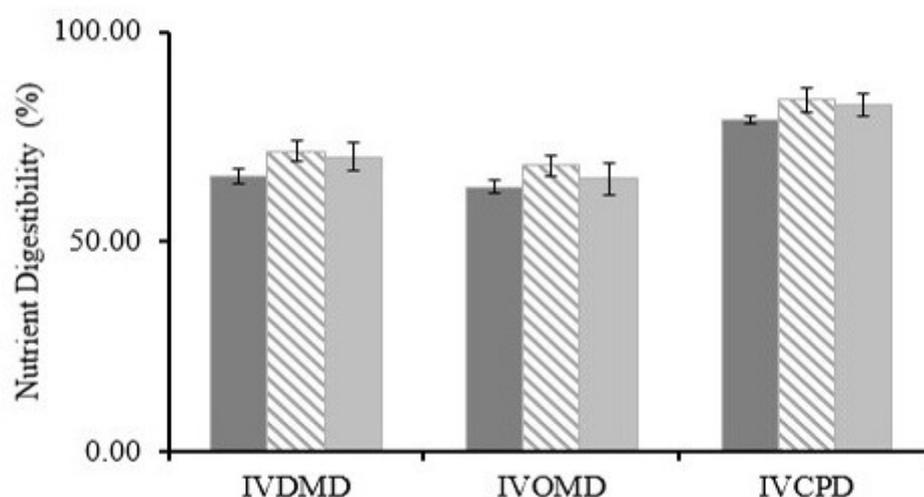


Figure 1. Nutrient Digestibility of Experimental Concentrate Feed. Error bars indicate standard error values for the concentrates feed.

(■) R1, concentrate feed without *I. zollingeriana* (control); (▨) R2, concentrate feed containing 20% DM *I. zollingeriana*; (■) R3, concentrate feed containing 40% DM *I. zollingeriana*.

concomitantly high fiber and low protein have been observed to be negatively correlated with digestibility coefficients in sheep, goat, cattle and buffalo (Riaz *et al.*, 2014). It is widely known that protein is more digestible as compared to fiber by rumen microbes. Although R3 had the highest inclusion level of *I. zollingeriana* than the other concentrate, it showed similar digestibility values to R1. It seems that secondary compounds present in *I. zollingeriana* such as tannins and saponins influence the digestibility by protecting protein and other nutrients from microbial degradation and fermentation in the rumen (Jayanegara *et al.*, 2009; Jayanegara *et al.*, 2014). It was analyzed that *I. zollingeriana* used in the present experiment contained 2.9 g/kg DM tannins and 2.6 mg/kg DM saponins.

The total gas production value describe the effectiveness of the fermentation process. The total gas production of each concentrate feed had similar values at any time of measurements (Figure 2). The values of total gas production of R1, R2, and R3 were 22.92±1.09, 24.83±4.42, and 20.31±4.22 ml/mg DM, respectively. This indicated that all of the concentrates had similar

in vitro fermentability values. There were no differences on total gas production in this study due to the concentrates feed have the similar crude fiber content (Table 2). Fiber fraction is a limiting factor on *in vitro* fermentation (Ndlovu and Nherera, 1997). The output of the carbohydrate fermentation is the volatile fatty acids (VFA) which is a source of energy for animals. The main component of VFA are acetic acid, propionic acid, butyric acid, and small amount of valeric acid. The partial VFA concentrations of each concentrates feed were not significantly different (Table 3), apparently because of each concentrate had similar proportions of nitrogen free extract (NFE). The inclusion of *I. zollingeriana* forage did not change the characteristics of concentrate. Therefore, *I. zollingeriana* forage can be used as a source of concentrate originally from forage. Acetic acid is required as a precursor of milk fat, therefore, the higher of acetate-propionat (A:P) ratio indicated ideal ration for dairy animals. Both acetate and propionate are required for milk production in dairy animals such us dairy cows and dairy goats; acetate is important for milk fat synthesis,

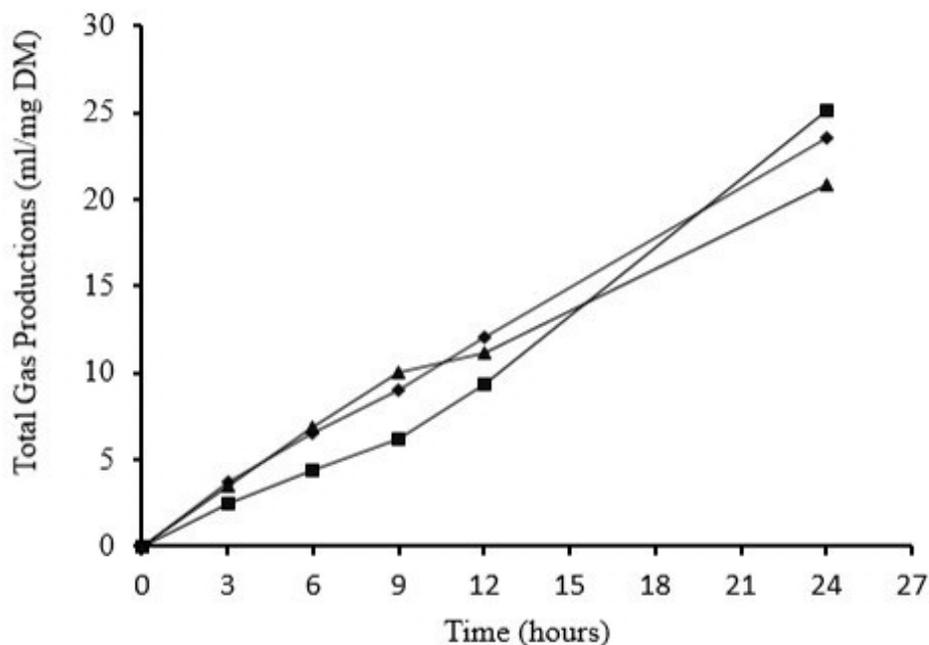


Figure 2. Effect of Different Levels of *Indigofera* in Experimental Concentrate Feed on Total Gas Production.

- ◆ R1, concentrate feed without *I. zollingeriana* (control);
- R2, concentrate feed containing 20% DM *I. zollingeriana*;
- ▲ R3, concentrate feed containing 40% DM *I. zollingeriana*.

Table 3. The Partial VFA and NH₃ Concentration of Experimental Concentrate Feed

VFA Partial (mM)	R1	R2	R3
Acetic acid	13.6±1.5	13.6±0.6	14.4±0.3
Propionic acid	4.40±0.4	3.76±0.1	4.40±0.4
Butyric acid	1.41±0.4	1.42±0.2	1.44±0.4
Iso-butyric acid	0.95±0.1	1.02±0.2	1.02±0.1
n-Valeric acid	0.34±0.1	0.34±0.1	0.33±0.1
n-Caproic acid	0.09±0.03	0.09±0.03	0.04±0.07
A-P ratio	3.08±0.08 ^b	3.62±0.27 ^a	3.27±0.29 ^{ab}
NH ₃ (mM)	4.21±0.1 ^b	3.87±0.1 ^c	5.23±0.2 ^a

^{a,b,c} Within rows, means followed by different superscripts are different at $P<0.05$; A-P ratio: Acetic acid Propionic acid ratio; R1, concentrate feed without *I. zollingeriana* (control); R2, concentrate feed containing 20% DM *I. zollingeriana*; R3, concentrate feed containing 40% DM *I. zollingeriana*.

whereas propionate is required for milk production (Sutton *et al.*, 2003; Serment *et al.*, 2011). The A:P ratio of R1, R2 and R3 in this study were 3.08, 3.62, and 3.29, respectively. The A:P ratio of R2 was higher ($P<0.05$) than R1, while R3 had similar value with R1 and R2. The lower of A:P ratio on R1 due to the absence of forage such as *I. zollingeriana*. The fermentation of forages in ruminal system is produce more acetate than propionate. The inclusion of *I. zollingeriana* increased A:P ratio on R2. The R3 concentrate showed similar A:P ratio to other concentrates, although it had the highest inclusion level of *I. zollingeriana*. It caused that *I. zollingeriana* contain secondary compound such us tannins and saponins which may affect the process of fermentation in the rumen. These substances may negatively influence digestion processes in ruminants by acting as anti-nutritional factors when present at high concentration, but low to moderate concentration of these compounds are desirable in order to positively modulate rumen fermentation for higher animal productivity and lower environmental pollution (Suharlina *et al.*, 2016). The concentrations of rumen NH₃ of each concentrates feed showed significantly differences ($P<0.05$). The concentration of rumen NH₃ of R2 was lower than R1 and R3 ($P<0.05$) even though the IVCPD of R2 was higher than R1. The lower concentration of rumen NH₃ of R2 could have been due to the rapid utilization of nitrogenous compound released from the legume by rumen

microbes in the presence of available energy and other nutrients (Khandaker *et al.*, 1998). Furthermore, the presence of tannins in the plant may also contribute to such lower rumen NH₃ as has been proven by a meta-analysis study conducted by Jayanegara and Palupi (2010).

CONCLUSION

The concentrate feed containing 20% of *I. zollingeriana* (R2) showed the highest *in vitro* dry and organic matter, and crude protein digestibility values in goat. It could be concluded that the inclusion of *I. zollingeriana* as concentrate feed ingredient at 20% maximum level.

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