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Assessment of *in vitro* ruminal fermentation characteristics, methane (CH₄) production, and associative effects between low-quality roughages and protein supplements

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Abstract

To evaluate the *in vitro* fermentation, CH₄ reduction, and associative effects between low-quality roughages and protein feeds, rice straw (RS), German grass (GG), Napier silage (NS), and maize silage (MS) were incubated alone or after replacement by Ipil-ipil or Gliricidia at levels of 10 %, 20 %, 30 %, and 40 %, respectively, using the Hohenheim Gas Test. An amount of 200 mg was incubated with rumen fluid-buffer solution for 72 h to measure gas production (GP) and calculate organic matter digestibility (dOM) and metabolisable energy (ME). Additionally, 120 mg of feed were incubated for 24 h to determine the CH₄ concentration in the GP. Associative effects between feed ingredients were calculated by comparing the measured and estimated values of the composite feed and the estimated values were the weighted mean of the values of fermentation of the individual feeds. The GP₂₄ and rumen fermentation rate increased ($p < 0.05$) as the level of Ipil-ipil added to RS was increased. Increasing the Ipil-ipil level resulted in a gradual increase in dOM and ME ($p < 0.05$) of the mix, reaching maximum values (59.1 % and 7.60 MJ ME/kg DM, respectively) and maximum reductions in CH₄ concentration and CH₄/dOM (6.89 % and 11.7 %, respectively) compared to the control, when Ipil-ipil was added at 40 % DM. When Ipil-ipil was added at 30 % to NS, the same trend was observed for dOM and ME (56.5 %, and 7.55 MJ ME/kg DM, respectively) and CH₄ concentration and CH₄/dOM (5.32 % and 13.3 %, respectively). No significant interaction was observed between Ipil-ipil and good quality roughages like GG and MS. The opposite trend was found for GP₂₄, dOM, ME, and CH₄ production when an increasing level of Gliricidia was added to the roughages. A positive associative effect was obtained for all mixed treatments ($p < 0.05$) and showed a maximum when 40 % Ipil-ipil was added to RS and 30 % Ipil-ipil to NS. In conclusion, based on *in vitro* rumen fermentation, CH₄ production, and associative effects, adding Ipil-ipil to low-quality roughages is superior to Gliricidia and demonstrated promising results and ranked as follows: RS + 40 % > NS + 30 % Ip > MS + 30 % Ip > GG + 10 % Ip.

Keywords: Associative effect, CH₄ emission, Grass, *In vitro* fermentation, Leguminous fodder, Rumen digestion