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**ENTERIC METHANE EMISSION ESTIMATED WITH THE SF<sub>6</sub> TRACER TECHNIQUE IS RELIABLE ON RUMEN CANNULATED SHEEP FED FORAGES SILAGES DIETS**

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**ABSTRACT:** The aim of this work was to study the effect of rumen cannulation on methane emission estimated with the SF<sub>6</sub> tracer technique in sheep fed forages silages diets based on mixtures of grass and legumes. Ten castrated male Texel sheep were allocated into 2 homogenous groups of 5 animals in which sheep were equipped with a rumen cannula (C+) or without (C-). Each group was fed ad libitum with five silages diets of pure timothy (T), mixture of T and sainfoin (T-SF, 50/50), T and red clover (T-RC, 50/50), SF-RC (50/50), T-SF-RC (50/25/25) in a 5x5 Latin square design. Dry matter intake (DMI) was recorded daily. Methane emissions using the SF<sub>6</sub> technique was performed on 4 successive days. Dry matter intake (DMI, kg/day) was significantly lower for sheep in the group C+ than in the group C- (-11% on average; P < 0.05), and similar among diets within each group. Methane emission (g/day) was significantly lower for sheep in group C+ than in group C- (-14% on average; P < 0.01), and similar among diets within each group. Methane yield (g/kg DMI) was similar between groups C+ and C-, but differed among diets for both groups (P < 0.01): values were higher (+17% on average) with T diet than with other diets which were similar among them. In our experimental conditions, it can be concluded that enteric methane emission estimated with the SF<sub>6</sub> tracer technique was reliable in rumen cannulated sheep. Forages silages diets including legumes (sainfoin and/or red clover) reduced methane yield in sheep compared to a diet of pure timothy grass.

**Keywords:** enteric CH<sub>4</sub>, SF<sub>6</sub>, sheep, rumen cannula, measuring method

**INTRODUCTION:** The use of the SF<sub>6</sub> tracer technique on rumen cannulated animals offers the attractive option for frequent sampling of rumen contents in order to study the rumen microbial metabolism in conjunction with the estimation of methane (CH<sub>4</sub>) emission. However, it has been suggested that estimation of CH<sub>4</sub> emission with the SF<sub>6</sub> technique could be biased on rumen cannulated animals because of non-uniform leakage of the different gases (SF<sub>6</sub> and CH<sub>4</sub>) via the cannula (Boland et al., 2014). Concerning feeding strategies to mitigate CH<sub>4</sub> emissions in ruminants, the effect of forages is less well documented than that of concentrates (Martin et al., 2010). The effect of forages on CH<sub>4</sub> production per unit of intake (CH<sub>4</sub> yield) is inconsistent probably because of differences in forages composition (stage of maturity, preservation mode, chemical composition, presence of bioactive compounds) and animal genotypes between studies. The aim of this work was to study the effect of rumen cannulation in sheep on CH<sub>4</sub> emission estimated with the SF<sub>6</sub> tracer technique. The impact of different forages silages diets based on mixtures of grass and bioactive legumes on CH<sub>4</sub> emissions was also evaluated.

**1. MATERIAL AND METHODS:** Ten castrated male Texel sheep (12 months old, initial BW of  $48.9 \pm 4.0$  kg and final BW of  $57.5 \pm 4.3$  kg) were allocated into 2 homogenous groups (5 animals each) in which sheep were equipped with a rumen cannula (C+) or without (C-). Each group was fed ad libitum with five silages diets (on a DM basis) of pure timothy (T), mixture of T and sainfoin (T-SF, 50/50), T and red clover (T-RC, 50/50), SF-RC (50/50), T-SF-RC (50/25/25) in a 5 x 5 Latin square design. Each experimental periods comprised an adaptation period to diets (8 days) followed by a measurement period (6 days). Dry matter intake (DMI) was recorded daily. Methane emissions using the SF<sub>6</sub> technique was performed on 4 successive days (Martin et al., 2008). Permeation rate of SF<sub>6</sub> from the tubes was similar between groups and averaged  $1.303 \pm 0.070$  mg/day and  $1.340 \pm 0.063$  mg/day for the group C- and C+, respectively. Permeation tubes were introduced in the rumen 9 days prior the beginning of measurements and remained throughout the experiment. Representative breath samples from each sheep were collected on 24 hours in pre-evacuated collection devices by a capillary tube fitted to a halter. Concentrations of SF<sub>6</sub> and CH<sub>4</sub> in gas samples (breath, ambient) were determined by gas chromatography using a GC fitted with a pulsed discharge electron capture detector for the SF<sub>6</sub>, or with a flame ionisation detector for the CH<sub>4</sub>. Daily CH<sub>4</sub> production for each sheep was calculated using the known permeation rate of SF<sub>6</sub> and the concentrations (above the background) of SF<sub>6</sub> and CH<sub>4</sub> in the breath samples:

$$\text{CH}_4 \text{ (g/day)} = \text{SF}_6 \text{ permeation rate (g/day)} \times \frac{[\text{CH}_4]/[\text{SF}_6]_{\text{breath-ambient}}}{[\text{CH}_4]/[\text{SF}_6]_{\text{molecular weight}}}$$

Data of the two Latin squares were analysed together using the Mixed procedure of SAS (SAS 9.1 release, SAS Inst. Inc., Cary, NC, USA). Model included period, diet, group and diet × group interaction as fixed effects, and the animal nested within diet as random effect.

**2. RESULTS:** Dry matter intake (kg/day) was significantly lower for sheep in the group C+ than in the group C- (-11% on average;  $P_{\text{group}} < 0.05$ ), and similar among diets within each group. Methane emission (g/day) was significantly lower for sheep in group C+ than in group C- (-16% on average;  $P_{\text{group}} < 0.01$ ), and similar among diets within each group. Methane yield (g/kg DMI) was similar between groups C+ and C-, but CH<sub>4</sub> yield differed among diets ( $P_{\text{diet}} < 0.01$ ): values were higher (+17% on average) with T diet than with other diets containing legumes which were similar among them, and that for both groups ( $P_{\text{group} \times \text{diet}} = 0.73$ ).

**3. DISCUSSION:** Daily CH<sub>4</sub> emission (g/day) was lower in rumen cannulated sheep than in sheep without cannula. This was related to differences in DM intake between groups of sheep, and not to the presence of the rumen cannula since, expressed per unit of intake (g/kg DMI), CH<sub>4</sub> yield was similar in sheep with or without rumen cannula. Gas leakages via the cannula cannot be excluded in our study, but the proportions of SF<sub>6</sub> and CH<sub>4</sub> gas lost through it appear similar and without consequence on the CH<sub>4</sub>/SF<sub>6</sub> ratios of concentration in breath gas samples. Our data support the fact that the rumen cannula does not have significant detrimental effects upon CH<sub>4</sub> yield estimation using the SF<sub>6</sub> technique (Boland et al., 2014). However, Beauchemin et al. (2012) pointed out that the type of rumen cannula can affect the extent of gas leakage from the rumen and the use of

the SF<sub>6</sub> technique in cannulated animals. The authors advise to use tight-fitting cannula to minimize gas leakage and to increase the number of experimental animals to overcome additional variability of the SF<sub>6</sub> tracer technique when working with cannulated ruminants.

We observed significant lower values of CH<sub>4</sub> yield when sainfoin and red clover were present in the diet compared to pure timothy grass silage. That means that the presence of these legumes, despite their dilution in mixtures, allowed to reduce methanogenesis in the rumen of sheep. This may be due to the bioactive compounds content of these two legumes (condensed tannins for sainfoin and polyphenol oxidase for red clover) which can potentially mitigate the rumen methanogenesis (Niderkorn et al., 2016). In direct comparisons on sheep, a decrease in CH<sub>4</sub> yield was reported only for pure red clover silage but not for mixtures with cooksfoot silage (Niderkorn et al., 2015). No change in CH<sub>4</sub> yield has been found comparing pure or mixtures of fresh white clover with fresh ryegrass (Niderkorn et al., 2017). All these results confirm that the potential effects of legumes on methanogenesis are inconsistent and need further research.

Table 1. Dry matter intake and methane (CH<sub>4</sub>) emissions in sheep equipped with a rumen cannula (C+) or without (C-) and fed five silages diets including timothy (T), sainfoin (SF) or red clover (RC)

Item	Dry matter intake (DMI, kg/day)			Methane emission (CH <sub>4</sub> , g/day)			Methane yield (CH <sub>4</sub> , g/kg DMI)		
	C+	C-	Mean	C+	C-	Mean	C+	C-	Mean
<b>T (100)</b>	0.95	1.12	<b>1.03</b>	33.8	39.2	<b>36.5</b>	36.0	35.5	<b>35.8</b>
<b>T-SF (50/50)</b>	1.07	1.21	<b>1.14</b>	30.8	38.7	<b>34.7</b>	28.9	32.1	<b>30.5</b>
<b>T-RC (50/50)</b>	1.14	1.19	<b>1.17</b>	31.9	37.7	<b>34.8</b>	28.6	32.0	<b>30.3</b>
<b>SF-RC (50/50)</b>	1.10	1.31	<b>1.20</b>	31.6	35.7	<b>33.7</b>	28.6	27.7	<b>28.1</b>
<b>T-SF-RC (50/25/25)</b>	1.18	1.22	<b>1.20</b>	33.7	36.7	<b>35.2</b>	28.6	30.1	<b>29.4</b>
<i>SEM</i>		0.083			2.59			1.96	
<i>P-value group</i>		< 0.05			< 0.01			0.29	
<i>P-value diet</i>		0.25			0.86			< 0.01	
<i>P-value group × diet</i>		0.81			0.90			0.73	

**CONCLUSION:** In our experimental conditions, it can be concluded that enteric methane emission estimated with the SF<sub>6</sub> tracer technique was reliable in rumen cannulated sheep. From the environmental perspective, forages mixtures including some legume species (sainfoin and/or red clover) reduced methane yield in sheep compared to a forage diet of pure timothy grass.

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## Posters

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The French partnership network on Livestock and Environment organized the 3rd edition of the EMILI conference (EMissions of gas and dust from Livestock) in Saint-Malo (France) from 21-24 May 2017.

This event constituted an opportunity to present the latest scientific advances from research on gas and dust emissions in animal agriculture and contributed to providing information that industry and governments need in order to achieve cost-effective gas and dust mitigation outcomes.

The Conference topics included:

- Measurement methods
- Emission factors and air quality
- Modeling
- Mitigation strategies
- Inventories/Environmental assessment

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