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# Research Article
# Nutritional Evaluation of Dairy Goat Rations Containing *Indigofera zollingeriana* by Using *in vitro* Rumen Fermentation Technique (RUSITEC)

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## Abstract
This study aimed to evaluate nutritional quality of total mixed rations containing *Indigofera zollingeriana* for dairy goats by using *in vitro* rumen fermentation technique (RUSITEC). Four rations with different levels of *I. zollingeriana* were tested, i.e., 20% of dry matter (R1), 40% (R2), 60% (R3) and 80% (R4). Each ration was repeated three times by following a randomized complete block design in which different batch of rumen fluid served as the block. Variables observed included chemical composition, *In Vitro* Dry Matter Digestibility (IVDMD) and *In Vitro* Organic Matter Digestibility (IVOMD), methane emission, Volatile Fatty Acid (VFA) profiles and protozoa population. Results revealed that R4 contained significantly higher crude protein content than that of R1-R3 ($p<0.05$). Rations containing higher proportions of *I. zollingeriana* (60 and 80% dry matter) had significantly higher IVDMD and IVOMD as compared to their lower proportions (20 and 40% dry matter, $p<0.05$). Methane concentration was lowest in ration containing the highest proportion of *I. zollingeriana* and it was accompanied with the lowest protozoa population. It can be concluded that higher inclusion levels of *I. zollingeriana* in rations improved their nutritional values while decreasing methane emission as a main greenhouse gas.

**Key words:** Dairy goat, *Indigofera zollingeriana*, *in vitro*, nutritional quality, IVDMD, IVOMD

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

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

## INTRODUCTION

Although milk production worldwide is dominated from dairy cow, some other species such as buffalo and goat also contribute to the supply of milk and they are significantly produced in some countries. With regard to goat milk, South Asian countries including India, Bangladesh and Pakistan are among the main producers of goat milk (Pal *et al*., 2011). Goat milk is believed to possess a number of advantages over cow milk, it provides more balance nutritional source for infants and can be used as a medicinal food due to its higher concentrations of health promoting components, which may be related to feeding pattern of goats in consuming large proportions of natural browses (Silanikove *et al*., 2010). It is also rich in beneficial fatty acid contents such as omega 3 fatty acids and trans-9 and trans-11 conjugated linoleic acids (Zervas and Tsiplakou, 2013; Chilliard *et al*., 2014). Thus, it is not surprising that the price of goat milk is much higher than that of cow milk and is considered as a premium food.

Dairy goats are important component of livestock and play a significant role in the socio-economic structure of rural poor. They are sometimes fed with poor quality feed given by small-holder farmers, which further lead to low milk production. Feeding of dairy goats with grasses is not sufficient to meet the nutritional requirement of high-producing dairy goats due to the low protein content in many grass species, particularly tropical grasses (Poppi and McLennan, 1995) in which the protein contents are normally ranging between 4-9% from Dry Matter (DM), while the protein requirement of dairy goat ration is 14% DM or more. Providing concentrate to fulfill the nutritional requirements of dairy goats may considerably improve their milk production (Bala *et al*., 2009; Giger-Reverdin *et al*., 2014; Gomes *et al*., 2015) but the price is unfortunately expensive and thus may not affordable for small-holder farmers. An alternative solution to reduce the use of concentrate for dairy goats is through utilization of 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). Another potential legume for feeding of dairy goats is a forage namely *Indigofera zollingeriana*. The plant has a rapid growth in the defoliation interval of 60 days with a production of 51 t $ha^{-1}$ $year^{-1}$ (Abdullah, 2010). *Indigofera zollingeriana* is very adaptive to low fertility rates, easy on maintenance and low prize and high seed production potential throughout the season (Abdullah and Suharlina, 2010).

Since the plant has been rarely investigated so far, therefore, the purpose of this study was to evaluate nutrient contents, rumen fermentation and methane emission of dairy goat rations when supplemented with different levels of *I. zollingeriana* as performed by using RUSITEC.

## MATERIALS AND METHODS

**Sample preparation:** All experimental procedures in the present study had been approved by the Faculty of Animal Science, Bogor Agricultural University, Indonesia. Leaf and twig samples of *Indigofera 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. Approximately, 3 kg dry matter of leaf and twig samples were collected from each plot. The samples were then air-dried in a greenhouse for 3 h and subsequently oven-dried at 70°C for another 3 h. The materials were ground to pass a 2 mm sieve size and mixed homogeneously for further *in vitro* RUSITEC incubation and chemical analyses.

***In vitro* incubation:** Prior to *in vitro* incubation, the ground *I. zollingeriana* samples were formulated into experimental rations at varying proportions, i.e., 20, 40, 60 and 80% DM and named as R1, R2, R3 and R4, respectively (Table 1). Other feedstuffs used to formulate the rations were napier grass, rice bran, ground corn, soybean meal, $CaCO_3$, NaCl and premix. The *in vitro* incubation was performed by using RUSITEC apparatus equipped with eight vessels (Sanshin, Tokyo, Japan) as described by Kajikawa *et al*. (2003). Experiment was performed in three periods and each period lasted for 10 days, comprised of 7 days adaptation period and 3 days data collection period. Rumen fluid was obtained from three lactating Etawa dairy goats and drawn before morning feeding through stomach tube technique. Rumen fluid was

Table 1: Feed ingredients of the experimental rations (in dry matter percentage)

| Item | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Indigofera | 20 | 40 | 60 | 80 |
| Napier grass | 45 | 23 | 5 | 6 |
| Rice bran | 26 | 5 | 3 | 2 |
| Corn | 2 | 30 | 30 | 10 |
| Soybean meal | 5 | 0 | 0 | 0 |
| $CaCO_3$ | 1 | 1 | 1 | 1 |
| NaCl | 0.5 | 0.5 | 0.5 | 0.5 |
| Premix | 0.5 | 0.5 | 0.5 | 0.5 |

R1: Ration containing 20% DM *Indigofera zollingeriana*, R2: Ration containing 40% DM *Indigofera zollingeriana*, R3: Ration containing 60% DM *Indigofera zollingeriana* and R4: Ration containing 80% DM *Indigofera zollingeriana*

filtered through four layers of gauze before being used. An amount of 15 g DM of each ration was put into a nylon bag (100 µm pore size). Each vessel was filled with 400 mL strained rumen fluid and 400 mL McDougall buffer and the ration was also put into the vessel. About 70 g of solid rumen content was put into a nylon bag, serving as microbial inoculum for the initial 24 h. After that, the samples were incubated for every 48 h and replaced with similar samples throughout the experimental period. During the data collection period, fluid samples were measured for VFA profiles and protozoa count and feed residual samples in the nylon bags were determined for degradability values (both IVDMD and IVOMD). Fermentation gas was collected in a gas-proof bag for each vessel and measured for methane concentration by using an infrared methane analyzer (Sable System MA-10a, USA).

**Pre and post-incubation analyses:** Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE) and Crude Fiber (CF) composition of the rations were determined by proximate analysis (AOAC., 2000). Feed residuals were analyzed for DM and OM to obtain IVDMD and IVOMD values, respectively. Analysis of VFA was performed by injecting fermentation fluid samples into a gas chromatograph by following the description of Jayanegara *et al.* (2015). Protozoa population was counted by using a burker counting chamber (Blau Brand, Wertheim, Germany).

**Statistical analysis:** The data obtained were analyzed using analysis of variance (ANOVA) with the following statistical model:

$$Y_{ij} = \mu+\tau_i+\beta_j+\varepsilon_{ij}$$

where, $Y_{ij}$ is the observed value, $\mu$ is the overall mean, $\tau_i$ is the effects of rations containing varying levels of *I. zollingeriana* (fixed effect), $\beta_j$ is the block effect (fixed effect) and $\varepsilon_{ij}$ is the random residual error. If there was a significant difference among the rations for a particular parameter at $p<0.05$, the analysis was continued a *post hoc* test i.e., Least Significance Difference (LSD) test. All the statistical analyses were performed by using SPSS statistical software version 20 (IBM Corp., Armonk, New York, USA).

## RESULTS AND DISCUSSION

The CP content of R4 was significantly higher than that of R1, R2 and R3 ($p<0.05$, Table 2). Rations of R1 and R2 had significantly higher CF contents as compared to R3 ($p<0.05$). Other chemical constituents were similar among the four rations. With regard to CP and CF contents, R3 and R4 are recommended to satisfy dairy goats nutrient requirements for optimal milk production due to high CP and low CF. Higher inclusion levels of *I. zollingeriana* apparently improve nutritional properties of rations especially the CP contents since the plant contained high level of CP i.e., 29.2% DM. Similar to the finding of high CP content in *I. zollingeriana*, Tscherning *et al.* (2006) reported that the plant species had 23.8% DM of CP. Other *Indigofera* species such as *I. arrecta* and *I. tinctoria* were also reported to have high CP contents i.e., 23.1 and 26.0% DM, respectively (Kaitho *et al.*, 1997; Bhatta *et al.*, 2013).

Table 2: Chemical composition of the experimental rations (in dry matter percentage)

| Item | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Dry matter | 95.70 | 94.80 | 95.70 | 94.80 |
| Ash | 7.43 | 10.30 | 7.79 | 8.83 |
| Crude protein | 15.30$^{a}$ | 16.50$^{a}$ | 17.90$^{a}$ | 21.50$^{b}$ |
| Crude fiber | 19.80$^{b}$ | 17.50$^{b}$ | 14.20$^{a}$ | 16.20$^{ab}$ |
| Ether extract | 2.60 | 3.99 | 2.41 | 3.26 |

$^{a,b}$: Different superscripts within the same row are significantly different at $p<0.05$, R1: Ration containing 20% DM *Indigofera zollingeriana*, R2: Ration containing 40% DM *Indigofera zollingeriana*, R3: Ration containing 60% DM *Indigofera zollingeriana* and R4: Ration containing 80% DM *Indigofera zollingeriana*

Table 3: Degradability, Volatile Fatty Acid (VFA) profiles and protozoa population of the experimental rations

| Item | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| **Degradability (%)** | | | | |
| Dry matter | 60.20$^{a}$ | 73.80$^{b}$ | 76.80$^{b}$ | 76.00$^{b}$ |
| Organic matter | 58.40$^{a}$ | 72.60$^{b}$ | 75.10$^{b}$ | 72.70$^{b}$ |
| **VFA (mmol L$^{-1}$)** | | | | |
| Total | 80.30$^{b}$ | 66.30$^{a}$ | 85.80$^{c}$ | 89.30$^{d}$ |
| Acetate | 56.60$^{b}$ | 44.20$^{a}$ | 58.80$^{b}$ | 61.30$^{c}$ |
| Propionate | 14.90$^{a}$ | 14.90$^{a}$ | 17.10$^{b}$ | 17.90$^{b}$ |
| Butyrate | 7.74$^{a}$ | 6.38$^{a}$ | 8.82$^{b}$ | 8.70$^{b}$ |
| Valerate | 1.09 | 0.79 | 1.04 | 1.43 |
| Protozoa ($10^4$ mL$^{-1}$) | 8.73$^{c}$ | 4.03$^{b}$ | 3.60$^{b}$ | 2.00$^{a}$ |

$^{a,b,c}$: Different superscripts within the same row are significantly different at $p<0.05$, R1: Ration containing 20% DM *Indigofera zollingeriana*, R2: Ration containing 40% DM *Indigofera zollingeriana*, R3: Ration containing 60% DM *Indigofera zollingeriana* and R4: Ration containing 80% DM *Indigofera zollingeriana*

The IVDMD and IVOMD values of R1 were lower than those of R2-R4 ($p<0.05$, Table 3). At low level of *I. zollingeriana* proportion as in R1, the ration contained high level of fiber at simultaneously low level of protein. Consistent with the results obtained in the present study, high fiber and low protein have been observed to be negatively correlated with digestibility coefficients in a number of domestic ruminant species i.e., sheep, goat, cattle and buffalo (Riaz *et al.*, 2014). It is well accepted that protein is more digestible as compared to fiber by rumen microbes. Since, *I. zollingeriana* is characterized by its high protein and low fiber contents, increasing amount of the plant within a ration leads to an increase in its digestibility. The study of Kaitho *et al.* (1997) revealed that degradability of

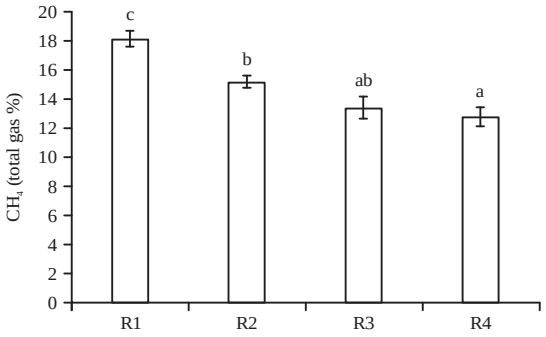


Fig. 1: Methane ($CH_4$) emission the experimental rations. Error bars indicate standard error values for the rations, different letters above the error bars are significantly different at $p<0.05$, R1: Ration containing 20% DM *Indigofera zollingeriana*, R2: Ration containing 40% DM *Indigofera zollingeriana*, R3: Ration containing 60% DM *Indigofera zollingeriana* and R4: Ration containing 80% DM *Indigofera zollingeriana* and DM: Dry matter

*Indigofera arrecta* measured at 24 h after incubation was higher than other commonly used high-protein legumes such as *Calliandra calothyrsus*, *Flemingia macrophylla*, *Gliricidia sepium* and *Leucaena leucocephala*. Further, increasing proportion of *I. zollingeriana* over *C. calothyrsus* increased the rate of *in vitro* gas production and apparently degraded N (Tscherning *et al*., 2006). These results may suggest the potentiality of Indigofera as a protein supplement in ruminant diets including those of dairy goats.

Production of total VFA was in line with IVDMD and IVOMD, in which higher proportion of *I. zollingeriana* led to an increase in total VFA production, especially the acetate and propionate (except for R2). Rumen VFA is produced as a result of microbial degradation and fermentation on feed entering the rumen. Thus it is unsurprising that VFA is positively correlated with feed degradability. Both acetate and propionate are required for milk production in dairy animals such as dairy cows and dairy goats; acetate is important for milk fat synthesis, whereas propionate is required for milk production (Sutton *et al*., 2003; Serment *et al*., 2011).

*In vitro* methane emission obtained in this study ranged from 12.8-18.2% from total gas, being highest in R1 and lowest in R4 (Fig. 1). The figure indicated that higher proportion of *I. zollingeriana* in diet led to a lower methane emission. This suggests that the plant is beneficial not only in term of improving animal production, but it also contributes to environmental conservation. Methane is considered as a main greenhouse gas after carbon dioxide although its capacity to retain heat is more than 20 folds than that of carbon dioxide. Accumulation of these greenhouse gas contribute to global warming and increase the earth's temperature (Moss *et al*., 2000; Hristov *et al*., 2013). Methanogenesis in the rumen occurs due to a reduction of carbon dioxide and hydrogen by the action of archaea methanogen (Morgavi *et al*., 2010). Part of the methanogen is living in symbiosis with protozoa especially on the surface of the fauna (Tokura *et al*., 1997; Xia *et al*., 2014), methanogen gets benefit from protozoa through inter-species hydrogen transfer. Thus among the strategies to mitigate ruminal methane emission are: (1) Decreasing population and/or activity of methanogen, (2) Lowering the utilization of carbon dioxide and hydrogen by methanogen and (3) Defaunation of protozoa (Hegarty, 1999).

Lower methane emission with increasing proportion of *I. zollingeriana* in ration is partly due to its lower fiber content. Lower fiber is associated with lower acetate to propionate (A:P) ratio since fiber fermentation leads to more acetate formation rather than that of propionate. In the present study, A:P ratio in R1 was 3.80 and decreased to 3.42 in R4, this supports the above explanation. Other explanation of lower methane emission by the plant was due to lower protozoa population in ration with high *I. zollingeriana* proportion as can be seen in Table 3. Protozoa population decreased by 53.8, 58.8 and 77.1% in R2, R3 and R4, respectively, in comparison with R1 ($p<0.05$). Anti-protozoa effect of *I. zollingeriana* might be due to the presence of secondary compounds in the plant. It was analyzed that the plant used contained tannins and saponins i.e., 2.9 g $kg^{-1}$ and 2.6 mg $kg^{-1}$ DM, respectively. Tannins and saponins have been known to possess anti-protozoa effects and may be used as defaunating agents (Wang *et al*., 2009; Bhatta *et al*., 2012; Jayanegara *et al*., 2014). Such amounts of tannins and saponins in *I. zollingeriana* are considered to be relatively low in comparison to other plants (Jayanegara *et al*., 2012, 2014). These substances may negatively influence digestion processes in ruminants by acting as anti-nutritive factors when present at high concentrations. Low to moderate concentrations of these compounds are desirable in order to positively modulate rumen fermentation for higher animal productivity and lower environmental pollution.

## CONCLUSION

Inclusion of *I. zollingeriana* improved nutritional value of dairy goat ration by increasing its crude protein content as well as its digestibility. Together with excellent agronomy characteristics of the plant, *I. zollingeriana* is a potent legume for supporting optimum production of dairy goat. It has been proven not only beneficial in term of animal production, but it also advantageous with regard to environmental conservation by mitigating enteric methane emission.

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