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Effects of forage chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*) on methane emissions *in vitro* and from sheep

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ABSTRACT

Published reports of CH_4 yields as g CH_4/kg dry matter (DM) intake suggest that emissions from sheep fed fresh forage chicory (*Cichorium intybus*) are about 30% lower than from those fed fresh ryegrass. In this study, 2 year old wethers (16; 54 ± 3.8 kg liveweight) were fed either mature chicory or perennial ryegrass at 1.3 times maintenance metabolisable energy requirements in the late spring/early summer of 2009. Methane emissions were determined using individual animal respiration chambers. Feeds differed in their chemical composition with chicory containing 856 g/kg organic matter (OM), 117 g/kg crude protein (CP) and 281 g/kg neutral detergent fibre (aNDF), whereas ryegrass contained 916 g/kg OM, 85 g/kg CP and 499 g/kg aNDF. The DM intake was similar for both forages at 0.76 kg/d, and CH_4 yields did not differ between forages being 22.8 and 23.8 g CH_4/kg DM intake for chicory and ryegrass, respectively. *In vitro* incubations of chicory and perennial ryegrass in the vegetative or mature states had similar CH_4 yields. Despite large differences in chemical composition, especially aNDF, chicory and ryegrass had similar CH_4 yields *in vitro* and *in vivo*. Chicory is not a viable alternative to perennial ryegrass for mitigating CH_4 in pastoral based sheep production systems.

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1. Introduction

Rumen fermentation produces CH_4, which is a greenhouse gas (GHG) emission. Methane accounts for 31% (CO_2-eqv) of the New Zealand GHG inventory and two-thirds of all agricultural emissions (New Zealand Ministry for the Environment, 2009). As CH_4 emissions from forage diets are typically 6–9% of gross energy intake (Johnson et al., 2000), its mitigation would lower GHG emissions and possibly provide more feed energy for meat and milk production.

Forage chicory, a member of the dicotyledons family Asteraceae, is being increasingly used as a forage herb in New Zealand. It is palatable, highly digestible and results in a higher voluntary feed intake and liveweight (LW) gain in ruminants

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; C2/C3, ratio of acetate to propionate; CP, crude protein; DM, dry matter; FID, flame ionisation detector; GC, gas chromatography; GHG, greenhouse gas; HWSC, hot water soluble carbohydrate; LW, liveweight; OM, organic matter; RFC, readily fermentable carbohydrate; SF_6, sulphur hexafluoride; VFA, volatile fatty acid.

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than perennial ryegrass based pasture (Barry, 1998). Chicory also produces a high dry matter (DM) yield, is drought tolerant and can be grown in regions throughout New Zealand (Li and Kemp, 2005).

Compared to perennial ryegrass, chicory has been reported to reduce CH_4 emissions by up to 30% as measured by the sulphur hexafluoride (SF_6) tracer technique (Waghorn et al., 2002; Swainson et al., 2008). Out objectives were to compare CH_4 emissions from wethers fed pre-flowering chicory or flowering perennial ryegrass at the same level of DM intake using respiration chambers. *In vitro* CH_4 emissions were also examined from the two forages at both the vegetative and flowering stages.

2. Materials and methods

2.1. In vivo experiment

2.1.1. Forages

Forage chicory (*cv*. Choice) was harvested just prior to flowering at a height of ~80 cm from a 120 m × 20 m plot at AgResearch Grasslands in Palmerston North (New Zealand). Stems accounted for 0.3 of harvested forage and leaves for 0.7. The chicory sward was established in a Manawatu fine sandy loam on 31 October 2008 and grazed regularly. Prior to harvest, the sward was heavily grazed on 23 September 2009, then fertilised with 75 kg/ha of a mixed fertiliser (ammonium sulphate:urea = 400:600; containing 307 g N/kg and 144 g S/kg) on 13 October 2009 and 60 kg/ha of urea on 28 October 2009.

Perennial ryegrass (*cv*. a mixture of Delish and Banquet) was harvested in the early flowering stage from Aorangi Experimental Station in Palmerston North. Prior to the experiment, 300 kg/ha of superphosphate was applied in autumn and grazed by the sheep up until 22 September 2009.

The study was in November 2009, with forages harvested daily in late morning (*i.e.*, 10:00–12:00 h) using a sickle bar mower and kept in a cold room at 4 °C until fed that afternoon and the following morning. Each day, 5 samples of ~200 g fresh weight each were dried, 3 at 105 °C for 24 h for DM determination and 2 at 65 °C for 48 h for chemical analysis. Samples from the 1st–2nd d and the 3rd–4th d during the CH_4 measurement period were separately pooled for chemical analysis.

2.1.2. Animals and feeding

Two year old Romney wethers (16; 54 ± 3.8 kg LW) were stratified by weight and randomly allocated to one of two groups ($n = 8$), with one group fed fresh chicory and other fresh perennial ryegrass, both at 1.3 times maintenance metabolisable energy requirements (Australian Agricultural Council, 1990). Wethers were drenched before the study with 5 ml of Scanda® (Schering-Plough Animal Health New Zealand Ltd., Wellington, New Zealand) to control intestinal nematodes. The study was in late spring/early summer from 18 November to 3 December 2009. During the adaptation period, wethers were fed the respective forage in two groups for 7 d and then individually in metabolic crates for 5 d with equal portions of forage provided at 09:00 and 16:00 h. Water was freely available at all times. Wethers were transferred to chambers for measurements of CH_4 and H_2. Prior to the study, animal ethics approval was obtained from the AgResearch Animal Ethics Committee.

2.1.3. Determination of methane emissions

An open circuit sheep respiration chamber system of 8 individual chambers was used to measure CH_4 emissions (Pinares-Patiño et al., 2008). Concentrations of CH_4 and H_2 were continuously measured for 48 h in conjunction with air flow. The product of flow rate and concentration per unit time was calculated for CH_4 and H_2 in inflow and outflow air with differences being indicative of emissions. Chambers were maintained at 21 °C with a relative humidity of 80% and the CO_2 concentration in the chamber was monitored continuously to ensure no accumulation.

Eight sheep, 4 from each group, were selected and transferred to individual respiration chambers in two batches and alternated every 3rd day. In the chambers, sheep were fed equal portions of forage at 09:00 and 16:00 h, with free access to water. Before each feeding, refusals were collected to determine dry weight (65 °C, 48 h), and urine collection trays and water were replaced so the chambers were open for about 30 min at each feeding. Gas measurements were made for about 23 h/d with the missing collection time being estimated by extrapolation.

2.1.4. Rumen sampling

Rumen fluid samples were collected using a stomach tube at 11:00 h after emission measurements in the chamber were completed. A subsample of 1.8 ml was centrifuged at 20,000 × *g* for 10 min at 20 °C, and 900 μl of supernatant was mixed with 100 μl internal standard containing 200 μl/ml ortho-phosphoric acid and 20.0 mM 2-ethyl butyric acid. The sample was stored at −16 °C. Prior to analysis, the sample was thawed, centrifuged as previously and supernatant used for volatile fatty acid (VFA) analysis.

2.2. In vitro experiment

2.2.1. Substrates

Samples of forages collected in the *in vivo* experiment were also used in an *in vitro* experiment. In addition, samples of vegetative chicory and perennial ryegrass were collected from the same paddock in April. Harvested forages (*i.e.*, 2 kg fresh weight) were stored at −16 °C. Two weeks before the *in vitro* experiment, half of the forage in each sample was freeze dried