Effect of sward composition and quality and supplementation on methane emission by grazing cattle

L. Mbanzamihigo[1], V. Fievez[1], L. Carlier[2] and D. Demeyer[1]

1. Motivation and project's objectives

Concern about global warming provoked by an increase in atmospheric concentration of greenhouse gases is growing and more attention is focused also on methane emissions (IPCC, 1996; Hansen et al., 1996, 1999). Due to its decay time (10 years), its global warming potential estimated to be 21 times greater than carbon dioxide, methane is an excellent target for control.

The contribution of agriculture, mainly cattle husbandry, is estimated to represent 45 and 60% of the total methane emissions in Europe and Belgium respectively (Organisation for Economic Co-operation and Development (OECD) 1998). Of course, these figures are based only on estimations as no method to quantify methane emission from ruminants on pasture was available. The set up evaluation of the method using the sulphur hexafluoride as a tracer gas (Ulyatt et al., 1999) and the micro-meteorological method (Harper et al., 1999) takes a long time (8 to 12 months). According to the Kyoto protocol (1997), European Union member countries should reduce the production of greenhouse gas by at least 8% during the first decade of the third millennium. Accordingly, the first objective of this project was the development of an easy, cheap and precise method for determination of ruminant methanogenesis.

As ruminant methane production represents a considerable feed energy loss, several antibiotics, ionophores and other chemical inhibitors of methanogenesis have been monitored and studied (Mbanzamihigo et al., 1995, 1996; Van Nevel and Demeyer, 1996). However, as the rumen is an integrated system, such manipulation of its metabolism is associated with negative effects on e.g. feed intake, fibre degradation and microbial growth efficiency whereas transient effects of additives have been reported (Russel and Martin, 1984; Immig et al., 1995). In addition, growing mistrust and reluctance of the public opinion concerning the utilisation of chemical products in animal nutrition increased the interest in both feeding and biological strategies to control methanogenesis. The use of lipids as methane inhibitors showed interesting results but their inclusion in the diet at levels above 5% inhibits fibre degradation in the rumen (Broudiscou et al., 1990; Machmuller et al., 1998). The Intergovernmental Panel on Climate Change (IPCC) (1996) stressed that improvements in animal feed quality and feeding system was one of the most attractive tools to reduce methane emissions from agriculture. Nitrogen supply in the pasture is used to increase plant biomass production and protein proportion in the grass. It has been shown *in vitro* in our laboratory (Demeyer and Van Nevel, 1979) and elsewhere (Cone and Van Gelder, 1999) that proteins produce less methane than carbohydrates. Hence, the increase in crude protein content of plant biomass through nitrogen supply to the pasture might be expected to decrease methane production in the rumen. Moreover, as organic matter degraded in the rumen is partitioned into microbial organic matter and fermented organic, matter generating methane, optimising microbial growth efficiency might redirect degraded organic matter from volatile fatty acids and methane production to microbial synthesis (Leng and Nolan, 1984; Beever, 1993). Hence, if synchronising nitrogen and energy would increase microbial growth (Sinclair et al., 1993), this result in a decrease of methane emissions. A second objective of this project was to support these hypotheses by experimental evidence.

2. Experimental design and activities

In this project, we intended to develop a simple and cheap method for quantification of methane emissions from grazing ruminants and to study these emissions in relation to:

* Nitrogen fertilisation rate: fertilisation might induce differences in chemical characteristics of the grass (proteins, sugars, fibres) inducing changes in methane production (Kirchgeβner et al., 1995).

* Growth stage and growing season: physical and chemical characteristics can change with growth stage and season (Peyraud and Astigarraga, 1998) inducing seasonal changes in methane emissions.

* Sward composition: fertilisation and period of the growing season may change sward composition of the pasture (Davies et al., 1991; Whitehead, 1995) (proportion ray grass/clover), resulting in different contents of secondary plant metabolites (e.g. saponins) in the pasture and hence in the diet, which might change efficiency of microbial growth (Beever et al., 1986) and protozoa numbers (Wallace, 1993) and hence eventually methane excretion figures.

* Supplementation and supplementation system: grass based diets of current production systems inducing a desequilibrium between energy and nitrogen supply for rumen microbial growth result in important urinary nitrogen losses (Peyraud and Astigarraga, 1998) supplementation of energy (e.g. maize silage) reduces this desequilibrium (Van Vuuren et al., 1993) and might optimise microbial growth efficiency (Kirchgeβner et al., 1995). Moreover,

supplying an energy source (maize silage) in a synchronised way might optimise microbial growth efficiency additionally (Sinclair et al., 1993). Finally, increased microbial growth efficiencies might decrease methane emissions (Leng and Nolan, 1984; Beever, 1993).

* In order not to shift from pollution with methane to pollution with nitrogen, next to methane emission, nitrogen utilisation efficiency has been monitored as well during this project.

The experiments ran during two years (1999-2000) and took place during two successive grazing seasons (June-July *vs* August-September). Sheep were used as model for methane emissions from ruminants. During the first experimental year (1999), effects of nitrogen fertilisation rate (150 kg/ha/year) on sward and chemical composition of a mixed pasture (ray grass / clover) and methane emissions were studied. In the second experimental year (2000), the same parameters mentioned above were monitored using sheep and a ray grass pasture supplied with two nitrogen fertilisation rates (200 and 400 kg N/ha/year). As it could be anticipated that the higher nitrogen fertilisation rate (400 kg N/ha/year) would induce higher urinary nitrogen excretions studied synchronised and non synchronised maize silage supplementation were studied. An integrated evaluation approach including plant yield and chemical composition, total and rumen digestibility of the ration, nitrogen utilisation efficiency (microbial growth rate and urinary excretion), methane emission and rumen fermentation pattern were studied. This approach is crucial for a good methane excretion evaluation, results interpretation and finally advising an optimal integrated production system: optimal plant production accompanied with a optimal utilisation of the diets with lower excretions (methane and nitrogen) by ruminants.

3. Material and methods

3.1. Dry matter production and sward composition

During the experimental period pasture were divided into two plots (first year: plot fertilised with 150kgN/ha/year fertilised and non fertilised plot, second year: plot fertilised with 200 and plot fertilised with 400kgN/ha/year). Grass samples were taken from each plot and effects of nitrogen fertilisation on sward composition (first year), total biomass production and chemical composition (first and second year) were evaluated. Chemical analysis including dry matter, ash, crude proteins, neutral detergent fibre, acid detergent fibre, crude fibre, crude fat, soluble sugars were done according to the association of official analytical chemists (AOAC) (1990). Nitrate concentration was measured chromatographycaly using an anion specific column (AS 4A, Dionex, Belgium).

3.2. Animal, diets and digestion parameters

For each year four different adult cross-breed rumen fistulated sheep were used. During the experimental periods, animals were housed indoors in metabolic cages to enable simultaneous measurements of feed intake, apparent total digestibility, nitrogen excretion in the urine, digestibility in the rumen and other rumen fermentation parameters. Experimental animal were fed *ad libitum* four times a day (10 a.m., 4 p.m., 10 p.m., 4 a.m). For feed intake measurement and total apparent digestibility determination, the feed offered, that refused and faecal material excreted were weighted fresh two times a week and a 50g sub-sample was used for DM measurement (70°C, 72h). This research protocol and animal treatment were approved by the Institutional Animal Care and Use Committee of the Ghent University (Belgium) (dossier number of the acceptance: 99/23).

3.3. *In vivo* methane production, pH, volatile fatty acids and ammonia concentration

Rumen fermentation parameters were monitored two times a week. Ethane, a gas behaving as methane was used as a tracer gas to evaluate methane production in the rumen as adapted from Moate et al. (1997). Ethane (Air Liquide, Belgium) was infused continuously through the rumen fistula during 6h at an exactly known infusion rate of 10ml/minute using a dosimeter (Brooks instruments B.V., G/T 1000, the Netherlands) between 10 a.m. and 4 p.m. or 10 p.m.. In preliminary *in vitro* incubations, it was shown that addition of ethane (% gas phase) has no effect on rumen fermentation pattern and was not metabolised or oxidised. Total gas expelled through the rumen fistula of each sheep was collected in large PVC columns (see Van Nevel et al. 1970b and Mbanzamihigo et al. 1995). At the end of the gas collection, rumen gas produced during 6h or 12h, mixed with ethane was sampled (1ml) using gas tight syringes and analysed for methane and ethane by chromatography. As ethane behaves as methane, the total methane produced in the rumen (MPR) can be calculated from the total ethane infused in the rumen (EIR) and the proportions of CH_4 (% v/v CH_4) and C_2H_6 (% v/v C_2H_6) in the collected gas as follows: MPR(l) = (% v/v CH_4 / % v/v C_2H_6) * EIR(l). Methane produced over the 6h or 12h was assumed to be a quarter or half of the daily production. Simultaneously with gas collection, rumen contents were sampled for pH and volatile fatty acids measurement. During the first season of each year, *in vitro* incubations were done to confirm *in vivo* results. Simultaneously with methane collection, rumen juice was sampled and pH, volatile fatty acids and ammonia concentration measured.

3.4. *In sacco* degradability and rumen passage rate

Once a week, *in sacco* degradability was measured by introducing 2.0g of sample in polyamide bags (Solana, Edegem, Belgium, pore size 37-50µm). One bag was not incubated and immediately washed for determination of the soluble fraction whereas the incubated ones were removed from the rumen at different periods and washed in a mini-washing machine, dried (65°C, 72h) and weighed for degraded dry matter determination. Rumen passage rate was determined using the Cr-mordanted hay procedure (Uden et al., 1980). From *in sacco* degradability and rumen passage rate, the effective degradability has been calculated according to Orskov and McDonald (1979).

3.6. Statistical analysis

Effect of nitrogen fertilisation on parameters studied was statistically evaluated using General Linear Model (GLM) procedures with fertilisation rate, season and animals as main factors and the Duncan test to distinguish statistically different groups of animals. In a second step, ANOVA was used to evaluate animal and season effects independently from the treatment (nitrogen fertilisation). All these tests were done using SPSS statistical program (SPSS, software for Windows release 9.0, (SPSS, Inc., USA)). Data are presented as means and standard deviations and significance is declared at $P<0.05$.

4. Results and discussion

4.1. First year (1999)

4.1.1. Dry matter production, sward and chemical composition of the pasture

Compared to the control (non fertilised pasture), nitrogen fertilisation increased the dry matter production per ha as well as the ray grass production at the expense of clover during the two seasons. However, the magnitude of the differences were season dependent. Hence, on average, in early summer (June-July: season 1), supplying 150kg nitrogen/ha/year increased dry matter production by 29%, and reduced by half the clover proportion in the total dry matter. In late summer (August-September: season 2), dry matter production was increased by 46% while the clover proportion was only 10 points lowered. The effect of season on biomass production was more outspoken than that of fertilisation, since dry matter produced per ha was more than twice as high in the first compared with the second season irrespective of the treatment.

For chemical composition of the swards, our attention was focused on key grass compounds susceptible to have a direct or indirect effect on methane production: crude protein, neutral detergent fibre, water soluble carbohydrate and crude fat percentages in the swards. Nitrogen fertilisation did not change clearly the crude protein percentage (season 1: from 12.2 to 13.8% ; season 2: from 21.9 to 23.2%) within each season while pasture total crude protein yield was increased by 46 and 53% in season 1 and 2 respectively. The season effect was more outspoken than the effect of nitrogen fertilisation, the crude protein percentage being higher in season 2 (22.5%) than in season 1 (13.0%) while the reverse occurred for total crude protein production. No significant effect of nitrogen fertilisation is observed on fibre content (season 1: from 49.5 to 49.4% ; season 2: from 58.7 to 60.8%). However, again, a strong season effect occurred. In all seasons, nitrogen fertilisation increased nitrate accumulation in the grass (season 1: from 55 to 250ppm ; season 2: from 1402 to 3475ppm) and this accumulation was higher in the second than in the first season. No effect of nitrogen fertilisation on water soluble carbohydrate concentration was observed (season 1: 13.4 to 14.1% ; season 2: from 5.0 to 4.9%), but again a strong seasonal effect ($P<0.001$) was apparent. On average the crude fat percentage was low (*c.a.* 4.5%). Neither nitrogen fertilisation rate nor season induced differences of this parameter.

4.1.2. Methane emissions and pasture digestion in the rumen

Neither level of nitrogen fertilisation, nor season affected *in vivo* rumen methane emission per kg of dry matter ingested and fermentation pattern volatile fatty acids, which has been confirmed through *in vitro* results. It is surprising that no difference in methane production between the two seasons was found despite the clear difference in crude protein percentage (13% in season 1 *vs* 22.5% in season 2). A significant ($P<0.05$) animal effect was observed however: on all diets, one animal produced on average less methane (20.3±3.1l /kg dry matter ingested) than the three others (27.6±4.1l/kg dry matter ingested). The lower methane production per kg of dry matter ingested observed in that sheep was accompanied by a lower daily average pH (5.92 *vs* 6.52), a higher total volatile fatty acids concentration (135.5 *vs* 97.6 mmols/l), a lower degradation rate (4.2 *vs* 7.2%/h) inducing a significantly ($P<0.001$**)** lower effective dry matter degradability in the rumen (61.7 *vs* 68.4%) while the degradation extend is comparable to that of other sheep (87.5 *vs* 87.1%). There is no difference between sheep concerning apparent total digestibility (76.2 *vs* 75.6%). One could expect that the lower methane produced by one sheep is only due to the lower effective dry matter degradability. Nevertheless, *in vivo* methane production calculated per kg of effectively degraded dry matter in the rumen shows persistent animal effects and animal effects tended to remain when *in* *vitro* incubations were performed suggesting animal differences in rumen microbial population. Methane released from each sheep per year was estimated and compared with methane calculated using equations recommended by the Intergovernmental Panel on Climate Change for national greenhouse inventory (IPCC, tier 1 and 2) (IPCC 1996). Although all the sheep received the same diet, one sheep produced considerably less methane. Relative differences between methane emission observed and methane calculated from IPCC values are considerably high for sheep A while for sheep B, C and D, both the default emission factor, IPCC tier 1 (8 kg methane / animal / year) as well as methane calculated using IPCC tier 2 (7.4-8.5kg methane / animal / year) is in the range of our methane production observed.

4.1.3. Nitrogen utilisation efficiency

A comparison between protein and dry matter degraded in the rumen gives an indication on possible imbalance between energy and nitrogen in the rumen. It has been evidenced in our experiment that no nitrogen lack occurred. Instead, a nitrogen surplus (>165g of protein incorporated per kg of dry matter degraded) higher in the late than in the early grazing season was observed. Hence, this nitrogen surplus induced a lower nitrogen utilisation efficiency through improved urinary nitrogen excretions.

4.1.4. Effect of clover saponins on methane production

In vitro trial with rumen contents were done with and without different doses of clover extract containing saponins. After incubation, methane, volatile fatty acids production were measured and protozoa counted. Results show exponential decrease of methane production relative to volatile fatty acids at low doses accompanied with a decrease in protozoa number for all doses. For doses higher than 6-9mg/ml, the volatile fatty acids and gas production decreased and even stopped at 24mg/ml. As clover saponins are known to be triterpenoid glycosides (Sakamoto et al., 1992), results contradict the general opinion that triterpenoidal saponins have no antibacterial activity (Hostettman and Martson, 1995; Lu and Jorgensen, 1987).

4.2. Second year (2000)

4.2.1. Dry matter production and chemical composition of the pasture

Compared to 200kg nitrogen/ha/year, supplying 400kg nitrogen/ha/year in a ray grass pasture increased the dry matter production in early summer (June) by almost 70% while in late summer (august), the biomass production was doubled. Irrespective of the fertilisation rate, a seasonal effect on dry matter production towards more dry matter production in early than in late summer season was observed. Indeed, dry matter production released after 22 days of regrowth in early summer only was reached after 39 days during the late summer season for the lowest fertilisation rate. For the higher fertilisation rate (400kg N/ha/year) 17 addition growth days in the second season induced an increase in the dry matter yield for 600kg/ha. The higher nitrogen fertilisation rate induced a higher crude protein percentage in the two seasons: (13.6 *vs* 20.1% in the first season and 13.1 *vs* 15.7% in the second season for 200kg nitrogen/ha/year and 400kg nitrogen/ha/year respectively. The increase in crude protein percentage was accompanied by a decrease in water soluble carbohydrates (season 1: from 10.8 to 7.3%, season 2: from 16.3 to 12.7%) and an increase in nitrate concentration (season 1: from 186 to 2697 ppm, season 2: from 211 to 2452 ppm) while neutral detergent fibre content did not change significantly (season 1: 49.7 *vs* 50.2%, season 2: 50.9 *vs* 52.7%).

4.2.2. Methane emissions and pasture digestion in the rumen

In season 1, compared to 400kg, 200kg nitrogen per ha per year was associated with an increase (20-40%) in methane production per kg of dry matter ingested for two sheep only. It is clear that the higher methane production observed here was linked with the lower ingestion rate as the two sheep ingested (25-70%) more dry matter when grass from the higher fertilisation rate was offered. The lack of nitrogen fertilisation rate effect on methane emission was confirmed by *in vitro* results. The higher ingestion rates induced higher passage rates and a lower methane productions in the rumen. Moreover, the shift of fibre digestion from the rumen to the hindgut induces a lower total methane emission as methane synthesis is partially replaced by acetate in the latter fermentation chamber. No clear change in volatile fatty acid concentration has been observed, confirming that the lower methane emission was effectively due to a shift of dry matter digestion from the rumen to the lower parts of the digestive system. There was no difference in methane emission between the two fertilisation rates in the second season where no difference in ingestion occurred.

4.3.3. Supplementation strategies and methane emissions

There is no difference between synchronised and non synchronised supplementation systems in methane emission per kg of ingested dry matter (27.6 *vs* 26.9 l for respectively synchronised and non synchronised supplementation system). The hypothesis was that by synchronising nitrogen and energy supply, more organic matter digested should be used for microbial growth decreasing the organic matter fermented and thus, generating less methane. The absence of synchronisation effect on methane production could be explained by the relatively slow rate degradation of maize silage, masking the difference between the synchronised and the non synchronised situations.

4.3.4. Nitrogen efficiency

Increased N fertilisation rates induce higher urinary N losses through inefficient utilisation of N in the rumen. These high urinary N losses could be predicted by the OEB-value of the diet. A reduced rumen degradable protein balance (OEB) either through feeding less fertilised or older grass or through supplementation of maize silage synchronously or asynchronously will induce a proportional decrease in urinary N excretion.. Moreover, animal differences in urinary urea excretions (at the same OEB level) could be observed. Hence, from an integrated evaluation of both the OEB-value of the diet and the milk urea content per cow or lactation group, dietary adaptations to improve N utilisation efficiency of the grazing dairy cow might be proposed. During this research no effect of synchronisation of energy and nitrogen supply in the rumen, on microbial growth efficiency could be observed. However, it is still unclear whether synchronisation is not important for high productive dairy cattle.

5. Conclusions and recommendations

5.1. Method for determination of methane emission.

A method for determination of rumen methane production using ethane as a tracer gas was perfected and used, giving results comparable with those obtained with other more expensive and complicate methods (sulphur hexafluoride as a tracer gas or micro-meteorological method). Determination of organic matter effectively degraded in the rumen, rumen concentrations of volatile fatty acids and methane production thus determined *in vivo* fit the stoichiometric model of rumen fermentation as developed from *in vitro* incubations (Demeyer, 1991).

5.2. Effects of nitrogen fertilisation on grassland production.

A moderate nitrogen fertilisation rate applied on a mixed pasture (ray grass/clover) increases the dry matter production but decreases clover proportion in a mixed pasture (ray grass / clover). In both experiments, nitrogen fertiliser is more efficiently utilised for biomass and protein production in early than in late summer.

5.3. Effect of nitrogen fertilisation on methanogenesis.

No clear effects of nitrogen fertilisation, and thus, of grass crude protein content, were observed on rumen methanogenesis, expressed per kg organic matter intake or organic matter totally digested (faeces), contrary to the hypothesis. In both experiment, rumen methanogenesis more determined by animal than by dietary effects. In the second experiment animal variability could be explained by differences in grass intake. A higher intake produces a shift in digestion from the rumen to the large intestine.

However, the increase in dry matter production induced by nitrogen fertilisation increases stocking rate and hence methane emission per ha. Individual (between animals) variation in methane emission is important and further insight in this animal variability concerning feed intake and side of digestion is needed both

- to understand differences in feed utilisation efficiency,
- to develop mitigation strategies to control methane emission by e.g. selection-breeding
- to improve the reliability of IPCC estimations of methane emissions from livestock. Incorporation of the level of feed intake in these estimations seems desirable.

In attendance of these possible refinements, IPCC (1996) estimations, expressed as kg methane production per animal per year, can be used. Indeed, both the average value for sheep, calculated from our experiments (11.1 kg animal-1 year-1) and the variability (variation coefficient = 19%) remain within the ranges proposed by the IPCC (1996): average of 11.7 and predicted variation coefficient of 20%. There is no reason to assume this conclusion to be different for other ruminants.

5.4. Grass feeding and efficiency of N utilisation

Concerning N utilisation efficiency of grass fed or grazing animals, CP levels of the diet should be pursued to ensure efficient N capture by the rumen microbes (balanced degradable protein balance, OEB = 0). Indeed, positive OEB-values are known and have been confirmed during our experiments to induce proportional urinary urea excretions. Rumen degradable protein balance of grass based diets could be reduced either by feeding less fertilised or older grass or by supplementing energy rich forage or concentrate (e.g. maize silage) synchronously or asynchronously.

In spite of a slight increase in rumen microbial growth yield at higher levels of intake, microbial protein supply to the small intestine - per kg OM apparently digested - decreased, suggesting the increased importance of post-ruminal digestion (in particular in the hindgut) at higher levels of intake. Hence, when protein supply is limiting milk production, this shift is not desirable from the ’N point of view’.

In order to reduce urinary urea excretions, increased crude protein concentration, as observed at the end of the grazing season, should be avoided, eventually through supplementation of energy rich-protein poor forage or concentrate.

5.3. Second year (2000)

The higher nitrogen fertilisation rate applied on a ray grass pasture induces a higher biomass production and higher crude protein contents. As observed in the first year, nitrogen fertilisation in more efficient for biomass production in early than in late summer. There is no effect of nitrogen fertilisation rate on methane emission, only an effect of ingestion level was apparent. A higher ingestion rate induces a beneficial shift of dry matter digestion from the rumen to the hindgut known to induce lower methane productions to the advantage of acetate, a high energy compound. This confirms that ingestion level should be incorporated in a model for estimation of methane emission from livestock in order to improve the IPCC estimations.

6. References

Association of Official Analytical Chemists, 1980. *Nutrition Research Reviews*, 11: 173-198. **Beever, D.E., 1993.** In : Forbes, J.M. and France, J. (Eds). Quantitative aspects of ruminants digestion and metabolism. Oxon, *CAB International*, 187-215. **Broudiscou, L. Van Nevel, C.J. and Demeyer, D.I., 1990.** *Arch. Tierernahr.*, 40: 329-337. **Cone, J.W. and Van Gelder, A.H., 1999.** *Anim. Feed Sci. Technol.* 76: 251-264. **Demeyer, D.I., 1991.** *In* , J.P. Jouany, ed., *Rumen Microbial Metabolism and Rumen Digestion*. INRA Editions, Paris, Cedex, 217-237. **Demeyer, D. and Van Nevel C. 1979.** *Ann. Rech. Vét.* 10: 277-279. **Hansen, J., Ruedy, R., Sato, M. and Reynolds., 1996.** *Res. Lett.*, 23: 1665-1668. **Hansen, J., Sato, M., Ruedy, R., Lacis, A. and Oinas, V., 2000.** *Proc. Natl. Acad. Sci.*, 97: 9875-9880. **Harper, L.A., Denmead, O.T., Freney, J.R. and Byers, F.M., 1999.** *J. Anim. Sci.*, 77, 1392-1401. Hostettmann, K. and Marston, A., 1995. *Cambridge: Cambridge University Press, UK*, 1-12; 19-75: 122-174. **Immig, I., Fiedler, D., Van Nevel, C. and Demeyer, D.I., 1995.** *Proc. Soc. Nutr. Physiol.*, 4: 68. **Intergovernmental Panel on Climate Change (IPCC).** 1996. *Technologies, policies and measures for mitigating climate change:* IPCC, technical paper 1. 99 pp. **Kirchgebner, M., Windisch, W. and Müller, H.L., 1995.** *Proc. of the 8th International Symposium on Ruminant Physiology*, Germany. **Kyoto protocol, 1997.** *Kyoto protocol to the united nations framework convention on climate change*. 11th December 1997, Kyoto, Japan. www.unfcc.de/. **Leng, R.A. and Nolan, J.V., 1984.** *J. Dairy Science*, 67, 1072-1089. **Lu, C.D. and Jorgensen, N.A., 1987.** *Journal of Nutrition*, 117: 919-927. **Machmuller, A., Ossowski, D.A., Wanner, M. and Kreuzer,**

M., 1998. *Anim. Feed Sci.Technol.*, 77: 117-130. **Mbanzamihigo, L., Van Nevel, C.J. and Demeyer , D.I. 1995.** *Reprod. Nutr. Dev.* 35: 353-365. **Mbanzamihigo, L., Van Nevel, C.J. and Demeyer , D.I. 1995.** *Anim. Feed Sci.Technol.*, 28: 215-228. **Moate, P.J., Clarke, T., Davis, L.H. and Laby, R.H., 1997.** *J. Agric. Sci., Cambridge*, 129, 459-469. **Organisation for Economic Co-operation and Development (OECD), 1998.***Agricultural practices to reduce greenhouse gas emissions: overview and survey instruments*. COM / ENV / POC / AGR / CA (98) 149. **Orskov, E.R. and McDonald, I. 1979.** *J. Agric. Sci., Camb*. 92: 499-503. **Peyraud and Astigarra, 1998.** *Anim. Feed Sci.Technol.,* 72: 235-259. **Russel, J.B. and Martin, S.A., 1984.** *J. Anim. Sci.*, 59: 1329-1338. **Sakamoto S., Kofuji, S., Kuroyanagi, A. and Sekita, S., 1992.** Phytochemistry, 31 (5): 1773-1777. **Sinclair, L.A., Garnsworthy, P.C. Newbold, J.R. and Buttery, P.J., 1993.** *J. Agric. Sci.*, 120: 251-263. **Uden, P., Colucci, P.E. and Van Soest, P.J., 1980.** *J. Sci. Food Agric*., 31, 625-632. **Ulyatt, M.J., Baker, S.K., McCrabb, G.J. and Lassey, K.R. 1999.** *Austr. J. Agric. Res.* 50: 1229-1334. **Van Nevel, C.J., Demeyer , D.I., Henderickx, H.K. and Martin, J.A. 1970.** *Zeitschr. Tierphys. Tierernähr. Futtermittelk.* 26: 91-100. **Van Nevel, C.J. and Demeyer, D.I., 1996.** *Environ. Monit. Assess*., 42: 73-97. Whitehead, D.C., 1995. Wallingford. *CAB International.* pp 397.