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Methane emissions and productivity of defaunated and refaunated sheep while grazing

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ABSTRACT

Rumen protozoa produce hydrogen, which can be utilised by methanogens to produce enteric methane (CH_4) that is a loss of digested energy and has an adverse environmental impact as a greenhouse gas. The aim of this study was to examine the effect of the absence of rumen protozoa on pasture intake, ruminal fermentation and enteric CH_4 production and performance of grazing sheep. An incomplete crossover experiment was conducted with eleven crossbred ewes (6 without [defaunated] and 5 with protozoa [refaunated]) on 2 × 2 ha pastures with daily CH_4 production (DMP) being measured by GreenFeed Emission Monitoring (GEM) units. Grazing defaunated sheep exhibited a lower concentration of rumen ammonia ($P = 0.01$), but similar concentrations of total rumen volatile fatty acids compared to refaunated sheep ($P > 0.05$). The molar proportion of acetate was decreased and butyrate proportion was increased by defaunation, while the proportion of propionate was unchanged. Estimated pasture intake was not different between defaunated and refaunated sheep ($P > 0.05$). Defaunated sheep tended to have a higher total dry matter intake (tDMI; $P = 0.06$), being the sum of pasture intake and pellet supplement intake. There was a tendency towards a lower CH_4 yield (g CH_4/kg tDMI; $P = 0.07$) in defaunated sheep, but no differences in average daily gain or wool growth occurred due to defaunation.

1. Introduction

The requirement to produce at least 70% more food in order to feed 9 billion people by 2050 (World Bank, 2008; Bijl et al., 2017) is a major challenge to animal production. The livestock population has surged in many developing countries in response to this rapid growing demand for livestock products and is forecast to rise further (FAO, 2006; Herrero et al., 2014). In association with an increased global human demand for food, an increased livestock products result in increased greenhouse gas (GHG) emissions which are expected to increase in coming years (van Beek et al., 2010). Recent meta-analysis confirms that removal of ciliate protozoa from the rumen of ruminants can increase livestock average daily gain (ADG) by 9% and reduce enteric methane (CH_4) emissions by 11% (Newbold et al., 2015). The positive effect of defaunation on animal growth is often seen with poor quality roughage diets that are low in nitrogen content and provide insufficient rumen degradable protein for the growth of rumen microbes (Bird and Leng 1978; William and Coleman, 1992). This may be advantageous in the tropics where forages are often deficient in protein and have higher fibre content than do temperate grasses (Minson, 1990). Since higher fibre content is associated with a great CH_4 yield (g CH_4/kg DMI; Margan et al., 1988; Pelchen and Peters 1998), a greater CH_4 yield as well as reduced animal performance can be expected to coincide if dietary fibre content increases. This suggests that elimination of rumen protozoa can improve growth and reduce CH_4 emissions of ruminants grazing on tropical or other low quality forages. However, there is little data from grazing animals available (Bird and Leng, 1984; Hegarty et al., 2000) and no grazing CH_4 production data. This study was conducted to quantify whether effects of defaunation on volatile fatty acid, intake, wool growth and CH_4 production observed in controlled feeding studies are also evident in grazing environments.

2. Materials and methods

2.1. Animals and experimental procedures

The animals and defaunation treatments were described previously (Nguyen et al., 2016). Briefly, twelve crossbred ewes (Border Leicester rams × Merino ewes) about 30 months of age, were defaunated by treatment with sodium 1-(2-sulfonatooxyethoxy) dodecane (Empicol ESB/70AV, Albright and Wilson Australia Ltd, Melbourne) administered at 10 g/day in a 10% v/v solution for three consecutive days.

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Table 1
Experimental schedule for animal rotation and pasture and animal data measurements.

Period	Day	Activity
	−14	Sheep were shorn and adapted to the pasture environment in 2 paddocks adjacent to experimental paddocks.
	−4	Rumen fluid for VFA, ammonia and protozoa were collected, liveweight and mid-side patches were measured.
Period 1	0–14	Defaunated and refaunated sheep grazed on experimental paddocks 1a and 2a respectively.
	14–28	Defaunated and refaunated sheep grazed on experimental paddocks 1b and 2b respectively.
	24–28	Faecal sampling
	28	Rumen fluid for VFA, ammonia and protozoa were collected and liveweight were measured
Period 2	28–42	Defaunated and refaunated sheep grazed on experimental paddocks 2a and 1a respectively.
	42–56	Defaunated and refaunated sheep grazed on experimental paddocks 2b and 1b respectively.
	49–53	Faecal sampling
	56	Rumen fluid for VFA, ammonia and protozoa were collected liveweight, mid-side patches and whole body fleece weight were measured

Daily methane production (DMP) was measured by GreenFeed emission monitors at all times but data for DMP was based on emissions measured in the final week of each period.

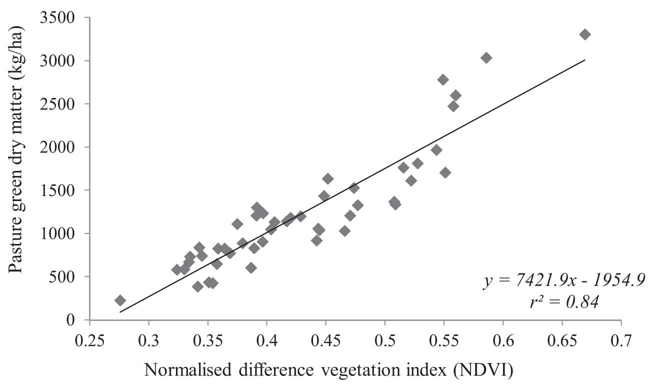

Fig. 1. The relationship between green dry matter biomass (GDM; kg DM/ha) measured on cut quadrats of pasture and normalised difference vegetation index (NDVI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

After defaunation one sheep was slow to recover appetite, so was removed from the study. Eighteen weeks after defaunation, 5 defaunated sheep were re-inoculated (50 mL per sheep per day on 2 consecutive days) with fresh mixed rumen fluid collected from five rumen cannulated sheep fed roughage.Rumen protozoal population from 5 refaunated sheep were plateaued after a period of 8 weeks.

Ewes with initial liveweight (± s.e.m) of 56.7 ± 1.9 kg (defaunated; n = 6) and 57.9 ± 2.0 kg (refaunated; n = 5) were adapted to the pasture environments in two paddocks adjacent to each other and immediately adjacent to the paddocks to be used in this experiment. After 2 weeks of adaptation to pasture, a 56 day grazing study was conducted with the two groups of sheep managed on 14 day rotation through 2 × 2 ha pastures in which each paddock (1,2) was partitioned into two halves (a, b; Table 1). This enabled sheep to use half of a paddock for 14d then to be moved to the other half for the following 14d when faecal samples were collected for marker analysis. This was done to ensure that the pasture on-offer and consumed in the second 14d was the same as had been available in the first 14d. There was a 14 day rest period for each paddock before it was re-grazed in the rotation and the rotation was arranged so that paddock did not bias the estimate of growth or intake by sheep over the 56 day rotational grazing study. Because two full rotations were made (each group grazed each paddock twice), pasture attributes were analysed for period 1 (rotation 1: d1-28) and period 2 (rotation 2: day 29–56) and the interaction of period × treatment tested (Table 1). Since pasture dry matter on offer was greater than 1 ton per ha, the slight difference in the number of sheep per group would not have influenced the potential intake of individual sheep (CSIRO, 2007). Animals were weighed at the start and end of each period and average daily gain (ADG) calculated.

Two GreenFeed Emissions Monitors (GEM; C-Lock Inc, Rapid City, SD, USA) were continuously used to measure daily methane production (DMP) and daily CO_2 production of individual sheep throughout the 56 day study, with the GEM units moved during rotation, so sheep had continuous GEM access. A wheat and barley based pelleted supplement (Pryde's EasiRide; Pryde's EasiFeed Gunnedah, NSW) to which chromic oxide has been included prior to pelleting was provided by the GEM to attract sheep to enable DMP measurements to be made.

2.2. Estimation of pasture green dry matter

Assessment of pasture green dry matter (pGDM; kg/ha) on offer was conducted on days −4, 17, 31 and 45 using a Crop Circle™ ACS 210 (Holland Scientific, Lincoln NE, USA) sensor coupled to a Trimble ProXRS differential receiver and Ranger data-logger. The Crop Circle™ sensor emits NIR (880 nm) and red (650 nm) light and measures the reflectance coming back from the plant canopy (Lamb et al., 2009). The values obtained from the device were calibrated against gravimetrically determined pGDM at the time of measurement by taking stationary readings of three 30 × 30 cm quadrats that were then cut, dried and DM/ha calculated. The Normalised Difference Vegetation Index (NDVI) was calculated from the individual light reflectance values (NDVI = $(NIR_{(reflectance)} - Red_{(reflectance)})/(NIR_{(reflectance)} + Red_{(reflectance)})$ and was correlated with total pGDM (Trotter et al., 2010). Estimates of pGDM for each NDVI were then calculated using the equation below developed over the same paddocks (McPhee et al., 2010) with the calibration data being shown in Fig. 1.

$$\text{Pasture green dry matter (kg/ha)} = 37.73 \times e^{5.86 \times NDVI} \quad (1)$$

As the standing dead portion of the sample does not contribute to the Crop Circle™ response, it was not measured by the NDVI, so three quadrat pasture samples in each paddock were also cut and dried in a fan-force oven at 60 °C until constant weight. These pasture samples were further partitioned into green and dead proportions to determine their individual biomass with samples of green and dead pastures. The green:dead ratio was multiplied by the NDVI measure for pGDM to estimate average pasture dead dry matter (pDDM) over the entire paddock and the quadrat samples analysed for chemical composition using near-infrared spectroscopy (AFIA, 2014; Table 2).

2.3. Predicted dry matter intake and dry matter digestibility

The GrazFeed modelling software is a computer programme that provides a calculation of the energy and protein requirements of sheep and cattle grazing and an estimation of the amount of food eaten that grazing animals are able to select from a particular pasture (Freer et al., 1997). This model was used to estimate probable pasture dry matter intake (pDMI) for sheep in this study based on the quality and quantity of pasture green and dead DM (Table 2) and animal information. GrazFeed also estimated likely pasture selection by sheep, and predicted dry matter digestibility (DMD), crude protein (CP) and metabolisable energy (ME) of pasture ingested are reported (Table 3). Total dry matter intake (tDMI) was calculated as the sum of pasture (pDMI)