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^{o}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^{o}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 $CH_4 \leq$ 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 $CH_4 \leq 105$ 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 aquea* (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.

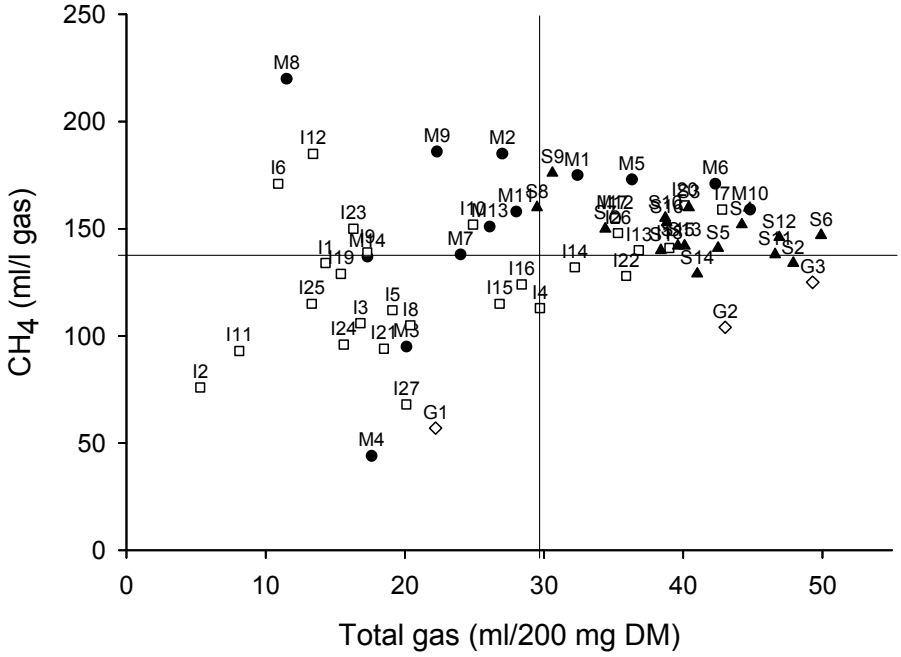

Figure 1: Bivariate approach for screening plants based on total gas produced and CH_4 emission
Vertical and horizontal lines show the average of total gas and CH_4 across all plants, respectively
I, plant samples from Indonesia (□); M, plant samples from Mongolia (●); S, plant samples from Switzerland (▲); 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 *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 *B. crassifolia* root (M4), *B. crassifolia* leaf (M3), *S. mahagoni* (I27), *C. hirta* (I8), *P. peltatum* (G1), *A. villosa* (I2) and *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 *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. *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

Bodas, R., Lopez, S., Fernandez, M., Garcia-Gonzalez, R., Rodriguez, A.B., Wallace, R.J. and Gonzalez, J.S. (2008): *In vitro* screening of the potential of numerous plant species as antimethanogenic feed additives for ruminants. *Anim. Feed Sci. Technol.* **145**: 245–258

Jayanegara, A., Togtokhbayar, N., Makkar, H.P.S. and Becker, K. (2009): Tannins determined by various methods as predictors of methane production reduction potential of plants by an *in vitro* rumen fermentation system. *Anim. Feed Sci. Technol.* **150**: 230–237

Jayanegara, A., Wina, E., Soliva, C.R., Marquardt, S., Kreuzer, M. and Leiber, F. (2011a): Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. *Anim. Feed Sci. Technol.* **163**: 231–243

Jayanegara, A., Marquardt, S., Kreuzer, M. and Leiber, F. (2011b): Nutrient and energy content, *in vitro* ruminal fermentation characteristics and methanogenic potential of alpine forage plant species during early summer. *J. Sci. Food Agric.* (in press)

Soliva, C.R., Zeleke, A.B., Clement, C., Hess, H.D., Fievez, V. and Kreuzer, M. (2008): *In vitro* screening of various tropical foliages, seeds, fruits and medicinal plants for low methane and high ammonia generating potentials in the rumen. *Anim. Feed Sci. Technol.* **147**: 53 – 71