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Estimation of Methane Production in Sheep Using Nutrient Composition of the Diet

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Abstract: This study was conducted to compare methane production in legume (two common Iranian alfalfa varieties) and grass (quackgrass; QCK) as forage sources in sheep nutrition by using nutrient composition of the diet. Each of the 2 hays of legume (Hamedani; HAM and Kareyonge; KAR varieties) and grass (QCK) were offered ad libitum to three Gezel rams. No significant difference found between Dry Matter (DM), Crude Protein (CP), Ash and Ether Extract (EE) of 2 alfalfa varieties, although the difference for Crude Fiber (CF) was significant ($p<0.01$). The Organic Matter (OM), DM, CP, CF, Ash contents of 2 species (legume and grass) were significantly different ($p<0.05$). Methane production (g per day, g per kg BW and g per kg $BW^{0.75}$) were similar in HAM and KAR hays ($p>0.05$), whereas methane production (g per day, g per kg BW and g per kg $BW^{0.75}$) in grass hay were significantly ($p<0.01$) higher than that of legume hay. Calculations based on nutrient composition of the diet indicate that grass hay can have a higher methane production than legume resulting to gross energy lost as CH_4.

Key words: Methane production, legume, grass, nutrient composition, sheep

INTRODUCTION

Methane, a powerful greenhouse gas contributing to global warming (Johnson and Johnson, 1995), is naturally produced in ruminants. Methane is produced as a result of anaerobic fermentation in the rumen and the hind-gut. Microbial enzymatic activity in the rumen (and salivary enzymes), hydrolyses much of the dietary organic matter to amino acids and simple sugars. These products are then an aerobically fermented to volatile fatty acids (VFA), hydrogen and CO_2. Some of the CO_2 is then reduced through combination with hydrogen to produce methane:

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$$

Alternatively, hydrogen can by used in the formation of some VFA or incorporated into microbial organic matter. The stoichometry of the formation of the main VFA is shown in the following equations:
2H producing reactions:

$$\text{Pyruvate} \rightarrow \text{acetate (C2)} + CO_2 + 2H$$

2H using reactions:

$$\text{Pyruvate} + 4H \rightarrow \text{propionate (C3)} + H_20$$

$$2\,C_2 + 4H \rightarrow \text{butyrate (C4)} + 2H_2O$$

From this it can be concluded that if ruminal fermentation patterns are shifted from acetate to propionate, both hydrogen and methane production will be reduced. This relationship between methane emissions and the ratio of the various VFA has been well documented (Hungate, 1966) and it provides opportunities to reduce methane emissions. Herein also lie the explanation as to why fibrous diets produce more methane than non-structural carbohydrate diets: the fibrous diets promote higher acetate, resulting in more hydrogen and thus more methane.

The methane in the rumen is produced by methanogenic bacteria and protozoa. The role of protozoa in methane formation is interesting. It has been established that virtually all of the bacteria attached to protozoa are methanogens (Vogels *et al*., 1980) and that these bacteria are responsible for between 0.25 and 0.37 of the total methane produced (Finlay *et al*., 1994; Newbold *et al*., 1995). By removing the protozoal population through defaunation, the ruminal bacterial population is modified, VFA production is shifted from

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acetate and butyrate towards propionate and methane emissions are decreased. There is also a negative impact on fiber digestion (Demeyer *et al*., 1982), so care must be taken not to unduly disrupt rumen metabolism by this route.

The hind-gut has been reported to account for between 0.13 and 0.23 of the total emissions by sheep (Murray *et al*., 1976; Kennedy and Miligan, 1978). However, it appears that most (0.89) of the methane produced in the hind-gut is absorbed through the gut wall and excreted via the lungs (Murray *et al*., 1976). In the hind gut, protozoa are absent and methane is produced by methanogenic bacteria. Methane emissions from the hindgut are lower than from the rumen and it has been speculated that this could be due to hydrogen removal by reductive acetogenesis rather than methanogenesis (De Grave and Demeyer, 1988).

High yielding dairy cows generally produce over 100 kg of methane/year from enteric fermentation. It is estimated that in cattle on average 8-12% of the digestible energy ingested is lost in the rumen as CH_4 (Tamminga, 1992). There is an inverse relation between feed intake and gross energy lost as CH_4 (Mills *et al*., 2003). This implies that at lower feed intakes, the effect of the nutrient composition of the ration, i.e. of single nutrients, is more distinct.

Thus, there have been a number of attempts to predict CH4 production from its relation to Body Weight (BW), level of feed consumption, dietary composition and crude nutrient intake (Wilkerson *et al*. 1995; Benchaar *et al*., 1998; Mills *et al*., 2001; 2003).

Therefore, it was the objective of this study to relate the total CH4 emission to the nutrient composition between two alfalfa varieties and legume to grass.

MATERIALS AND METHODS

Forages: Three forage species, alfalfa (Hamedani and Kareyonge varieties) and Quackgrass (Agropyron repens) were used in the experiment. Samples of the forages were collected near Urmia, Miandoab and Karaj, Iran. Samples harvested at late maturity (mid to late bloom), were collected randomly from ten. Collected samples were air-dried and ground (1 and 5 mm screen) for chemical analysis.

Chemical analysis: Dry Matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as N×6.25. Nitrogen-Free Extract (NFE) was calculated as (OM- CP-CF-EE) (NRC, 2001).

Animals: Forages (HAM, KAR and QCK) were offered *ad libitum* and separately (each forage for 10 days) to 3 Gezel rams (1.5 year old, average initial BW 55 kg) kept in metabolism cages to enable accurate determination of feed intake and allow easy collection of faeces. The hay was fed twice daily at 08: 30 and 16: 30 h and fresh drinking water and mineral salt licks were freely available.

Methane production equations: Methane production was estimated by below equations by using nutrient composition of the diet, dry matter Intake (I) or the number of kcal feed ingested (X) values:

$$CH_4\ (g/d) = 63 + 79CF + 10NFE + 26CP - 212EE$$
(all nutrients in kg/d) (Kirchgessner *et al*., 1995) (1)

$$CH_4\ (g/day) = 18 + 22.5\ I$$
(Blaxter and Clapperton, 1965) (2)

$$CH_4\ (kcal/100\ kcal\ feed) = 6.8 + (243/X)\ I$$
(Blaxter and Clapperton, 1965) (3)

$$CH_4\ (kcal/day) = -494 + 0.629\ I - 25.0\ I^2\ I$$
(Blaxter and Clapperton, 1965) (4)

In this study, the methane production values of HAM, KAR and QCK hays samples were calculated using equation of Kirchgessner *et al*. (1995).

Statistical analysis: All of the data were analyzed by using software of Statistical Analysis Systems and means were separated by independent-samples t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The chemical compositions of HAM and KAR hays are presented in Table 1. The CF content of KAR hay was higher than that HAM hay (34 vs. 29.2). The methane production (g per day) of KAR hay was higher than that of HAM hay ($p>0.05$). On the other hand, methane production (g per kg BW and g per kg $BW^{0.75}$) were 1.667 and 1.712 for HAM and 4.539 and 4.663 for KAR hays, respectively. The fiber fraction which is an important part of the ruminant diet has the greatest impact on CH_4 production in accordance with results from Moe and Tyrrell (1979).

There was variation between forages in terms of chemical composition and methane production of Legume and Grass hays (Table 2). Grass hay was significantly ($p<0.05$) higher in DM, CF, Ash than legume, but the OM and CP of legume hay were higher than that of grass hay ($p<0.05$). The EE and NFE were similar ($p>0.05$) in legume

Table 1: Chemical composition and methane production in 2 legume (alfalfa) varieties

Component	HAM	KAR	SEM	Sig.
DM (%)	92.93	93.46	0.133	NS
(%DM)				
OM	89.66	89.66	0.333	NS
CP	15.80	12.50	0.819	NS
CF	29.20	34.00	0.519	**
EE	1.33	1.33	0.334	NS
Ash	10.33	10.33	0.493	NS
NFE	43.30	41.84	1.195	NS
Methane production				
g per day	91.7	94.2	0.921	NS
g per kg BW	1.667	1.712	0.016	NS
g per kg $BW^{0.75}$	4.539	4.663	0.045	NS

HAM = Hamedani alfalfa; KAR = Kareyonge; the data are mean value of three replicate; DM = Dry matter; OM = Organic matter; CP = Crud protein; CF = Crud fiber; EE = Ether Extract; NFE = Nitrogen-free extract; SEM = Standard Error of Means; NS = Non-significant; $^{**}p<0.01$

Table 2: Chemical composition and methane production in legume and grass hays

Component	legume	grass	SEM	Sig.
DM (%)	93.2	95	0.278	*
(%DM)				
OM	89.66	88.7	0.215	*
CP	15.83	8.9	1.125	*
CF	31.6	34.3	0.433	*
EE	1.33	1.44	0.181	NS
Ash	10.33	11.2	0.215	*
NFE	42.53	44.35	0.507	NS
Methane production				
g per day	92.94	95.9	0.504	*
g per kg BW	1.689	1.743	0.009	*
g per kg $BW^{0.75}$	4.600	4.747	0.025	*

All values for legume obtained from means values of HAM = Hamedani alfalfa and KAR = Kareyonge; Grass = Quackgrass the data are mean value of three replicate; DM = Dry matter; OM = Organic matter; CP = Crud protein; CF = Crud fiber; EE = Ether Extract; NFE = Nitrogen-free extract; SEM = Standard Error of Means; NS = Non-significant; $^{*}p<0.05$

and grass hays. The methane production (g per day, g per kg BW and g per kg $BW^{0.75}$) was significantly ($p<0.05$) higher than legume hay. Wainman *et al*. (1978) reported that dry forages, methane losses are a little higher than with grass silages.

Typically, methane emissions from enteric fermentation represent about 6% of dietary gross energy, but this varies with diet from about 2% (cattle in feedlots) to 12% (animals eating very poor quality forage) according to Johnson and Johnson (1995).

The proportion of concentrate within the diet has been reported to be negatively correlated with methane emissions (Holter and Young, 1992; Kurihara *et al*., 1998; Yan *et al*., 2000). Concentrates contain less structural carbohydrates than forages and the effect of increasing the proportion of concentrates in the diet on ruminal VFA concentrations is well documented, with an increase in the proportion of propionate and a decrease in the proportion of acetate (and sometimes butyrate). This would be expected to impact on methane production. Also, increasing the proportion of concentrate in the diet will generally reduce rumen pH and as methanogens are pH sensitive, this will also tend to reduce methane emissions. Sometimes the effect of concentrate proportion is compounded by increases in total intake, but when expressed as a proportion of gross energy intake, reductions in methane production are generally found as the proportion of concentrate increases, with these reductions being most dramatic when concentrates form the major proportion of the diet (Johnson and Johnson, 1995). Increased use of concentrates also increases animal performance and this will further reduce emissions as outlined above. Ovenell-Roy *et al*. (1998) reported differences in methane production from 4 cultivars of barley fed to lambs. When seven different diets were given to both sheep and cattle no significant differences between the two species were found in the amounts of CH_4 produced at the maintenance level (Blaxter and Clapperton, 1965).

Increasing forage digestibility increases daily methane emissions because of increased intake. However, at high intake levels, the proportion of energy lost as methane decreases as the digestibility of he diet increases (Johnson and Johnson, 1995). In addition, improving forage digestibility will improve productivity because DM and energy intake are increased. Therefore the gains outlined above due to increased productivity should materialize. Indeed increasing the digestibility of pasture for grazing ruminants has been proposed as the most practical means of reducing their methane emissions (Hegarty, 1999a). However, he later points out that if animal numbers do not decrease in response to the improved productivity, then emissions from the sector will increase rather than decrease (Hegarty, 2002).

Legumes generally have higher intakes and digestibility than grass swards and thus give rise to higher productivity. This should reduce methane emissions. However, it as also been reported that legumes give rise to reduced methane emissions when fed at comparable intake levels (Beever *et al*., 1985). McCaughey *et al*. (1999) speculated that the reduced emissions could result from a modified ruminal fermentation pattern combined with higher passage rates as reported by Minson and Wilson (1994).

There are substantial differences in the carbohydrate fractions of forages such as grass silage maize silage or whole crop wheat silage, which will affect their methanogenic potential. In addition, these forages can give rise to differences in productivity: e.g. maize silage supports higher intake and performance than grass silage.

Within a forage species, there may be potential to select cultivars that result in reduced methane production. Recent *in vitro* work (Lovett *et al*., 2003) has demonstrated differences between cultivars of perennial ryegrass in their methanogenic potential. The differences were significantly related to chemical composition of the cultivars, but differences between cultivars could also be due to differences in contents of organic acids.

There have been many strategies proposed that could reduce methane emissions and these have been comprehensively reviewed by Moss (1994).

- Increased animal productivity
- Effect of Longevity in Dairy Cows
- Effect of Concentrate Proportion in the Diet
- Effect of Concentrate Type
- Effect of Forage Quality
- Effect of Forage Type
- Use of Ionophores
- Effect of Dietary Oil Supplementation

Increasing animal productivity will generally reduce methane emissions per kg of product (milk or meat) because the emissions associated with maintenance are spread over a larger amount of product. However, daily emissions and thus emissions per animal per year are usually increased because the higher productivity is usually associated with higher intake. Methane production is closely related to Dry Matter (DM) intake. Kirchgessner *et al.* (1995) reported that increasing milk yield from 4000-5000 kg/year increases annual methane emissions, but will decrease emissions per kg of milk by 0.16 for a 600 kg cow.

The longer that cows stay in a herd, the lower the number of replacements enquired and thus the lower the total farm methane emissions.

The review of NRC (2001) outlined increases in milk production, better feed conversion efficiency, reduced acidosis, ketosis and bloat resulting from the feeding of ionophores. Intake is also reduced in many experiments, with O'Kelly and Speirs (1992) calculating that this is responsible for 0.55 of the decline in methane emissions following monensin application. However, several researchers have reported that the effects on methane production are transient (Rumpler *et al*., 1986; Abo-Omar, 1989; Carmean, 1991; Johnson *et al*., 1991; Saa *et al*., 1993) indicating that microbial adaptation occurs.

Defaunation or removal of protozoa from the rumen is one method which could reduce methane emissions. One method by which defaunation can be brought about is the addition of certain oils/fats (Machmüller *et al*., 1998). In the absence of protozoa, rumen CH_4 output is reduced by 0.13 on average, although this varies with diet (Hegarty, 1999b).

CONCLUSION

In an overall conclusion it seems that, methane production from ruminant diets which have high inclusion of grass hay was higher than that of legume hays. Thus using high inclusion rate of grass hay in ruminant diets can be lead to high energy loss as well as increasing methane emission in the environment as greenhouse gas contributing global warming.

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