Comparison of statistical screening methods for methane mitigating properties of tannin-containing plants

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Introduction
There has been a growing interest in the finding of forage plants that are not only good in quality but also are able to mitigate methane (CH$_4$) formation in the rumen. Screenings of plants that possess CH$_4$ mitigating properties have been conducted using different statistical methods, such as using univariate approach (Soliva et al., 2008), bivariate (Bodas et al., 2008) and multivariate (Jayanegara et al., 2011a). However, no studies so far have attempted to address these different methods simultaneously when screening the plants and to compare the results obtained, which then become the main objective of the present study. A particular interest is to observe whether these methods will lead to a different conclusion when screening the plants.

Materials and Methods
A database was constructed from plant samples obtained from various collection sites in different countries, i.e. Indonesia (n = 27 species), Mongolia (n = 14), Switzerland (n = 16) and Germany (n = 3). The data were based on our previous studies (Jayanegara et al., 2009, 2011a, 2011b). All plant samples were oven dried at 50-60°C and ground to pass 1 mm sieve. Samples were determined for their chemical composition. In vitro incubation of the plants was done by using the Hohenheim gas test, incubated at 39°C for 24 h. After the incubation was terminated, total gas production was recorded as an indicator of feed quality and CH$_4$ emission was measured. Univariate screening was based on CH$_4$ ≤ average CH$_4$ across all plants minus 1 × standard deviation. Bivariate screening was based on a two-dimensional plot between total gas and CH$_4$. Multivariate screening was based on the loading and score plots of principal component analysis (PCA), where Eigenvalue ≥ 1.0 was used to extract the principal components (PC) without rotation method. Each plant was encoded by its country of origin, i.e. I, M, S and G for plants obtained from Indonesia, Mongolia, Switzerland and Germany, respectively, and numbered according to alphabetical order within country.
Results

Across all plants, the average CH$_4$ emission after 24 h *in vitro* incubation was 137 (±32) ml/l gas. Therefore, for the screening based on univariate approach, the promising plants were those which produced CH$_4$ ≤ 105 ml/l gas. These plants were (ordered from lowest to highest CH$_4$) *Bergenia crassifolia* root (M4), *Peltiphyllum peltatum* (G1), *S. mahagoni* (I27), *A. villosa* (I2), *Eugenia aqua* (I11), *Myristica fragrans* (I21), *B. crassifolia* leaf (M3), *Pithecellobium jiringa* (I24), *R. typhina* (G2) and *C. hirta* (I8). Screening based on bivariate approach is presented in Figure 1. It appeared that, generally, plants possessed low CH$_4$ production had low quality, i.e. below the average of total gas production. Among plants that produced low CH$_4$, only *R. typhina* (G2) resulted in a high total gas production, i.e. 43 ml/200 mg DM. *Salsola laricifolia* (M8), *Hibiscus tiliaceus* (I12) and *Canna indica* (I6) had simultaneously high CH$_4$ emission and low total gas.

![Figure 1: Bivariate approach for screening plants based on total gas produced and CH$_4$ emission](image)

Vertical and horizontal lines show the average of total gas and CH$_4$ across all plants, respectively. I, plant samples from Indonesia (□); M, plant samples from Mongolia (●); S, plant samples from Switzerland (▲); G, plant samples from Germany (◊).

In relation to screening plants based on the multivariate approach, the PC1 and PC2 explained 42.3% and 23.3% of the total variation, respectively. Based on the PCA loading plot, it is clear that all phenolic fractions were in the opposite direction with CH$_4$ and total gas production; variable CH$_4$ and total gas production appeared to be in close direction. However, less clear relationships
were observed between the other chemical constituents (CP, EE, NDF and ADF) and the \textit{in vitro} fermentation variables. Based on the PCA score plot, some plants were clustered together in reverse direction to that of CH$_4$. These plants were \textit{B. crassifolia} root (M4), \textit{B. crassifolia} leaf (M3), \textit{S. mahagoni} (I27), \textit{C. hirta} (I8), \textit{P. peltatum} (G1), \textit{A. villosa} (I2) and \textit{R. typhina} (G2).

**Conclusion**

Plant phenolic contents may be used as an indicator for CH$_4$ mitigating properties of the plants, although they are also reversely related to the forage quality. Different approaches, i.e. univariate, bivariate and multivariate (PCA) for screening of tannin-containing plants in relation to their ruminal CH$_4$ emission \textit{in vitro} led to similar results to certain extent. However, screening based on univariate approach has to be conducted cautiously since it does not consider the forage quality, which is also quite an important factor. The bivariate and multivariate approaches may overcome such problem by considering the forage quality related variable. Although almost all tannin-containing plants that possessed low CH$_4$ emission had low quality in the present study, still, a good quality plant could be obtained, i.e. \textit{R. typhina} (G2). This is possible since great variation have occurred in the structure and activity of each plant phenolics. The searching of plants with both desired characteristics is subjected to further research.

**References**


