THE USE OF PRINCIPAL COMPONENT ANALYSIS IN IDENTIFYING AND INTEGRATING VARIABLES RELATED TO FORAGE QUALITY AND METHANE PRODUCTION

A. Jayanegara [1], H. P.S. Makkar [2] and K. Becker [2]
[1] Department of Nutrition and Feed Technology, Faculty of Animal Science
Bogor Agricultural University, Jl. Agatis Kampus IPB Darmaga Bogor 16680 – Indonesia
[2] Department of Animal Production in the Tropics and Subtropics (480b)
University of Hohenheim, Fruwirthstrasse 12, 70593 Stuttgart, Germany
Corresponding E-mail: anu_jayanegara@yahoo.com

Received Sept 16, 2009; Accepted November 19, 2009

ABSTRACT

This research was aimed to explore the use of multivariate statistics i.e. principal component analysis (PCA) in identifying and integrating variables related to forage quality and ruminal methane production, and in classifying forage species into both characteristics. Seventeen plants were used as a database for the above mentioned purposes. Plant samples were determined for their chemical composition, cumulative gas production (represents the nutrient degradation) and methane production after 24 hours of fermentation period using the Hohenheim gas test. The results showed that the PCA could clearly identify factors related to forage quality and methane production and separated them into different principal components (PC). The obtained PC1 was related to methane production and substantially influenced positively by crude protein, NDF, ADF (positive), total phenols, total tannins, condensed tannins and tannin activity (negative). On the other hand, the obtained PC2 was related to cumulative gas production (forage quality) and substantially influenced by crude protein (positive), NDF, ADF and condensed tannins (negative). Classification and screening of forages that have high quality and low methane production are possible using the PCA technique. *Rhenum undulatum*, *Peltiphyllum peltatum* and *Rhus typhina* were found to have such desired characteristics.

Key words: PCA, multivariate, gas, quality, methane, forage

INTRODUCTION

Productivity of ruminants is affected by the quality of forage consumed. Several factors related to quality of forage are energy content, fiber, protein, anti-nutritional compounds and the digestibility of forage in gastro-intestinal tract. On the other hand, the concern on global warming due to accumulation of green-house gases has increased in the last decades, especially for CO_2 (carbon dioxide), CH_4 (methane) dan N_2O (dinitrogen oxide). Ruminants are among major contributors of methane accumulation in the atmosphere which is generated from microbial fermentation in the rumen. Methane produced by ruminants contributes to 95% of the anthropogenic total methane and 18% of the total green-house gases in the atmosphere (Kreuzer & Soliva, 2008). Methane emission is not only associated with environmental problem but also reflects the loss of energy from animal and, hence, can not be utilized for production purpose. Approximately 6–10% of the gross energy of the feed consumed by ruminants is lost as methane (Jayanegara, 2008a).

The above-mentioned factors have led to the exploration of forages that not only good in quality but also able to reduce methane formation in the rumen. Statistical evaluation on forage quality and ruminal methane emission so far is limited to univariate analysis, especially the analysis of variance (ANOVA) and followed by post-hoc tests after ANOVA such as Fischer's test (LSD, least significant difference), Duncan's test and Tukey's test (honestly significant difference). Orthogonal contrast and polynomial orthogonal have also been used for analyzing structured treatments, both structured qualitatively and quantitatively. However, the use of multivariate statistics for data exploration that involved many variables simultaneously was scarcely done.

This paper was aimed at applying a method of multivariate statistics i.e. principal component analysis (PCA) in identifying and integrating

variables related to forage quality and methane emission. It was expected that by the use of PCA, variables determining forage quality and methane emission can be identified simultaneously as well as their relationships. Moreover, PCA technique is possible to be used for classification of forages according to their intrinsic characteristics. In this case, the author was interested to obtain forage species possessing high quality and low methane formation in the rumen.

MATERIALS AND METHODS

Forage and Data Sources

The data used in the present study were from Jayanegara *et al.* (2009a) with total of 17 forage species (Table 1). All samples except *Salix alba*, *Rhus typhina* and *Peltiphyllum peltatum* were collected from Mongolia. The plants collected from Mongolia were used locally in the region of production as medicinal plants. *Salix alba* and *R. typhina* were collected from the Botanical Garden of the University of Hohenheim in Stuttgart (Germany) and *P. peltatum* was provided by Dr. John Wallace from the Rowett Research Institute in Aberdeen (UK). Prior to chemical composition analysis, all forages were air-dried, ground and passed to a 1 mm sieve.

Chemical Composition Analysis of Forage

Forage samples were analyzed for their dry matter (DM) content using oven at 100 ºC for 16 h. Crude protein (CP) and ether extract (EE) were analyzed according to AOAC (1990), whereas neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Van Soest *et al.* (1991). Total phenols (TP) and total tannins (TT) were determined using Folin-Ciocalteu method, while condensed tannins (CT) using the butanol-HCl-Fe method (Makkar, 2003). Total phenols and total tannins were expressed as tannic acid equivalent, while condensed tannins was expressed as leucocyanidin equivalent. Biological activity of tannins was determined based on the *in vitro* gas production increase with or without polyethylene glycol (PEG) addition

Table 1. Forage database used in principal component analysis (PCA)

Forage species	CP	EE	NDF	ADF	TP	TT	CT	TA	Gas	CH_4
			---------- % DM ----------					%	ml/200mg	% gas
Artemisia frigida[1]	9	1.4	56.1	42.7	2.2	0.4	0.08	0	32.4	17.5
Tanacetum vulgare	15.2	3.5	46.2	41.6	3.8	0.6	0.04	0	28	15.8
Iris lactea	10.3	1.5	49.5	43.6	3.8	2.9	1.43	0	42.3	17.1
Rhenum undulatum	10.5	0.6	22.8	16.9	7.7	5.6	0.71	92.7	24	13.8
Thymus gobicus	10.4	2.3	54.1	44.3	3.6	1.2	0.02	33.4	26.1	15.1
Seratula centauroides	11.5	3.5	62.6	50.9	5.8	4.6	0.05	7.2	22.3	18.6
Stellera chamaejasme	13.4	2.8	39.1	31.2	4.4	1.5	0.03	0.4	44.8	15.9
Taraxacum officinale	24.6	2.6	31.8	27.1	2.3	0.7	0.03	11	35.1	15.5
Delphinum elatum	13.6	2.2	51.5	38.7	2.2	0.9	0.07	4.1	36.3	17.3
Artemisia frigida[2]	15.6	1.9	54.8	43.1	2	0.5	0.03	4.3	27	18.5
Vaccinium vitis idea	6.3	3.3	47.6	35.4	24.3	17.5	14.9	96.3	17.3	13.7
Salsola laricifolia	9.5	1.2	57.4	38	6.5	3.2	2.8	17.7	11.5	22
Bergenia	6.3	2.4	26.1	19	32	17.8	1.41	169.8	20.1	9.5
Bergenia[x]	3.3	0.8	22.6	19.7	30.9	16.5	3.31	204.7	17.6	4.4
Salix alba	16.9	1.7	32.2	18.5	5.7	3.6	1.45	0.7	49.3	12.5
Rhus typhina	14	5.6	22	17.4	22.2	20.9	0.08	45.7	43	10.4
Peltiphyllum peltatum	11.3	2	19.1	18.3	20	14.7	1.57	122.6	22.2	5.7
SEM									1.85	0.98
LSD									2.11	1.14

CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; TP, total phenols; TT, total tanins; CT, condensed tannins; TA, tannin activity

[1] Cut on 20 August 2007

[2] Cut on 20 July 2007

[x] Root sample

Source: Jayanegara et al. (2009a)

after 24 h fermentation (Jayanegara and Sofyan, 2008).

Determination of forage quality and methane production

Forage quality was determined *in vitro* using Hohenheim gas production method (Menke *et al.*, 1979). Detailed description of this method can be seen in Jayanegara & Sofyan (2008). Forage quality was associated with total gas production due to very high correlation (r^2 = 0,92) between total gas production *in vitro* and organic matter digestibility of forage in sheep *in vivo* (Menke *et al.*, 1979). Methane production was measured using infrared methane analyzer (Pronova Analysentechnik GmbH & Co. KG, Berlin, Germany), calibrated against pure methane at 10.6% concentration (Goel *et al*., 2008). After recording the total gas production, the outlet of *in vitro* syringe was inserted to the inlet of methane analyzer. Data obtained was percentage of methane in the total gas.

Statistical Analysis

Principal component analysis (PCA) was applied to the obtained data using SPSS software version 17.0. The PCA technique transforms the number of original variables into few new variables called principal components (PC). The result was presented as loading plot (the relationship of variables) and score plot (used in the classification of forage).

RESULTS AND DISCUSSION

Reduction of Variables into Principal Components

Principal component analysis or PCA is a multivariate statistics method that involved data reduction from correlated variables (Fievez *et al.*, 2003). Ian (2005) stated that when large multivariate datasets are analyzed, it was often desirable to reduce their dimensionality using PCA technique. It replaces the original variables by a smaller number of derived variables, the principal components, which are linear combinations of the original variables. Often, it is possible to retain most of the variability in the original variables with very few derived variables. The PCA technique so far is scarcely applied in the field of animal science. However, there were some authors reported the use of PCA in their data analysis such as Destefanis *et al.* (2000), Fievez *et al.* (2003) and Kadegowda *et al.* (2008).

Reduction of variables using PCA started by examining Eigen values. The Eigen value of each principal component (PC) is presented in Table 2, arranged from the highest to the lowest values. Kaiser (1960) recommended to explore only the PC whose Eigen values higher than 1.0 since they reflect considerable amount of variation to the total variation. In the present study, there are three PC whose Eigen values > 1.0, i.e. PC1, PC2 and PC3. PC1 has Eigen value of 5.180, whereas PC2 and PC3 have Eigen values of 2.193 and 1.294, respectively. PC1 to PC3 represent 51.81%, 21.93% and 12.94% of the total variation, respectively, and all of them contribute 86.67% to the total variation. Therefore, only these three PC which are used further for derivatizing factor loading.

Factor Loading and Relationship between Variables

Factor loading is the correlation between the original variable and the factor derived from PCA (Field, 2005), and the values range from –1 to 1. A value close to –1 or 1 shows a strong correlation between a variable and a factor, whereas a value close to 0 shows a weak correlation. As a rule of thumb, Field (2005) recommended to interprete only the factor loading whose absolute value higher than 0.4 which explains 16% of the total variation. Factor loading of each variable in the PC whose Eigen value > 1.0 is presented in Table 3.

Table 2. Eigenvalue of Each Principal Component (PC)

PC	Eigen value		
	Total	% total variance	% cumulative
1	5.18	51.81	51.81
2	2.19	21.93	73.73
3	1.29	12.94	86.67
4	0.57	5.72	92.39
5	0.45	4.53	96.92
6	0.18	1.82	98.74
7	0.06	0.61	99.35
8	0.04	0.35	99.70
9	0.02	0.22	99.92
10	0.01	0.08	100.00

PC1 can be interpreted as the factor related to methane formation in the rumen *in vitro* (factor loading of methane production in PC1 = 0.881).

Crude protein (CP), NDF and ADF contribute to the increase of methane production with the factor loading of 0.556, 0.758 and 0.745, respectively. Phenolic compounds in forage i.e. total phenols (TP), total tannins (TT), condensed tannins (CT) and tannins activity (TA) lead to the decrease of methane with the factor loading of –0.959, –0.916, –0.420 and –0.945, respectively. The next component i.e. PC2 can be interpreted as the factor related to forage quality (factor loading of cumulative gas production = –0.753). Crude protein contribute positively to the forage quality with factor loading of –0.672, while NDF, ADF and CT contribute negatively to the forage quality with factor loading of 0.599, 0.552 and 0.531, respectively. However, PC3 is not easy to be interpreted since there is only ether extract (EE) component whose factor loading higher than 0.4 i.e. 0.913.

The relationship between variables is presented in Figure 1. It is the plot of factor loading in PC1 (variables related to methane production) and PC2 (variables related to forage quality). Component NDF and ADF contribute in increasing methane production in the rumen. Higher fiber content increases methane production by shifting short chain fatty acid proportion towards acetate which produces more hydrogen as a substrate in the methanogenesis pathway (Beauchemin *et al*., 2008). Therefore, one approach to reduce methane emission in ruminants is by increasing concentrate proportion which means increasing soluble carbohydrate fraction in the ration (Kreuzer & Soliva, 2008).

Table 3. Factor Loading of Each Variable in the Principal Components (PC) whose Eigenvalue > 1.0

Variable	PC1	PC2	PC3
CP	556	–0,672	98
EE	–-0,003	–0,304	913
NDF	758	599	184
ADF	745	552	208
TP	–0,959	103	186
TT	–0,916	0	353
CT	–0,420	531	386
TA	–0,945	174	–0,199
Gas	371	–0,753	132
CH_4	881	309	91

This approach seems to be not practical in the developing countries, however, since it increases production cost which may not be compensated by the increase of animal's productivity.

Phenolic compounds in the form of TP, TT and TA have considerable contribution in the reduction of methane emission, while weaker effect is of CT. This is in agreement with some previous reports, both tannin phenolics (Carulla *et al*., 2005; Puchala *et al*., 2005) and non tannin phenolics (Jayanegara, 2009b). The mechanisms of phenolic compounds in inhibiting methane formation in ruminants have been proposed by Tavendale *et al*. (2005), i.e. (1) indirectly, through reduction in fibre digestion, which decreases H_2 production, and (2) directly, through inhibition of the growth of methanogens. It seems that the condensed tannins decrease methane more

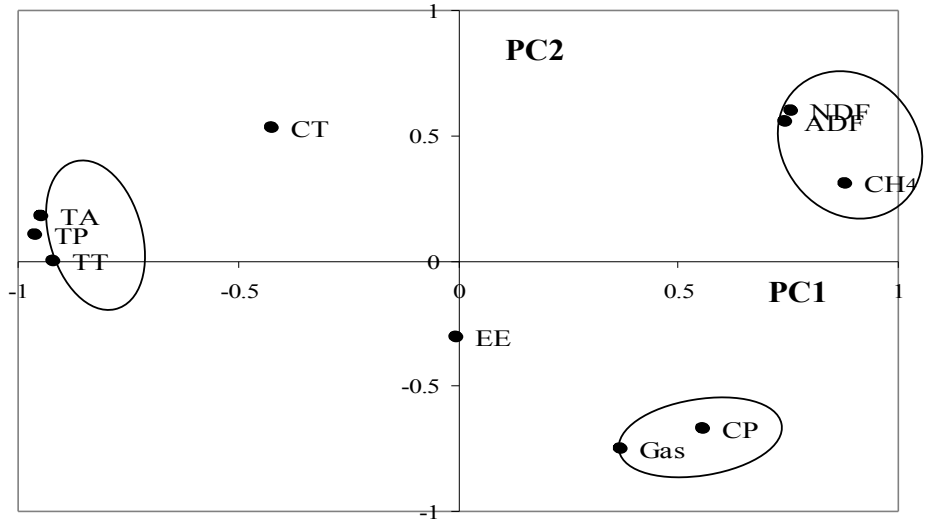

Figure 1. Relationship between variables in PC1 (x) and PC2 (y).

through the first mechanism, while hydrolysable tannins act more through the second mechanism and/or through inhibition of hydrogen producing microbes. Additionally, tannins could decrease the activity of methanogens. Furthermore, tannins are known to decrease protozoal number (Makkar *et al.*, 1995) and as discussed above, the decrease in methane production could be mediated through decrease in protozoal number.

Factors that influence forage quality can also be identified in Figure 1. Crude protein positively contributes to forage quality, whereas CT, NDF and NDF negatively influence the forage quality. Protein is easily digested fraction by rumen microbes (Fox *et al.*, 2004) and, hence, increasing cumulative gas production. On the other hand, the relationship between highly fiborous materials and low digestibility has been well recognized. Structural components of plant such as cellulose, lignin, cell wall, NDF and ADF negatively influenced nutrient digestibility of sheep *in vivo*, while soluble carbohydrate (starch) and crude protein increased the digestibility of nutrient (Fonnesbeck *et al*.,1981; De Boever *et al*., 2005). Condensed tannins were also known for their negative effect on digestibility of ruminants, especially on fiber digestibility (Beauchemin *et al.*, 2007).

Factor Score and Forage Quality Classification

Factor score is a relative score of each object (in this case the object is forage species) in a principal component (Field, 2005). Factor score can be used for forage quality classification multivariately by plotting PC1 as horizontal axe (x) and PC2 as vertical axe (y) in two dimension. The result of forage classification based on factor score is presented in Figure 2. In PC1 (horizontal axe), direction to the positive value (right) shows high methane production. On contrary, direction to the negative value (left) shows low methane production. In PC2 (vertical axe), direction to the positive value (up) shows low cumulative gas production which means low forage quality. Conversely, direction to the negative value (down) shows high forage quality (high cumulative gas production). Therefore, forages can be classified into four quadrants (Figure 2), i.e. quadrant I (forages with high methane and low quality), quadrant II (forages with low methane and low quality), quadrant III (forages with low methane and high quality) and quadrant IV (forages with high methane and high quality).

Figure 2 can be interpreted differently according to different purposes. One may have interest in screening forage species possessing low methane production and high quality, i.e. forages in quadrant III. One may also want to avoid the use of forages possessing high methane production and low quality, i.e. forages in quadrant I. There are three forages in quadrant III, i.e. *Rhenum undulatum*, *Peltiphyllum peltatum* and *Rhus typhina*. This means that these forages have high potential to be widely used as ruminants' feed since they possess not only high quality for production purpose, but also have

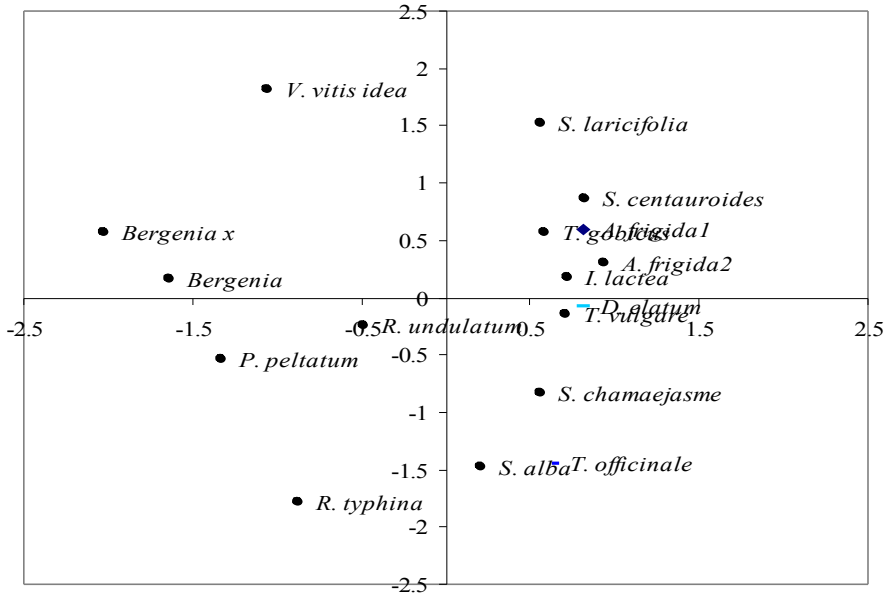

Figure 2. Factor score of each forage species in PC1 (x) and PC2 (y).

ability to reduce methane emission compared to other forages and give additional benefit by decreasing the rate of methane accumulation in the atmosphere and global warming.

CONCLUSION

Principal component analysis (PCA) technique may be used in identifying and integrating variables related to forage quality and ruminal methane production simultaneously. Components CP, NDF and ADF increase methane production, while phenolic compounds like TP, TT, CT and AT decrease the methane production. Component CP also positively related to forage quality, while NDF, ADF and CT negatively related to the forage quality. Moreover, PCA may be used in forage classification, and in this case is used for screening of forages possessing high quality and low methane production. In the present study, based on the PCA technique, *Rhenum undulatum*, *Peltiphyllum peltatum* dan *Rhus typhina* were found to have such desirable characteristics. Nevertheless, there is a need to conduct such screening approach using larger databases to obtain most promising multi purpose forages in a wider scale.

REFERENCES

AOAC. 1990. Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Beauchemin, K.A., S.M. McGinn, T.F. Martinez and T.A. McAllister. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85: 1990–1996.

Beauchemin, K.A., M. Kreuzer, F. O'Mara and T.A. McAlister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21–27.

Carulla, J.E., M. Kreuzer, A. Machmüller and H.D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Aust. J. Agric. Res. 56:961–970.

De Boever, J.L., J.M. Aerts, J.M. Vanacker and D.L. De Brabander. 2005. Evaluation of the nutritive value of maize silages using a gas production technique. Anim. Feed Sci. Technol. 123: 255–265.

Destefanis, G., M.T. Barge, A. Brugiapaglia and S. Tassone. 2000. The use of principal component analysis (PCA) to characterize beef. Meat Sci. 56: 255–259.

Field, A. 2005. Discovering Statistics using SPSS. Second Edition. SAGE Publications, London.

Fievez, V., B. Vlaeminck, M.S. Dhanoa and R.J. Dewhurst. 2003. Use of principal component analysis to investigate the origin of heptadecenoic and conjugated linoleic acids in milk. J. Dairy Sci. 86: 4047–4053.

Fonnesbeck, P.V., J.L. Christiansen and L.E. Harris. 1981. Factors affecting digestibility of nutrients by sheep. J. Anim. Sci. 52: 363–376.

Fox, D.G., L.O. Tedeschi, T.P. Tylutki, J.B. Russel, M.E. Van Amburgh, L.E. Chase, A.N. Pell and T.R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112: 29–78.

Goel, G., H.P.S. Makkar and K. Becker. 2008. Effect of *Sesbania sesban* and *Carduus pycnocephalus* and Fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage- and concentrate-based feeds to methane. Anim. Feed Sci. Technol. 147: 72–89.

Ian, J. 2005. Principal Component Analysis. John Wiley and Sons, Ltd., Aberdeen, UK.

Jayanegara, A. 2008a. Reducing methane emissions from livestock: nutritional approaches. Proceedings of Indonesian Students Scientific Meeting (ISSM), Institute for Science and Technology Studies (ISTECS) European Chapter, 13-15 May 2008, Delft, the Netherlands: 18–21.

Jayanegara, A. and A. Sofyan. 2008b. Penentuan aktifitas biologis tannin beberapa hijauan secara *in vitro* menggunakan 'Hohenheim Gas Test' dengan polietilen glikol sebagai determinan. Med. Pet. 31 (1): 44–52.

Jayanegara, A., N. Togtokhbayar, H.P.S. Makkar and K. Becker. 2009a. 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. 2009b. Ruminal methane production on simple phenolic acids addition in *in vitro* gas production method. Med. Pet. 32 (1): 53–62.

Kadegowda, A.K.G., L.S. Piperova and R.A. Erdman. 2008. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. J. Dairy Sci. 91: 749–759.

Kaiser, H.F. 1960. The application of electronic-computers to factor-analysis. Educ. Psychol. Meas. 20: 141–151.

Kreuzer, M. and C.R. Soliva. 2008. Nutrition: key to methane mitigation in ruminants. Proc. Soc. Nutr. Physiol. 17: 168–171.

Makkar, H.P.S., M. Blümmel & K. Becker. 1995. *In vitro* effects of and interactions between tannins and saponins and the fate of tannins in the rumen. J. Sci. Food Agric. 69: 481–493.

Makkar, H.P.S. 2003. Quantification of Tannins in Tree and Shrub Foliage, A Laboratory Manual. Kluwer Academic Publishers, p. 102.

Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. The estimation of the digestibility and metabolisable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93: 217–222.

Puchala, R., B.R. Min, A.L. Goetsch and T. Sahlu. 2005. The effect of a condensed tannin-containing forage on methane emission by goats. J. Anim. Sci. 83: 182–186.

Tavendale, M.H., L.P. Meagher, D. Pacheco, N. Walker, G.T. Attwood and S. Sivakumaran. 2005. Methane production from *in vitro* rumen incubation with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol. 123/124: 403–419.

Van Soest, P.J., P. Robertson and J.B. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non–starch carbohydrates in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597.