The Inclusion Effects of *Indigofera zollingeriana* in Oil Palm Fronds Based Diet on Rumen Fermentation Kinetics and Microbial Yields *In Vitro*

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ABSTRACT

Oil palm fronds (OPF) cannot be used as a single feed for ruminants because its low rumen degradability due to low crude protein (CP) and energy content. The aim of this study was to investigate the inclusion effect of indigofera into OPF ration on rumen fermentation kinetics and microbial biomass production. The OPF and indigofera were milled through a 1 mm screen. Samples from individual OPF and indigofera and their combinations of 0.75:0.25, 0.50:0.50 and 0.25:0.75 (DM basis) were accurately weighed (1.0 g ± 0.0010 fresh weight in total) directly into Duran bottles. Samples were incubated in buffered rumen fluid at 39 °C for 72 h. Gas production was measured at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72 h post incubation. Cumulative gas volume (three duplicate runs) were fitted to the exponential equation \[ y = a + b \left(1-e^{-x}\right) \]. At 8 h, microbial biomass (MBO) and microbial nitrogen (MN) were estimated. Data were subjected to ANOVA based on CRD, and data showing significance between treatment means \((P \leq 0.05)\) were separated with orthogonal polynomial contrasts. The contrasts used were linear (L), quadratic (Q), or deviated from both L and Q (Dev). Significance was accepted at \( P \leq 0.01 \). There were highly significant effects \((P<0.01)\) of feed mixtures on gas production profiles and microbial yields in the rumen. Increasing indigofera levels in the diet increased (quadratic effect, \(P<0.01\)) the gas asymptote and fermentation rate of the mixtures. Both MBO and MN had also the quadratic effects for the mixtures of OPF and Indigofera where the highest microbial yield was found in the combination of 25% OPF + 75% indigofera. It can be concluded from the present experiment that the optimal combination of OPF and indigofera was 25% and 75%, respectively.

Keywords: Oil palm fronds, *Indigofera zollingeriana*, Fermentation, Microbial yields, Rumen

INTRODUCTION

OPF cannot be used as a single feed for ruminants because its low rumen degradability due to low crude protein (CP) and energy content and high in fibre contents (Dahlan, 2000). It is usually combined with commercial concentrate feeds but the price is unfortunately expensive resulting a low animal farm efficiency. An alternative solution to reduce the use of concentrate is through utilization of legume such as *Indigofera zollingeriana*. It is highly nutritional having a CP content of 27-31% and high dry matter digestibility (78%). The plant has a rapid growth in the dofoliation 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 price (Abdullah and Suharlina, 2010). The use of indigofera as feed for goats is popular (Suharlina et al., 2016) but its utilization is still limited for beef cattle. The
aim of this study was to investigate the interaction effects between OPF and indigofera on rumen fermentation kinetics and microbial biomass production.

**MATERIALS AND METHODS**

**Sample preparation.** Fresh OPF were chopped to 1–2 cm lengths and air dried at ambient temperature (30–35 °C). Leaf and twig samples of *I. zollingeriana* were collected from the Faculty of Animal Science Farm, University of Jambi, Jambi, Indonesia. The samples were obtained from five plants growing at the same area. Approximately 500 g DM leaves and twigs were collected from each plant. The samples were air-dried in a greenhouse. Both dried OPF and *I. zollingeriana* samples were hammer milled (Christy Norris Ltd., UK) to pass through a 1 mm screen. Representative sub-samples of each raw material were analysed for DM content and total ash. Mixtures were made containing OPF and *I. zollingeriana* in combinations of 25 : 75, 50 : 50 and 75 : 25.

**In vitro incubation.** Samples from each feedstuff and their combinations of 0.75:0.25, 0.50:0.50 and 0.25:0.75 (DM basis) were accurately weighed (1.0 g ± 0.0010 fresh weight in total) directly into Duran bottles. Duplicate bottles for each combination and individual feed were prepared, one was terminated at 8h and the second bottle was stopped at 72 h and both were used for OMD. In addition, one blank for each time period and each parameter measured was also prepared making in total 12 incubation bottles prepared in each run. The above was repeated on three separate occasions. Similar preparation was done for each feed mixture. Samples were incubated in buffered rumen fluid as described by Mauricio *et al.* (1999) at 39 °C for 72 h. Gas production was measured at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72 h post incubation. At the end of each incubation period, the fermentation was stopped by addition of 15 ml 1 M orthophosphoric acid. Residues from 8 h incubation were recovered for apparent and true OMD determination, both were used for the calculation of microbial biomass production (MBO) (Blümmel *et al.*, 1997) and microbial nitrogen (MN) (Demeyer, 1991). Cumulative gas volume (three duplicate runs) were fitted to the exponential equation

\[ y = a + b (1 - e^{-dt}) \]

of McDonald (1981).

**Statistical analysis.** Data were subjected to analysis of variance (ANOVA) based on a completely randomized design (CRD), and data showing significance between treatment means \((P \leq 0.05)\) were separated with orthogonal polynomial contrasts (SAS Institute, 2008). The contrasts used were linear (L), quadratic (Q), or deviated from both L and Q (Dev). Significance was accepted at \(P \leq 0.01\).

**RESULTS AND DISCUSSION**

**Gas production profiles.** There were highly significant effects \((P<0.01)\) of feed mixtures on gas production profiles. Increasing indigofera levels in the diet increased (quadratic effect, \(P<0.01\)) the gas asymptote and fermentation rate of the mixtures (Figure 1).
Microbial yields. There were highly significant effects (P<0.01) of feed mixtures on microbial yields in the rumen (Table 1). Both MBO and MN had also the quadratic effects for the mixtures of OPF and Indigofera where the highest microbial yield was found in the combination of 25% OPF + 75% indigofera.

**Table 1.** Microbial yields (g kg⁻¹OM) and kinetics fermentation of feeds and simple mixtures

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Indigofera : OPF ratio (%)</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 : 100</td>
<td>25 : 75</td>
</tr>
<tr>
<td>a + b (ml g⁻¹ OM)</td>
<td>110</td>
<td>147</td>
</tr>
<tr>
<td>c (h⁻¹)</td>
<td>0.020</td>
<td>0.038</td>
</tr>
<tr>
<td>MBO (g kg⁻¹ OM)</td>
<td>102</td>
<td>251</td>
</tr>
<tr>
<td>MN (g kg⁻¹ OM)</td>
<td>9.2</td>
<td>22.6</td>
</tr>
</tbody>
</table>

**P<0.01; NS, non-significant

CONCLUSIONS

The inclusion effects of indigofera into the OPF based diet were non-additive on ruminal gas production and microbial yields. The optimal combination was found on 75% indigofera and 25% OPF. These results are needed to be validated *in vivo.*

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REFERENCES