Ministry for Primary Industries
Manatū Ahu Matua

# Forage brassicas: a tool for the mitigation of methane and nitrous oxide?

## Final Report

## Effect of forage rape on GHG emissions from sheep

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# Contents

# Public summary

We evaluated the potential of forage rape (*Brassica napus* L.) to mitigate animal-level methane and nitrous oxide emissions when fed to growing sheep. In a previous study, funded by the PGgRc, feeding forage rape to sheep reduced methane emissions per unit of feed eaten by 25% compared with those from sheep fed ryegrass. The current research programme had the following objectives: 1) to confirm the results from our previous study, 2) to evaluate the mitigation potential of forage rape if fed to growing lambs over an extended period of time (15 weeks) and 3) to assess the nitrous oxide emissions from urine and dung of sheep fed the brassica crop, ensuring that reductions in one greenhouse gas (methane) are not counteracted by increased in the other (nitrous oxide).

Methane emissions from lambs were measured in two periods, after 7 or 15 weeks of continuous feeding with diets consisting of 100% forage rape or perennial ryegrass. Methane production was measured using the open-circuit respiration chambers for sheep at the New Zealand Ruminant Methane Measurement Centre, in AgResearch Grasslands, Palmerston North. The results of the animal trials indicated that sheep fed forage rape emitted 30% less methane per unit of dry matter intake than those fed perennial ryegrass in the first period (7 wks), and 20% less in the second period (15 wks). This reduction was also observed when values were expressed in terms of methane emissions per unit of organic matter or digestible dry matter. These results confirmed our previous report and also suggested that the effect of forage rape feeding on enteric methane emissions is persistent for at least ~3 months.

The proportion of dietary nitrogen intake that was partitioned to urine was similar for both forage rape and ryegrass, suggesting that animal N use efficiency was similar for both forages. Samples of urine and dung were collected for measuring nitrous oxide emissions from sheep excreta applied to soils. The duration of nitrous oxide flux peaks from the application of urine from sheep fed forage rape was much shorter than that from urine of sheep fed ryegrass. This difference in duration was associated with differences in the concentrations of mineral nitrogen in the soil after application of urine from sheep fed the two types of forage. Consequently, the nitrous oxide emission factor (EF3) of urine from sheep fed forage rape (0.110%) was 59% lower than that of urine from sheep fed ryegrass (0.269%). Sheep dung N transformation rates in the soil were slightly slower for dung from sheep fed forage rape compared with dung from sheep fed ryegrass.

The mechanisms behind the measured reduction in methane and nitrous oxide emissions from sheep fed forage rape are not yet fully elucidated. Further research on understanding these mechanisms is required and would be useful to ensure that the potential of forage rape as a greenhouse mitigation tool is realised in a consistent and predictable manner.

The reduction in methane emissions per unit of intake, together with the measured reduction in nitrous oxide emission factor suggest that forage rape could be a valuable option to reduce greenhouse gas emissions from livestock production systems in New Zealand. Grazing experiments to confirm the results obtained in respiration chambers, together with the evaluation of other brassica types and ruminant livestock classes are required before the results from the current programme can be implemented in practice.

# Methane ($CH_4$) emissions from sheep fed fresh rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.)

By Xuezhao Sun and David Pacheco

## SUMMARY

Forage rape (*Brassica napus* L. var. Titan) was compared with perennial ryegrass (*Lolium perenne* L. var. Ceres One 50 containing endophyte AR1) to confirm the potential of rape to mitigate methane emissions from sheep, as identified in a previous study (Sun et al., 2012b).

Forty-two, 9-month-old Romney male lambs were randomly allocated to either forage rape or perennial ryegrass feeding. After adaptation to the diets under grazing conditions, the sheep were transferred indoors and fed at 1.5 times their metabolic energy maintenance requirement. Methane emissions were measured using open circuit respiration chambers following consumption of the designated diets for 7 weeks (the first period of measurement) and 15 weeks (the second period). Apparent digestibilities and metabolisable energy (ME) contents of the diets and rumen fermentation and blood profile parameters were also determined. Conventional chemical composition, nitrate, sulphur, sulphate, glucosinolates and S-methyl cysteine sulfoxide (SMCO) concentrations in forage were analysed for their effects on methane emissions.

The main findings include:

- Sheep fed forage rape emitted 30% less methane per unit of dry matter intake than those fed perennial ryegrass in the first period (7 weeks), and 20% less in the second period (15 weeks). This confirms reported findings (Sun et al., 2012b) from our previous PGgRc-funded project and also suggests a persistent effect of rape feeding on enteric methane emissions for up to 3 months.
- Despite similar organic matter contents, rape contained more readily fermentable carbohydrates and less structural carbohydrates than ryegrass. The crude protein content of rape was slightly higher than, or similar to, ryegrass.The chemical composition of forages did not appear to be related to methane yield, which is consistent with previous studies at AgResearch with alternative forages including chicory and white clover.
- The apparent digestibilities of dry matter and organic matter were greater for rape than for ryegrass, resulting in greater ME content in rape than in ryegrass (11.0 *versus* 8.6 MJ/kg DM in the first period, 12.4 *versus* 10.9 MJ/kg DM in the second period, respectively).
- Sheep fed forage rape had lower molar proportions of acetate and more propionate in their rumen fluid than sheep fed ryegrass, which might be associated with the lower methane emissions of rape.
- Sheep fed forage rape did not exhibit any health problems.

In conclusion, compared with perennial ryegrass, forage rape reduced methane emissions from sheep by 20-30% for up to 3 months. This suggests that forage rape, and possibly other forage brassicas, could be a potential mitigation tool for pastoral based sheep production systems. The reasons for the reduction in methane emissions measured from forage rape are not clear yet and need to be elucidated.

## INTRODUCTION

Methane ($CH_4$) accounts for 37% of total national greenhouse gas (GHG) emissions in New Zealand (Ministry for the Environment, 2011) of which 85% originates from enteric

fermentation in grazing ruminants. Some options have been proposed to mitigate enteric $CH_4$ emissions, including rumen microbial manipulation, dietary additives, animal selection and livestock system improvements (Cottle et al. 2011). New Zealand agriculture is based on pastoral animal production systems. Therefore, some of these mitigation options have limited practical application in New Zealand.

Forage-based mitigation tools would most likely be accepted and incorporated into current pastoral livestock systems. Attempts to find low $CH_4$ forages were initially made with chicory and white clover, but the results reported have not been consistent (Waghorn et al., 2002; Swainson et al., 2008; Sun et al., 2011, 2012a; Hammond et al. 2011). Recently, brassica forages were found to reduce $CH_4$ emissions from sheep, with the mitigation effect being largest for forage rape (Sun et al., 2012b). In the latter study, sheep fed forage rape emitted 25% less methane per unit of dry matter intake compared with sheep fed perennial ryegrass. However, this result was observed in a single trial, with sheep fed rape for 6-7 weeks.

The objective of this study was to confirm the previous finding (Sun et al. 2012b) that $CH_4$ emissions per unit of dry matter eaten by sheep fed forage rape were reduced compared with sheep fed perennial ryegrass and to test whether this effect might persist after longer feeding periods representative of those used in practical farming (e.g. 3 months).

## MATERIALS AND METHODS

### Experimental design

The experiment was conducted at AgResearch Aorangi Experimental Station and AgResearch Grasslands, Palmerston North, New Zealand. The trial compared the methane emissions from lambs fed forage rape with those fed perennial ryegrass. Forty two sheep were used in the study with 24 fed forage rape and 18 fed ryegrass. We used a larger number of animals in the forage rape group to account for the greater variability in methane emissions measured in our previous study (Sun et al. 2012b). Acclimation to diets was initiated at AgResearch Aorangi Experimental Station, then at AgResearch Grasslands for further adaptation to indoor conditions. Animal ethics approval was obtained in advance from AgResearch Grasslands Animal Ethics Committee (Approval no. 12320).

All lambs were grazing a ryegrass pasture before the trial. Lambs allocated to the rape treatment grazed rape for 8 h a day in the first two days, for 16 h in the second two days and thereafter full time, with the balance of time spent grazing ryegrass-based pasture. During this time, lambs allocated to the ryegrass treatment remained grazing on ryegrass sward. After 41 days adaptation to diet treatments, well adapted sheep were transferred to AgResearch Grasslands for indoor trials (27 June – 14 July 2011). Sheep were considered well adapted based on general appearance and liveweight gain achieved during the adaptation period. The animals were fed in groups (8-9 sheep each group) in pens for 3 days and then individually in metabolic crates for 5 days for acclimatisation to housing conditions. On the second day in metabolic crates (day 45 of the trial), rumen fluid and blood samples were taken after feeding. Methane emissions were then determined for 2 consecutive days in respiration chambers> Methane measurements were conducted in two batches, with 12 sheep from the rape treatment and 9 from the ryegrass treatment in each batch. After the methane measurement in chambers, additional rumen samples were taken before feeding on days 51 and 53. Six sheep were randomly selected from each forage treatment and used to determine apparent total tract digestibility and metabolisable energy in metabolic crates for 7 d.
After the first period of measurements, sheep were returned to grazing paddocks of their designated forage at the Aorangi Station for 38 days before returning indoors for a second period of measurements, which followed similar sequence of events as described above.

## Forages

Forage rape (*Brassica napus* L. *var.* Titan) and perennial ryegrass (*Lolium perenne* L. *var*. Ceres One 50 containing endophyte AR1) swards grazed at the AgResearch Aorangi Station were pure swards and in the vegetative state.

The forage rape harvested for the indoor experimental periods was established in a paddock (150 m x 20 m) at AgResearch Grasslands on 3 March 2011 in Manawatu fine sandy loam soil with sowing rate at 4.7 kg/ha and diammonium phosphate applied at the rate of 140 kg/ha at sowing.

The perennial ryegrass harvested for the indoor experimental periods was established in autumn 2008. The paddock received 200 kg/ha of superphosphate containing P 93 g/kg, S 108 g/kg and Ca 200 g/kg (Ravensdown Limited, Hornby, New Zealand) on 12 April 2011 and 60 kg/ha urea containing 460 g N/kg on 5 May 2011. Before harvest, the paddock was grazed by sheep on 9 May 2011.

Forages fed to sheep during the indoor trial periods were harvested daily in mornings (10:30 to 12:00 h) using a sickle bar mower. Both rape and perennial ryegrass were in the vegetative state. Rape plant height was *ca*. 70 cm, stem height *ca*. 20 cm in the first period; and *ca*. 68 cm and *ca*. 27 cm, respectively, in the second period, with stubble height *ca*. 10 cm after cutting. Harvested rape contained 76% green leaves, 16% stems and 8% dead leaves with weeds less than 0.5% in the first period and 66% green leaves, 24% stems and 9% dead leaves with weeds less than 0.1% in the second period. Perennial ryegrass was harvested at *ca*. 25 cm in height in the first period and *ca*. 34 cm in the second period with a stubble height at *ca*. 5 cm. Harvested ryegrass had no broadleaf weeds.

After harvest, forage was stored in a cold room (4 °C) for the afternoon meal on the day of harvest and morning meal the next day. Triplicate samples (*ca*. 50 g in fresh each) of each forage were dried at 105 $^{o}$C for dry matter (DM) determination, and one sample dried at 65 $^{o}$C for 48 h in preparation for subsequent chemical analysis. During the methane measurement period, an additional forage sample was collected daily and frozen for later analysis of SMCO after freeze-drying.

## Animals and feeding

Forty two healthy 9-month-old Romney male lambs (32.4 ±0.6 kg, mean ± S.D.) were drenched before the first period on 17 June 2011with a dose of 4 ml anthelmintic containing 8 mg Abamectin, 320 mg Levamisol, 20 mg cobalt and 4 mg selenium (Converge, Intervet Schering-Plough Animal Health Ltd., Wellington, New Zealand). Lambs were then randomly allocated to two groups. Lambs in the rape group were given intramuscular injections of 1.5 ml iodised peanut oil containing 390 mg of organically bound iodine (Flexidine, Bomac Export Limited Auckland, New Zealand) and dosed orally with a copper capsule containing 4 g of CuO (Bayer New Zealand Limited, North Shore, New Zealand) in order to prevent mineral deficiencies in this group. During the grazing periods, salt blocks were supplied to the sheep in both forage treatments. Live weight was monitored weekly throughout the grazing period.

After 41 days grazing adaptation to the designated forages on paddocks at the AgResearch Aorangi Experimental Station, lambs (40.0 ± 2.1 kg for the rape group; 40.7 ± 1.6 kg for the ryegrass group) were transferred to AgResearch Grasslands for the indoor trial. All animals were drenched again with 9 ml of anthelmintic containing 9 mg abamectin, 360 mg levamisole, and 204 mg oxfendazole (Merial New Zealand Ltd, Auckland, New Zealand). During the indoor acclimatisation and experimental periods, the animals were provided fresh

forage at a feeding level of 1.5 times maintenance energy requirements (Australian Agricultural Council, 1990) using the metabolic energy (ME) content of forage estimated using near infrared reflectance spectroscopy (NIRS; Bruker Optics, model MPA, Ettlingen, Germany). Forage was provided in two equal meals at 09:00 and 16:00 h with free access to water at all times.

### Determination of methane and hydrogen emissions

The sheep respiration chamber facility at the New Zealand Ruminant Methane Measurement Centre (AgResearch Limited, Palmerston North, New Zealand) was used to determine methane and hydrogen emissions. The chamber design and operation of this facility has been described in detail by Pinares-Patiño et al. (2011). Briefly, each chamber is 1.8 m long, 0.85 m wide and 1.2 m high, with a fresh air inlet in the front and an exhausted air outlet in the back. The facility comprises three clusters, each of them including 8 chambers. Air flow through the chamber is achieved by tandem air pumps (UNI-JET 40, ESAM, Parma, Italy) blowing fresh air to the inlet at a constant flow rate which is measured continuously by differential pressure using a custom made Venturi flow meter fitted with Vaisala PTB 100 barometric sensors (Vaisala Oyj, Helsinki, Finland). Each system is equipped with a multi-gas analyser (4900C Continuous Emission Analyser, Servomex Group Ltd., Crowborough, East Sussex, UK) and an electrochemical hydrogen detector (7HYT Citicel, City Technology Ltd., Portsmouth, Hampshire, UK) to measure methane and hydrogen concentrations in the outflow air. The sensitivity of the measurement is 0.5 and 5 μl/L, detection range 0-200 and 0-50 μl/L, recovery rate 98.2 ±0.60 and 100.5 ± 4.01, for methane and hydrogen, respectively. The outflow air from each chamber in a system as well as ambient air is sequentially measured in a cycle. Each cycle takes 6.8-7.2 min. Just before the chamber doors were closed for emission determinations, each system was calibrated using standard gas mix containing 200±4 ppm $CH_4$, 2000±20 ppm $CO_2$, 21.1±0.1% $O_2$ and 50±1 ppm $H_2$ with $N_2$ balance (BOC Limited, Auckland, New Zealand). The environmental parameters, including temperature, relative humidity, and $CO_2$ concentration inside the chambers, were monitored to comply with animal welfare regulations. Ventilation rate was monitored to ensure the value in the range of 250-265 L/min for reliable data output. Emissions of $CH_4$, $H_2$ and $CO_2$ were calculated from the product of flow rate and the differences in concentrations between the outflow and inflow air.

Each individual chamber allows for measurement of methane emissions from individual sheep. The chamber door was opened for feeding at 8:00 and 16:00 h for *ca*. 15 min each ocassion. At the same time, feed refusals were collected, chambers cleaned, faeces and urine trays under the metabolic crates replaced with clean ones and drinking water changed. The data lost whilst chamber doors were opened were estimated using the mean value of the previous 10 readings immediately before opening of doors.

### Measurement of apparent digestibility and metabolisable energy

After methane measurements, 6 random animals of each dietary treatment were used to measure apparent total tract digestibility and metabolisable energy in metabolic crates. Methods used to determine apparent digestibility and metabolisable energy are described by Sun et al. (2012b). In brief, forage offered and refused and faeces excreted were collected daily for 7 consecutive days and dried at 65 $^{o}$C for 48 h. Forage offered was pooled for each treatment group. Refusal samples were weighed after drying and pooled for each animal. Faeces were collected using faecal bags, subsampled for drying, and pooled for each animal. Urine was collected in a bucket with sulphuric acid (100 ml, 6 M) added to minimise nitrogen (N) losses due to ammonia volatilisation. Urine was weighed daily, subsampled and pooled for each animal. All solid samples were ground through to a 1 mm screen using a Wiley mill for analyses of chemical composition and gross energy. Urine samples were analysed for N content and gross energy. Energy loss from methane was estimated for the digestibility period

using the methane emissions per unit of feed intake directly determined during the respiration chamber measurement period.

### Rumen fluid and blood sampling

The collection of rumen fluid samples (*ca*. 50 ml) was conducted using stomach tubing both before feeding (9:30-10:00 h) on the day 45 of the trial and 2-3 h after feeding (10:30-11:30 h) on days 51 and 53. An aliquot (1.8 ml) of the sample was immediately treated with ortophosphoric acid containing ethyl-butyric acid as internal standard (Sun et al. 2011) for subsequent volatile fatty acid (VFA) determination. In the second period of the experiment, rumen samples were collected at 9:00 h (before feeding), 11:00, 13:00, 15:00; 17:00; 19:00 and 21:00 h on the day and 9:00 h (before feeding) next day.

Blood samples were taken from the jugular vein on day 45 for determination of methaemoglobin and haemoglobin concentrations and haematocrit. The samples for methaemoglobin determination were taken randomly from 5 lambs in the rape group and 3 in the ryegrass group at 10:30 h 1.5 h after the feeding. Blood collection evacuated tubes with lithium heparin anticoagulant (Vacutainer, BD, Franklin Lakes, NJ, USA) were filled, immediately stored over ice in an insulated bin and dispatched for analyses within 1 h of sampling. The analysis was conducted by visible optical spectroscopy using a blood gas analyser (ABL 800 FLEX; Radiometer, Copenhagen, Denmark) by Medlab Central Limited, (Palmerston North, New Zealand). Blood samples for haemoglobin and haematocrit determinations were taken at 14:30 h from all animals into Vacutainers containing EDTA (BD, Franklin Lakes, NJ, USA). The samples were stored at room temperature and analysed on the same day (New Zealand Veterinary Pathology Ltd., Palmerston North, New Zealand). In the second period, the blood samples were taken from 6 rape-fed and 4 ryegrass-fed sheep for methaemoglobin and from all lambs for haemoglobin and haematocrit determinations.

### Laboratory analyses

Forage samples (n=3, 2 from methane measurement period, 1 from the digestibility period) in both periods were analysed for gross energy, neutral detergent fibre (aNDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude protein (CP), lipid, ash, hot water-soluble carbohydrate (HWSC) and pectin, refusal samples (n=6) and faecal samples (n=6) for gross energy, aNDF, ADF, ash and nitrogen, and urine for gross energy and nitrogen. Gross energy was measured using the total combustion bomb calorimetry method (Method 968.06, AOAC, 2000) and nitrogen in urine determined using a Leco analyser (AC350, Leco Corporation, St. Joseph, MI, USA). Other analyses were conducted as described by Sun et al. (2011).

Forage samples (n=2) collected during the methane measurement period were analysed for the concentrations of nitrate, sulphur and sulphate using methods described by Cataldo et al. (1975), Araújo et al. (2002) and Miller (1998), respectively.

Ground (1 mm) freeze dried feed samples collected during the methane measurement periods were extracted in hot water and supernatants used for determination of glucosinolates and SMCO using HPLC-MS in a method described previously (Sun et al., 2012b).

### Statistical analyses

Forage composition, digestibility, ME and animal blood profile parameters were analysed using one way ANOVA (SAS, 2003) to compare forage rape and perennial ryegrass. Methane emission data were analysed using the linear mixed-effects model with forage treatment as a fixed effect and measurement batch and chamber system as random effects. Rumen fermentation parameters were analysed with sampling time as repeated measurements. The

correlation of methane yield between two experimental periods was analysed using PROC CORR (SAS, 2003). Significant differences between two forages were declared at P<0.05.

# RESULTS

## Forage composition

In the first period, forage rape had a similar organic matter (OM; P=0.834) and lignin (sa) (P=0.323) content to perennial ryegrass (Table 1), but 19% more crude protein (CP; P=0.058) and 17% less lipid (P=0.015) than ryegrass. Hot water-soluble carbohydrates (P=0.023) and pectin contents (P<0.001) were both higher in rape than in ryegrass.

**Table 1: Mean (±SEM) chemical composition of forage rape (*Brassica napus* L.) and perennial ryegrass (*Lolium perenne* L.).**

| Chemical constituent (g/kg DM except as noted) | Forage rape (*n*=3) | | Perennial ryegrass (*n*=3) | | *P* |
|---|---|---|---|---|---|
| **Period 1** | | | | | |
| Dry matter (g/kg)[a] | 131 | ±2.9 | 148 | ±4.8 | 0.005 |
| Organic matter | 852 | ±16.4 | 842 | ±38.4 | 0.834 |
| Crude protein | 215 | ±11.0 | 181 | ±6.3 | 0.058 |
| Lipid | 34 | ±0.9 | 41 | ±1.7 | 0.015 |
| Hot water-soluble carbohydrates | 142 | ±11.9 | 83 | ±11.3 | 0.023 |
| Pectin | 76 | ±2.8 | 9 | ±0.6 | <0.001 |
| Readily fermentable carbohydrates[b] | 218 | ±13.9 | 92 | ±11.8 | 0.002 |
| aNDF | 209 | ±17.5 | 464 | ±23.7 | <0.001 |
| ADF | 161 | ±16.4 | 242 | ±26.1 | 0.059 |
| Hemicellulose | 48 | ±1.2 | 222 | ±5.0 | <0.001 |
| Cellulose | 124 | ±14.6 | 215 | ±25.0 | 0.035 |
| RFC:SC[c] | 1.31 | ±0.214 | 0.21 | ±0.038 | 0.007 |
| Lignin (sa) | 38 | ±9.1 | 27 | ±1.5 | 0.323 |
| **Period 2** | | | | | |
| Dry matter (g/kg)[a] | 142 | ±2.1 | 198 | ±4.6 | <0.001 |
| Organic matter | 917 | ±1.9 | 901 | ±1.9 | 0.003 |
| Crude protein | 158 | ±2.1 | 160 | ±5.5 | 0.742 |
| Lipid | 34 | ±0.9 | 35 | ±1.0 | 0.374 |
| Hot water-soluble carbohydrates | 240 | ±2.4 | 123 | ±8.4 | <0.001 |
| Pectin | 75 | ±1.7 | 11 | ±0.3 | <0.001 |
| Readily fermentable carbohydrates[b] | 315 | ±2.6 | 134 | ±8.4 | <0.001 |
| Andf | 170 | ±4.4 | 445 | ±6.0 | <0.001 |
| ADF | 123 | ±3.5 | 231 | ±3.2 | <0.001 |
| Hemicellulose | 47 | ±0.9 | 214 | ±2.8 | <0.001 |
| Cellulose | 86 | ±5.4 | 214 | ±2.7 | <0.001 |
| RFC:SC[c] | 2.38 | ±0.120 | 0.31 | ±0.017 | <0.001 |
| Lignin (sa) | 37 | ±2.6 | 17 | ±0.6 | 0.002 |

DM = dry matter; aNDF = neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; ADF = acid detergent fibre expressed inclusive of residual ash.
Mean±SEM.
[a]*n* = 18.
[b]Hot water-soluble carbohydrates plus pectin.
[c]Ratio of readily fermentable carbohydrates : structural carbohydrates (hemicellulose + cellulose).

As a result, rape contained 2.37 times the readily fermentable carbohydrates as ryegrass (P=0.002). In contrast, aNDF (P<0.001), ADF (P=0.059), hemicelluloses (P<0.001), and cellulose (P=0.035) contents were all lower in rape than in ryegrass. Thus, the ratio of readily fermentable carbohydrates to structural carbohydrates was higher for rape (1.31) than for ryegrass (0.21, P=0.007).

In the second period, forage rape had a slightly higher OM content (by 1.8%; P=0.003) than ryegrass. The contents of CP (P=0.742) and lipid (P=0.374) were similar for both forages. Forage rape had almost double the concentration of hot water-soluble carbohydrates compared with ryegrass (P<0.001). Pectin content was also higher (P<0.001) in rape than in ryegrass. Total readily fermentable carbohydrates were 2.35 times higher in rape than in ryegrass (P<0.001). All measurements related to the structural carbohydrates (aNDF, ADF, hemicelluloses and cellulose) were much lower in rape than in ryegrass (P<0.001). For example, aNDF content in rape was less than half that in ryegrass. As a result, the ratio of readily fermentable carbohydrates to structural carbohydrates was much higher for rape (2.38) than for ryegrass (0.31, P<0.001). Although lignin(sa) content in rape was similar to that in the first period, it was higher than in ryegrass (P=0.002) in the second period.

### Methane emissions

During the first period of methane measurements, the same amount of feed was provided to sheep in both forage treatments, but sheep refused more ryegrass (8.7% of DM offered) than rape (4.1%; P=0.007). As a result, DM intake of sheep fed rape was slightly higher (P=0.006) than of sheep fed ryegrass (Table 2). However, $CH_4$ production (g/d) was lower (P<0.001) for rape than for ryegrass. In terms of $CH_4$ yield (g/kg DM intake), rape (13.6) was lower by 30% (P<0.001) compared with ryegrass (19.5). This led to a lower proportion of energy loss from $CH_4$ emissions in total gross energy intake for rape than for ryegrass (P=0.002). $H_2$ was also emitted at twice the rate from sheep fed rape compared with those fed ryegrass (P=0.109). $CO_2$ released from sheep was similar for both treatments, but the ratio of $CH_4$ to $CO_2$ was lower for rape than for ryegrass (P<0.001).

**Table 2: Mean (±SEM) methane, hydrogen and carbon dioxide emissions from sheep fed fresh forage rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.).**

| | Forage rape (n=24) | | Perennial ryegrass (n=18) | | *P* |
|---|---|---|---|---|---|
| **Period 1** | | | | | |
| DM intake (g/d) | 862 | ±8.1 | 792 | ±25.9 | 0.006 |
| $CH_4$ (g/d) | 11.7 | ±0.48 | 15.4 | ±0.97 | <0.001 |
| $CH_4$ (g/kg DM intake) | 13.6 | ±0.52 | 19.5 | ±1.14 | <0.001 |
| $H_2$ (g/kg DM intake) | 0.026 | ±0.004 | 0.010 | ±0.001 | 0.109 |
| $CO_2$ (g/kg DM intake) | 1005 | ±6.8 | 1019 | ±22.2 | 0.480 |
| $CH_4/CO_2$ | 0.014 | ±0.0005 | 0.019 | ±0.0011 | <0.001 |
| $CH_4$ energy loss/gross energy intake | 0.050 | ±0.0019 | 0.063 | ±0.0039 | 0.002 |
| **Period 2** | | | | | |
| DM intake (g/d) | 896 | ±8.4 | 929 | ±20.8 | 0.116 |
| $CH_4$ (g/d) | 16.0 | ±0.60 | 21.2 | ±0.50 | <0.001 |
| $CH_4$ (g/kg DM intake) | 17.8 | ±0.64 | 22.9 | ±0.45 | <0.001 |
| $H_2$ (g/kg DM intake) | 0.037 | ±0.008 | 0.033 | ±0.006 | 0.746 |
| $CO_2$ (g/kg DM intake) | 1190 | ±10.7 | 1065 | ±16.3 | <0.001 |
| $CH_4/CO_2$ | 0.015 | ±0.0005 | 0.021 | ±0.0004 | <0.001 |
| $CH_4$ energy loss/gross energy intake | 0.058 | ±0.0021 | 0.073 | ±0.0014 | <0.001 |

DM = dry matter.

During the second period, sheep refused a similar amount of ryegrass (8.8% of DM offered) and rape (7.4%; P=0.638) and DM intake was similar (P=0.116). Both $CH_4$ production (g/d) and yield (g/kg DM intake and % of gross energy intake) were lower for rape than for ryegrass (P<0.001).

$CH_4$ yields of individual animals were analysed to determine the relationship between the two periods of measurements (Figure 1). The results indicated that $CH_4$ yields were highly correlated between two periods (r= 0.792, P< 0.001).

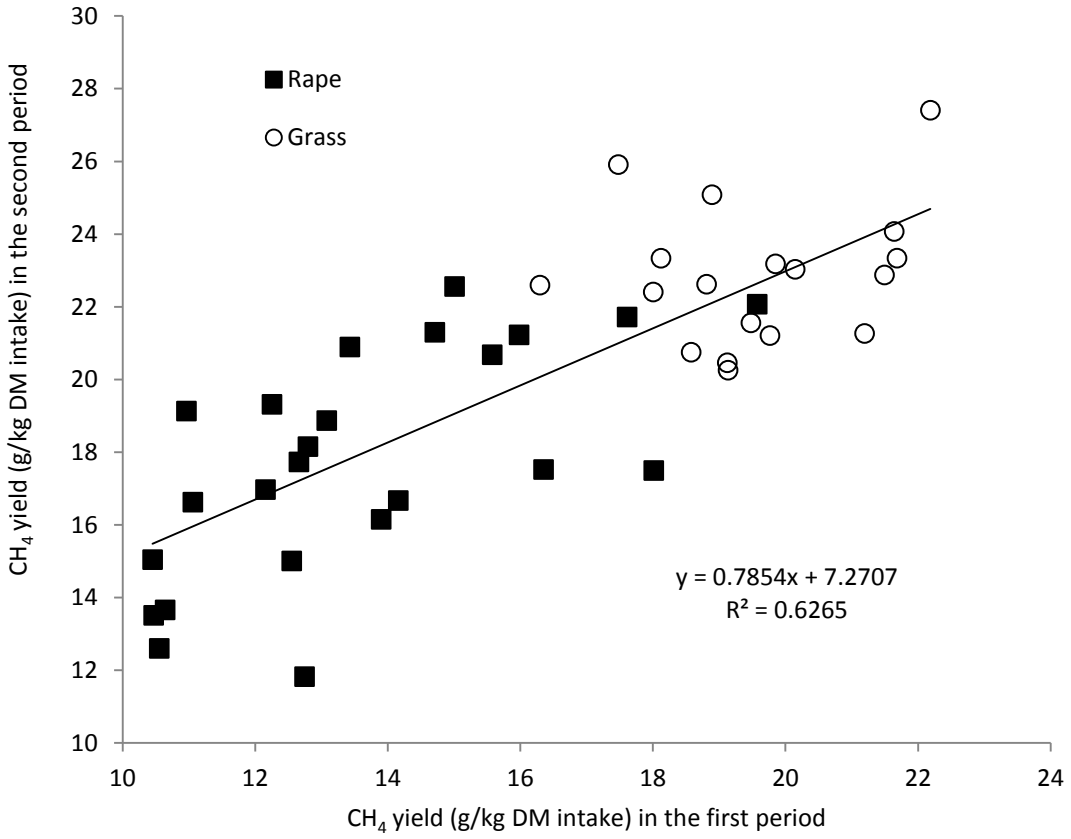


**Figure 1: Scatterplot of individual methane emissions (g/kg DM intake) from sheep fed fresh forage rape or perennial ryegrass during the two measurement periods (period 1= 7 weeks feeding period, period 2=15 weeks feeding period.**

### Apparent digestibility and metabolisable energy

During the first period of digestibility measurements, sheep ate 895 g rape and 826 g ryegrass DM per day (Table 3) and refused only 1.5% of DM offered for rape and 5.6% for ryegrass. Digestibility of forage rape was higher than ryegrass. Forage rape had 23.8% higher DM digestibility, 13.2-16.2% higher OM, CP and aNDF digestibilities and 37.9% higher ADF digestibility than ryegrass (P<0.01).

Sheep obtained only 0.1 MJ/kg DM higher energy intake from rape compared with ryegrass. But energy losses from faeces, urine and methane were 43, 22 and 20% less (P<0.001), respectively, for rape than for ryegrass (Table 3). As a result, DE and ME were 16 and 22% higher (P<0.001) for rape than for ryegrass.

In the second period, sheep refused 8.7% of rape and 6.0% of ryegrass DM offered and ate 932 g rape and 1041 g ryegrass DM per day (Table 3). Digestibilities of DM, OM and CP were 8.5-13.7% higher (P<0.001) for rape than for ryegrass, but rape had 10.3-16.4% lower (P<0.001) ADF and aNDF digestibilities than ryegrass.

**Table 3: Mean (±SEM) dry matter (DM) intake and apparent digestibility of constituents and energy in sheep fed either fresh forage rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.).**

| | Forage rape (*n*=6) | | Perennial ryegrass (*n*=6) | | *P* |
|---|---|---|---|---|---|
| **Period 1** | | | | | |
| DM intake (g/d) | 895 | ±2.1 | 826 | ±6.4 | <0.001 |
| Apparent digestibility (g/kg DM) | | | | | |
| Dry matter | 800 | ±4.9 | 646 | ±11.3 | <0.001 |
| Organic matter | 873 | ±2.8 | 751 | ±5.3 | <0.001 |
| Crude protein | 837 | ±3.3 | 736 | ±6.2 | <0.001 |
| aNDF | 660 | ±15.1 | 583 | ±16.3 | 0.006 |
| ADF | 670 | ±14.9 | 486 | ±23.8 | <0.001 |
| Energy digestion (MJ/kg DM intake) | | | | | |
| Intake energy | 14.8 | ±0.01 | 14.7 | ±0.03 | <0.001 |
| Faeces energy | 2.4 | ±0.04 | 4.2 | ±0.08 | <0.001 |
| Urine energy | 0.7 | ±0.03 | 0.9 | ±0.02 | <0.001 |
| Methane energy | 0.8 | ±0.07 | 1.0 | ±0.04 | 0.011 |
| DE (MJ/kg DM intake) | 12.5 | ±0.04 | 10.5 | ±0.10 | <0.001 |
| ME (MJ/kg DM intake) | 11.0 | ±0.07 | 8.6 | ±0.11 | <0.001 |
| **Period 2** | | | | | |
| DM intake (g/d) | 932 | ±16.6 | 1041 | ±12.9 | <0.001 |
| Apparent digestibility (g/kg DM) | | | | | |
| Dry matter | 821 | ±2.3 | 750 | ±5.5 | <0.001 |
| Organic matter | 850 | ±2.9 | 771 | ±5.8 | <0.001 |
| Crude protein | 772 | ±6.4 | 679 | ±5.2 | <0.001 |
| aNDF | 632 | ±12.4 | 756 | ±5.7 | <0.001 |
| ADF | 685 | ±12.0 | 764 | ±8.8 | <0.001 |
| Energy digestion (MJ/kg DM intake) | | | | | |
| Intake energy | 17.2 | ±0.04 | 17.5 | ±0.01 | <0.001 |
| Faeces energy | 3.1 | ±0.07 | 4.7 | ±0.09 | <0.001 |
| Urine energy | 0.7 | ±0.04 | 0.7 | ±0.02 | 0.259 |
| Methane energy | 1.0 | ±0.08 | 1.2 | ±0.02 | 0.051 |
| DE (MJ/kg DM intake) | 14.1 | ±0.04 | 12.8 | ±0.09 | <0.001 |
| ME (MJ/kg DM intake) | 12.4 | ±0.08 | 10.9 | ±0.10 | <0.001 |

DM = dry matter; aNDF = neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; ADF = acid detergent fibre expressed inclusive of residual ash; DE, digestible energy; ME, metabolisable energy.

Rape provided slightly less ( P<0.001) gross energy per kg of DM intake than ryegrass, but sheep fed rape lost less energy from faeces, hence DE was higher for rape than ryegrass (P<0.001). Energy loss from urine was similar (P=0.259) between sheep fed the two forages, but sheep fed rape lost less energy from methane (P=0.051) than ryegrass. As a result, ME was 1.5 MJ higher (P<0.001) for rape than ryegrass in the second period.

### Rumen fermentation parameters

Total VFA concentrations before the morning feeding were similar between forage rape and ryegrass in the first period of the experiment (P>0.05), but after the morning feeding, sheep fed forage rape had a higher total VFA concentration than those fed ryegrass (Table 4). Both forages had a higher total VFA concentration after feeding than before feeding (P<0.001).

The proportions of acetate and propionate in total VFA were similar between before and after feeding for ryegrass (P>0.05), but the acetate proportion was higher and the propionate proportion lower, before feeding than after feeding for rape (P<0.001). The average proportion of acetate in rumen fluid of sheep fed rape (56.7 mol/100 mol) was lower (P<0.001) than for sheep fed ryegrass (67.8 mol/100 mol), whereas the converse true for the proportion of propionate in rumen fluid (26.7 *versus* 18.5 mol/100 mol; P<0.001). The ratio of

acetate to propionate in rumen fluid was much lower (P<0.001) for sheep fed rape than ryegrass (2.27 versus 3.71, respectively). This ratio was less (P<0.001) before feeding than after feeding for both forages, but this difference before and after feeding was larger for rape than for ryegrass.

**Table 4: Mean (±SEM) concentration of total volatile fatty acids (VFA), the molar proportions of individual VFAs and the ratio of acetate to propionate (C2/C3) in the rumen fluid of sheep fed fresh rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.). (Period 1).**

| | Forage rape (n=24) | | | | Perennial ryegrass (n=18) | | | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre[a] | | Post[a] | | Pre[a] | | Post[a] | | Forage | Time[a] | Forage× Time |
| Total VFA (mM) | 51.2 | ±2.65 | 104.1 | ±2.70 | 51.3 | ±3.15 | 74.5 | ±3.06 | <0.001 | <0.001 | <0.001 |
| VFA (mol/100 mol) | | | | | | | | | | | |
| Acetate | 60.0 | ±0.72 | 53.4 | ±0.74 | 67.7 | ±0.86 | 67.9 | ±0.84 | <0.001 | <0.001 | <0.001 |
| Propionate | 22.4 | ±0.65 | 31.0 | ±0.66 | 17.6 | ±0.77 | 19.4 | ±0.74 | <0.001 | <0.001 | <0.001 |
| *n*-butyrate | 10.0 | ±0.40 | 12.5 | ±0.41 | 9.4 | ±0.47 | 8.6 | ±0.46 | <0.001 | 0.010 | <0.001 |
| Minor VFA[b] | 7.6 | ±0.30 | 3.1 | ±0.30 | 5.3 | ±0.35 | 4.0 | ±0.34 | 0.040 | <0.001 | <0.001 |
| C2/C3 | 2.75 | ±0.094 | 1.77 | ±0.096 | 3.88 | ±0.111 | 3.53 | ±0.108 | <0.001 | <0.001 | 0.003 |

[a] time samples were taken, pre and post (before and after) morning feeding.
[b] Minor VFA includes *iso*-butyrate, *iso*-valerate and *n*-valerate.

In the second period, rumen fluid samples were taken before the morning feeding and 2, 4, 6, 8, 10, 12, and 24 h after the morning feeding. Rumen pH (Figure 2a) in the sheep fed rape was lower (P<0.001) than that in those fed ryegrass at every time point, averaging 6.02 for rape and 6.71 for ryegrass across 24 h, respectively.

The total VFA concentration (Figure 2b) at each sampling was always higher (P<0.001) for rape than for ryegrass. The proportion of acetate in total VFA (Figure 2c) was lower (P<0.001) for rape (0.54) than for ryegrass (0.64), and the proportion of propionate (Figure 2d) higher (P<0.001) for rape (0.30) than for ryegrass (0.22). As a result, the ratio of acetate to propionate (Figure 2e) was lower for rape (1.89) than for ryegrass (2.94). The proportion of *n*-butyrate (Figure 2f) was higher (P<0.01) between 2 and 12 h after morning feeding for rape than for ryegrass, but was similar between the two forages before feeding.

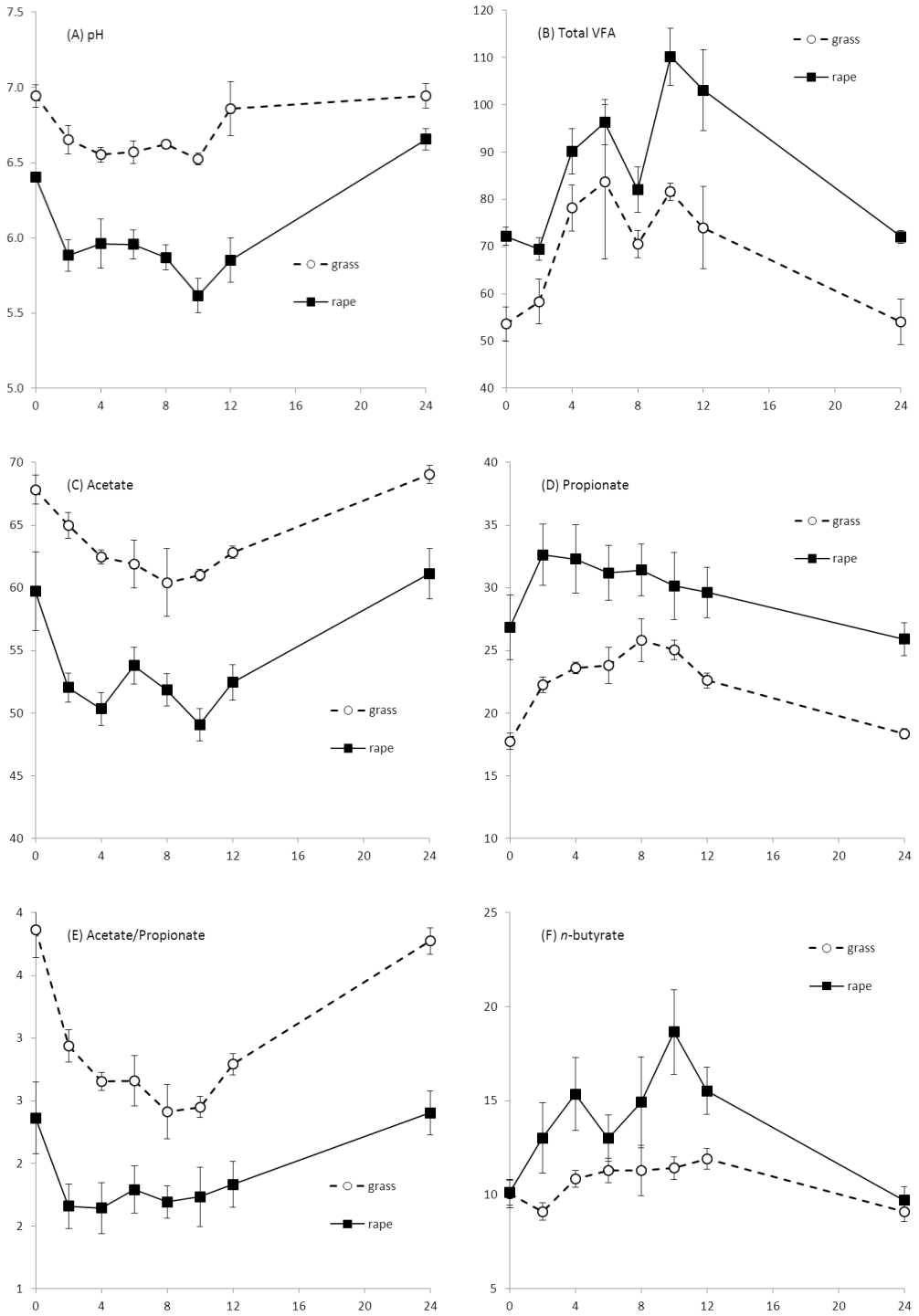


**Figure 2. pH (a) and the concentration of total volatile fatty acids (VFA) (b), the molar proportions of acetate (c), propionate (d), *n*-butyrate (f) and the ratio of acetate to propionate (e) in the rumen fluid of sheep fed fresh rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.). Bar, standard error of mean. (Period 2)**

### Nitrate, sulphur, sulphate, glucosinolate, and SMCO

Forage rape contained 10 times more nitrate-N (P=0.004) and 0.75 g/kg DM more sulphate-S (P=0.015) in the first period (Table 5). In the second period, nitrate-N in forage rape was below the limit of detection (<100 mg/kg DM), whereas perennial ryegrass contained 204 mg/kg nitrate-N. Sulphate-S content was also lower in rape compared with ryegrass (P=0.011) in period 2.

**Table 5: Mean (±SEM) nitrate, sulphur (S) and sulphate concentrations in forage rape (*Brassica napus* L.) and perennial ryegrass (*Lolium perenne* L.) and potential methane reduction from nitrate and sulphate.**

| | Forage rape (*n*=4) | | Perennial ryegrass (*n*=4) | | *P* |
|---|---|---|---|---|---|
| **Period 1** | | | | | |
| Nitrogen (N, g/kg) | 35.3 | ±0.85 | 29.5 | ±0.29 | <0.001 |
| Nitrate-N (mg/kg) | 1895 | ±364.6 | 188 | ±42.0 | 0.004 |
| Sulphur (S, g/kg) | 4.83 | ±0.312 | 3.32 | ±0.025 | 0.003 |
| Sulphate-S (g/kg) | 2.00 | ±0.220 | 1.25 | ±0.029 | 0.015 |
| $CH_4$ (g/kg DMI) | 13.6 | | 19.5 | | |
| $CH_4$ reduction(g/kg DMI) compared with perennial ryegrass | 5.9 | | | | |
| Potential maximum $CH_4$ reduction (g/kg DMI) from nitrate | 1.3 | | | | |
| Potential maximum $CH_4$ reduction (g/kg DMI) from sulphate | 0.3 | | | | |
| Unexplained $CH_4$ reduction (g/kg DMI) | 4.3 | | | | |
| **Period 2** | | | | | |
| Nitrogen (N, g/kg) | 24.8 | ±0.48 | 24.8 | ±0.25 | 1.000 |
| Nitrate-N (mg/kg)* | <100 | | 204 | ±33.3 | 0.004 |
| Sulphur (S, g/kg) | 3.13 | ±0.075 | 2.73 | ±0.048 | 0.004 |
| Sulphate-S (g/kg) | 0.90 | ±0.041 | 1.08 | ±0.025 | 0.011 |
| $CH_4$ (g/kg DMI) | 17.8 | | 22.9 | | |
| $CH_4$ reduction(g/kg DMI) compared with perennial ryegrass | 5.1 | | | | |
| Potential $CH_4$ reduction(g/kg DMI) from nitrate | -0.1 | | | | |
| Potential $CH_4$ reduction(g/kg DMI) from sulphate | -0.1 | | | | |
| Unexplained $CH_4$ reduction(g/kg DMI) | 5.4 | | | | |

1 mol nitrate-N (or 1 mol sulphate-S) theoretically reduces 1 mol methane, *i.e.* 14 g nitrate-N (or 32 g sulphate-S) reduces 16 g CH4 (van Zijderveld et al., 2010).
*Detection limit is 100. Assume the values of nitrate-N were 50 mg/kg for calculation *P* value when the concentration was less detection limit.

Forage rape contained a greater concentration of glucosinonates and SMCO than ryegrass in both experimental periods (Table 6). Epiprogoitrin, glucobrassicanapin and glucobrassicin were the major glucosinonates in rape.

## Animal health

No visible abnormal behaviours and symptoms were observed in both experimental periods. Although all blood parameters (WBC, RBC, HB, HCT, MCV, MCH and MCHC) examined were lower for sheep fed rape than for those fed ryegrass, parameters for all animals remained within normal ranges (data not shown).

**Table 6: Relative concentrations of glucosinolates and S-methyl cysteine sulfoxide (SMCO) in forage rape (*Brassica napus* L.) and perennial ryegrass (*Lolium perenne*) fed to sheep during the methane measurement periods (Values are peak areas obtained by chromatography).**

| | Period 1 | | Period 2 | |
|---|---|---|---|---|
| | Rape (n=6) | Ryegrass (n=6) | Rape (n=4) | Ryegrass (n=4) |
| Glucoerucin | 0 | 0 | 1029 | 0 |
| Gluconasturtiin | 781246 | 394 | 962381 | 239 |
| Glucoiberin | 16 | 25 | 274 | 23 |
| Progoitrin | 293 | 0 | 149 | 23 |
| Glucobrassicanapin | 2880894 | 2806 | 6714513 | 2123 |
| Sinalbin | 19 | 8 | 0 | 0 |
| Glucotropaeolin | 0 | 559 | 29 | 1046 |
| Gluconapin | 749492 | 899 | 1632059 | 26 |
| Glucobarbarin | 332 | 7 | 103 | 11 |
| Gluconapoleiferin | 1124926 | 1463 | 1581775 | 99 |
| Sinigrin | 73 | 0 | 1614 | 0 |
| Glucoalyssin | 428272 | 345 | 952517 | 109 |
| Glucosibarin | 17 | 0 | 39 | 0 |
| Glucobrassicin | 2238466 | 4331 | 900338 | 27 |
| Glucoraphenin | 0 | 0 | 0 | 0 |
| Epiprogoitrin | 5274992 | 10035 | 8812177 | 2792 |
| Glucoraphanin | 152628 | 15 | 391969 | 13 |
| 4-hydroxyglucobrassicin | 225 | 0 | 12143 | 0 |
| SMCO | 550398 | 1222 | 581339 | 84 |

# DISCUSSION

### Methane emissions from forage rape

The present study found that sheep fed forage rape for 7 and 15 wks emitted 30% and 20% less methane per unit of feed eaten, respectively, in comparison with perennial ryegrass. These results confirmed findings from our previous study (Sun et al., 2012b), in which forage rape reduced $CH_4$ emissions by 25% from sheep, compared with perennial ryegrass.

New Zealand agriculture is mainly based on pastoral production systems. Using forage as a tool for mitigation of enteric $CH_4$ emissions is therefore a practicable approach for these systems. Previous attempts have been made to identify forages with low methane emissions when digested. Forage species such as chicory (*Cichorium intybus*) (Waghorn et al., 2002; Swainson et al., 2008; Sun et al., 2011, 2012a), white clover (*Trifolium repens*) (Hammond et al., 2011) and Persian clover (*Trifolium resupinatum*) (Margan et al., 1988) have been touted as potential methane mitigation options. However, consistent $CH_4$ mitigating effects have not been observed. Forage brassicas were examined recently for the first time and forage rape and swedes were found to reduce $CH_4$ emissions by 23-25% when fed to sheep in comparison with perennial ryegrass (Sun et al., 2012b).

New Zealand has recently grown 300 000 ha of forage brassicas per annum, with 20% of the area being forage rape (A.V. Stewart, PGG Wrightson Seeds, Christchurch, New Zealand; pers. comm.) and 0.44 M t rape DM is estimated to be ingested annually by ruminants. Forage rape has a high nutritive value (Sun et al., 2012b), a high DM yield (Garcia et al., 2008) and supports fast animal growth (Campbell et al., 2011). Thus, forage rape, and possibly other brassicas such as swedes, for which the $CH_4$ mitigation potential has not yet been confirmed, could have benefits for $CH_4$ mitigation of grazing ruminants in addition to their current use and value as forage crops to fill the ruminants' dry matter requirements. However, use of

alternative forages for mitigating $CH_4$ in New Zealand's pastoral production systems must be fully evaluated in terms of the whole farm system and wider environmental impacts.

### Long term effects on methane emissions with forage rape

The results in the present study indicated that reductions in $CH_4$ emissions attributed to forage brassicas may be long term. Some methane mitigating agents, such as monensin (G.C. Waghorn, DairyNZ, Hamilton, New Zealand, pers. comm.) and acetylene (S. Muetzel, AgResearch Limited, Palmerston North, New Zealand, pers. comm.), do not persist in inhibiting methanogens over a long period, presumably due to the adaptation of rumen microbes to the inhibitors. Although the $CH_4$ reduction persisted in the present study, the reduction decreased from 30% at 7 wks to 20% at 15 wks. This could suggest that the rumen methanogen populations may have partially adapted to the rape diet as a result of the long exposure to this diet. This hypothesis could be examined using DNA evidence from microbial fingerprinting to assess the effect of forage rape feeding on methanogen communities. Samples were collected for future analysis, but such work was out of the scope of the current project. Another possible explanation is variation in plant secondary compounds which could be involved in $CH_4$ mitigation between the experimental periods, as illustrated by the change in concentration in nitrate and sulphate, two compounds with putative $CH_4$ mitigation properties. Nevertheless, in commercial operations, forage rape is often used for finishing lambs, deer and beef cattle for about 3 months, or used for wintering capital stock for 2-4 months. Thus, in spite of the reduction in the mitigation effect of forage rape after 15 wks, this forage seems to be a valid $CH_4$ mitigation tool.

### Potential mechanisms for reduced methane emissions with forage rape

The mechanisms by which forage rape reduced $CH_4$ emissions from sheep compared with perennial ryegrass in this and the previous study are unknown, although differences between the two forages in chemical composition and rumen fermentation and/or the presence of secondary compounds in rape are suspected.

The wet chemical composition in forage rape (Table 1) differed considerably to that in ryegrass, with much more readily fermentable and less structural carbohydrates in the former. Chicory (Sun et al., 2011, 2012a), white clover (Hammond et al., 2011) and forage brassicas (Sun et al. 2012b) had contrasting composition relative to perennial ryegrass as well. However, no consistent effect on $CH_4$ emissions has been demonstrated with most of these forages (Hammond et al., 2011; Sun et al., 2011, 2012a). Furthermore, conventional chemical constituents of forages have been demonstrated to have minimal explanatory effect on $CH_4$ emissions from ryegrass (Hammond et al. 2009). In our previous study, when more than one brassica was studied, the $CH_4$ reductions by forage brassicas were not explained by chemical composition (Sun et al. 2012b). Thus, rape chemical composition in this study is unlikely to contribute to the reduced $CH_4$ emissions.

The ratio of acetate to propionate in the rumen affects hydrogen (H) availability for $CH_4$ formation (Janssen, 2010). Lower ratios of acetate to propionate associated with reduced methane emissions in the present study and Sun et al. (2012b) feeding brassicas and the wider literature (e.g. Beauchemin and McGinn, 2005 feeding grain diets to cattle), indicate that volatile fatty acid production is related to $CH_4$ emissions. But the exact mechanisms involved in the shift towards reduced acetate to propionate ratios from feeding forage rape is yet to be fully elucidated.

Nitrate and sulphate are both H sinks and divert some H towards reducing compounds other than $CO_2$ in the rumen (van Zijderveld et al., 2010), thereby decreasing $CH_4$ formed in the rumen. The maximum potential $CH_4$ reductions were estimated in Table 5. In the first period,

73% of $CH_4$ reduction could not be explained by diversion of H by nitrate and sulphate and in the second period, $CH_4$ reductions by nitrate and sulphate concentrations were negligible.

In the present study, forage rape contained more glucosinonates and SMCO than ryegrass. In our previous study (Sun et al., 2012b) comparing methane yields of different forage brassica *spp*. with ryegrass, only sinalbin (a glucosinolate present in small quantities in the brassicas) tended to be negatively correlated with $CH_4$ production and positively associated with H emissions. In the present study, progoitrin, glucobarbarin and glucobrassicin concentrations decreased from the first period to the second period when $CH_4$ mitigation potential reduced. In similar fashion to sinalbin, progoitrin and glucobarbarin are glucosinolates present in very small concentrations in rape, suggesting high bioactivity if these compounds do indeed contribute to the $CH_4$ mitigation properties of forage rape. In the present study, the lack of considerable spread in glucosinolates as a result of having only one brassica representative on 2 measurement periods limits the potential to prove the hypothesis that glucosinolates have inhibitory properties against methanogens. Thus, it is recommended to conduct more research, to determine if members of the glucosinolate family of compounds have inhibitory effect against methanogens, as well as the mode of action of this effect. The positive identification of causal mechanisms behind the reduction in $CH_4$ production measured in this trial will enable implementation of brassicas as a mitigation tool with consistent and predicable responses. Further, research on the role of glucosinolates as methanogen inhibitors will enable alternative implementation of these compounds in livestock operations, such as dosing of animal with the active compounds.

### Animal health

Feeding forage brassicas can result in animal health problems (Nichol, 2007). By following best practice described for brassicas use (e.g. Agricom , 2008), such as gradual introduction to the crop and supplementation with Cu and I following determination of the mineral status of the sheep, these problems were controlled. No adverse effects of forage rape feeding on animal health were observed in this study, as evidenced by normal blood parameters observed even after grazing of forage rape for long periods of time.

## CONCLUSION

Sheep fed forage rape emitted 20-30% less $CH_4$ per kg DM eaten than those fed ryegrass and the reduction persisted for 15 wks. This confirms that forage rape, and maybe other brassicas such as swedes could be viable, practical methane mitigation tools for pastoral-based sheep production systems. The mechanisms behind the reduction in methane emissions with forage rape feeding may not be associated with conventional parameters of chemical composition *per se*. The reductions in $CH_4$ production could not be explained by the concentration of nitrate and sulphate in the forage rape. There is evidence that digestion of forage rape elicited changes in the rumen fermentation conducive to lower $CH_4$ production, such as a lower ratio of acetate to propionate in the rumen fluid, compared with perennial ryegrass. The role of glucosinolates as a contributor to the reductions in $CH_4$ emissions cannot be ruled out from this experiment, and could be a factor to be considered in addition to the digestion characteristics of the crop. Further focussed, in-depth studies are required to elucidate the mechanism(s) behind the reduction in methane emissions from sheep fed forage rape.

Although this and the previous study both indicate that feeding forage rape reduces $CH_4$ emissions compared with feeding perennial ryegrass, these studies were conducted indoors. Before extrapolating these effects to practical farming conditions, it is necessary to confirm under grazing conditions the results reported herein. Factors such as animal feeding and ruminating behaviour and diet selection, for example, may affect methane emissions and may differ between indoor housing with feeding of harvested forage and the grazing situation. In

addition, nitrous oxide emissions from animal excreta and soil cultivation should be included for an integrated evaluation of feeding forage rape on total whole-farm greenhouse gas emissions.

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# Nitrous oxide ($N_2O$) emissions from excreta of sheep fed forage rape (*Brassica napus* L.) or perennial ryegrass (*Lolium perenne* L.)

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## SUMMARY

A field plot trial was conducted to quantify nitrous oxide ($N_2O$) emissions and emission factors (EF3) associated with excreta of sheep fed with either winter forage rape or ryegrass. Major findings included:

- Urine N output per unit of N intake was similar for both forage rape and ryegrass, suggesting that animal N use efficiency was similar for both forages.
- Initial urine N transformation rates were higher when urine from sheep fed forage rape was applied to the soil, than when urine from sheep fed ryegrass was applied to the soil.
- The duration of $N_2O$ flux peaks from the application of urine from sheep fed forage rape was much shorter than that from urine of sheep fed ryegrass.
- This difference in duration was associated with differences in the concentrations of mineral N in the soil after application of urine from sheep fed the two types of forage.
- Consequently, the $N_2O$ emission factor (EF3) of urine from sheep fed forage rape (0.110%) was 59% lower than that of urine from sheep fed ryegrass (0.269%).
- Sheep dung N transformation rates in the soil were slightly slower for dung from sheep fed forage rape compared with dung from sheep fed ryegrass.
- The duration of $N_2O$ flux peaks from application of dung from sheep fed forage rape was longer than that from application of dung from sheep fed ryegrass.
- As for the urine treatments, this difference in duration was associated with differences in the concentrations of mineral N in the soil after application of dung from sheep fed both forages.
- The $N_2O$ emission factor for dung from sheep fed forage rape was greater than that with feeding ryegrass. However, the difference was not statistically significant.
- As observed in other trials, the $N_2O$ emission factor for sheep dung was much lower than that for sheep urine.
- Further studies are required to confirm the findings from this initial trial in which we found reduced $N_2O$ emissions from excreta of sheep fed forage rape.

## INTRODUCTION

In New Zealand, agriculture is predominantly based on outside pasture-based grazing systems. Agricultural soils have been identified as a major source of nitrous oxide ($N_2O$) emissions (Ministry for the Environment, 2006) which contributes to global warming as a greenhouse gas and it is also involved in the destruction of stratospheric ozone (McTaggart et al., 1997).

In New Zealand, $N_2O$ emissions from agricultural soils account for approximately one-third of all greenhouse gas emissions from the agricultural sector (Ministry for the Environment, 2011). $N_2O$ emissions in New Zealand, are generally higher in winter when soils are wet and

emissions will increase after grazing, due to the large amount of nitrogen (N) in the dung and urine that is excreted by grazing animals (Luo et al., 2008a, b).

Forage brassicas are widely used in animal agriculture due to their fast growing ability, high dry matter yield and high nutritional value (Belesky et al., 2007). Forage brassicas also have less neutral detergent fibre and more non-structural carbohydrates than perennial ryegrass (Sun et al., 2011). The lower crude protein content and the greater readily fermentable carbohydrate content of brassicas compared with ryegrass is expected to improve the efficiency of N utilisation in the rumen and consequently reduce N losses.

The aim of this experiment was to quantify $N_2O$ emissions associated with feeding winter forage rape and comparing these emissions with those from excreta of sheep fed conventional ryegrass pasture.

## METHODOLOGY

### Experimental design

A field plot trial was established at the Ruakura Farm (AgResearch Ltd., Hamilton, NZ). The experimental site contained perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens*) pasture, on a poorly-drained silt-loam soil (Te Kowhai soil). The Te Kowhai soil is classified as a Typic Orthic Granular soil in the Soil Classification system (Hewitt, 1998).

Excreta (urine and dung) were separately collected from 24 lambs that had been fed forage rape (*Brassica. napus* L.) and from 18 lambs fed perennial ryegrass (*Lolium perenne* L.) at Grasslands Research Centre (AgResearch Ltd. Palmerston North, NZ). The chemical composition of the forage rape and perennial ryegrass was analyzed as described in detail by Sun et al. (2011) (Table 7). Excreta was collected twice a day (morning and afternoon) prior to feeding for 2 consecutive days. After collection, urine and dung were kept in sealed containers in a cool room (4$^{o}$C) and transferred to the experimental site in iced chilly bins before application to the pasture. Urine destined for soil studies was not acidified, in contrast to urine collected for measurement of nitrogen partitioning within the sheep.

**Table 7: Chemical composition (g/kg dry matter) of forage rape and perennial ryegrass**

| | Period 1 | | Period 2 | |
|---|---|---|---|---|
| **Category** | **Rape** | **Ryegrass** | **Rape** | **Ryegrass** |
| Organic matter | 860 | 863 | 921 | 903 |
| Crude protein | 194 | 169 | 156 | 150 |
| Readily fermentable carbohydrates* | 195 | 71 | 319 | 149 |
| Neutral detergent fibre | 243 | 506 | 163 | 450 |
| Lignin | 50 | 30 | 36 | 17 |

* Readily fermentable carbohydrates include hot water-soluble carbohydrates plus pectin.

The treatments included:

i. urine from sheep fed forage rape,
ii. urine from sheep fed ryegrass,
iii. dung from sheep fed forage rape,
iv. dung from sheep fed ryegrass,
v. control (without urine or dung).

The plots used were approximately 0.5 m x 0.5 m set-up in a block design, 4 blocks with 5 plots each. The individual treatments were randomly assigned to the treatment plots in a block. Separate plots were established for destructive soil sampling. These plots received the same treatments (fresh sheep dung and urine) as for $N_2O$ measurement.

Sheep urine was applied at a rate equivalent to 4 L $m^2$. The application rate of urine for the sheep fed ryegrass was 441 kg/ha and for the sheep fed forage rape was 155 kg/ha. The urine was evenly applied to the entire 24 cm diameter area of the gas chamber. Sheep dung was applied at a rate equivalent to 5 kg $m^{-2}$. The application rate of dung for sheep fed ryegrass was 430 kg/ha and for sheep fed forage rape was 890 kg/ha. The dung was evenly spread to a plot 20 cm in diameter inside the 24 cm diameter gas chamber.

### Measurement of $N_2O$ fluxes

Following the treatment application in early September 2011, $N_2O$ emission measurements were carried out at least weekly. More frequent sampling occurred in the first month and following rainfall. On each sampling day, headspace gas samples were taken from each chamber during a cover period of 60 minutes. Gas sampling from the two urine treatments was completed in December 2011 and sampling from the dung treatments continued until May 2012 when return to background levels was reached for both dung treatments.

A soil chamber technique was used to measure $N_2O$ emissions, and the methodology was based on Luo et al. (2008c). The sampling chambers were modified PVC "sewer-hatches" attached to a section of PVC pipe. The chambers were 200 mm deep and with a 240 mm internal diameter. The "sewer-hatch" rim had an internal half-turn locking system and a greased rubber O-ring, which formed a gas-tight seal. Chambers were inserted 50-100 mm into the soil one day before excreta application. Chambers remained in place throughout each individual measurement period. Chamber heights were measured and the volume of each chamber calculated. On each sampling day, the chamber was closed with a lid for 1 hour, and the air above the soil surface was sampled through a three-way tap on the chamber lid, using a 60-ml syringe. A 25-ml air sample was taken from each chamber at 0 minutes ($T_0$) and 60 minutes ($T_{60}$).

Gas samples were analysed using a gas chromatograph equipped with a $^{63}$Ni-electron capture detector. The measured hourly $N_2O$ fluxes were calculated for each chamber, from the linear increase in head space $N_2O$ concentration over the sampling time. The hourly fluxes were integrated over time to estimate the total daily emission and the emissions over the measurement period.

Emission factors ($N_2O$-N emitted as percent of urine-N or dung-N applied: EF3) were then calculated from the difference in total emissions from each excreta treatment and the control treatment, divided by the rate of urine N or dung N applied, as described in equation 1:

$$EF3 = \frac{N_2O \text{ total (urine/dung; kg N ha}^{-1}) - N_2O \text{ total (control; kg N ha}^{-1})}{\text{Urine/Dung N applied (kg N ha}^{-1})} \times 100\% \quad (1)$$

### Soil and climatic parameters

Soil samples (75 mm deep, 25 mm diameter) were taken from all plots for determination of soil nitrate, ammonium and water content. Immediately after sampling the hole was back-filled with sealed PVC tubes to minimise any effects on soil aeration. Back in the laboratory on the same day or the following day, the samples were thoroughly mixed and about 15 g of

fresh soil (about 10 g dry soil equivalent) was extracted for 1 hour in 100 ml of 2 M KCl. The filtered (using filter paper No. 42 or equivalent) solutions were then frozen until analysed for nitrate (plus nitrite) and ammonium using a Skalar SAN$^{++}$ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). The nitrate method involves cadmium reduction to nitrite followed by diazotisation with sulphanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form an azo die measured colourimetrically at 540 nm. The ammonium method is based on the modified Berthalot reaction. Ammonia is chlorinated to monochloramine which reacts with salicylate and is then oxidised to form a blue/green coloured complex which is measured colourimetrically at 660 nm.

The remainder of the mixed soil was dried at 105ºC for 24 hours, to determine gravimetric soil water content. Water-filled pore space (WFPS) was calculated by dividing volumetric water content by total porosity. Total porosity was calculated as follows: 1–(bulk density/particle density). Volumetric water content was calculated by multiplying gravimetric water content by bulk density. Air and soil temperatures (at 5 cm depth) were monitored on each sampling day and rainfall was monitored continuously over the sampling period.

## RESULTS AND DISCUSSION

### Partitioning of excreta N between urine and dung

In combination with digestibility measurements described by Sun and Pacheco in the first part of this report, N excretions were measured during both animal experimental periods from 6 sheep per treatment to partition N between urine and dung (Table 8).

**Table 8: N excretions into urine and dung, and N balance from sheep fed fresh forage rape or perennial ryegrass.**

| | Period 1 | | | Period 2 | | |
|---|---|---|---|---|---|---|
| | Rape | Ryegrass | P | Rape | Ryegrass | P |
| **Feed** | | | | | | |
| DM intake (kg/d) | 0.895 | 0.826 | <0.001 | 0.932 | 1.041 | 0.003 |
| N content (g/kg DM) | 31 | 27 | | 25 | 24 | |
| N intake (g/d) | 27.9 | 22.7 | <0.001 | 24.1 | 24.7 | 0.266 |
| **Urine** | | | | | | |
| Urine output (L/d) | 4.89 | 3.98 | <0.001 | 4.89 | 3.4 | 0.001 |
| N content (g/L) | 4.55 | 4.75 | 0.494 | 2.43 | 3.48 | 0.002 |
| N output (g/d) | 22.2 | 18.8 | 0.021 | 11.9 | 11.8 | 0.475 |
| **Dung** | | | | | | |
| Dung output (g/d) | 0.179 | 0.293 | <0.001 | 0.167 | 0.261 | <0.001 |
| N content (g/kg DM) | 25.4 | 20.5 | <0.001 | 33 | 30.5 | 0.085 |
| N output (g/d) | 4.5 | 6 | <0.001 | 5.5 | 8 | <0.001 |
| **N partitioning** | | | | | | |
| Proportion of urine N in total N excretions | 0.83 | 0.76 | 0.001 | 0.68 | 0.59 | 0.001 |
| **N balance** | | | | | | |
| Urine N (g/kg N intake) | 797 | 831 | 0.505 | 493 | 476 | 0.297 |
| Faeces N (g/kg N intake) | 163 | 264 | 0.001 | 228 | 321 | <0.001 |
| Retention N (g/kg N intake) | 40 | -94 | 0.014 | 279 | 202 | 0.193 |

* DM = dry matter. n=6 each treatment each period. 6M $H_2SO_4$ was added into urine sample to prevent ammonia evaporation during collection.

Urine N output per unit of N intake was lower for ryegrass fed sheep in the first period (P<0.05) compared with forage rape fed sheep, but it was similar in the second period for sheep fed either forages (Table 8). Excretion of urine N was much higher in the first period than in the second period. Dung N output from sheep fed forage rape was slightly less (P<0.001) than that from those fed ryegrass. This suggests that the animals N use efficiency

was equivalent for forage rape and ryegrass. Values for excreta N concentration and partitioning were used to determine excreta application rate and volume for the field $N_2O$ measurements.

In general, results from a number of New Zealand studies suggest that in grazed pastures it is the animal excreta deposited in the form of dung and urine, along with N fertiliser, which provides high concentrations of available N in the soil. This high available N is a source for potential losses and is also the principle cause of enhanced $N_2O$ production and emissions.

Studies have found that $N_2O$ emission increases after animal excreta application and the increase is due to the enhanced denitrification activity as a result of increased nitrogen and carbon availability (Egginton and Smith, 1986; Sharpe and Harper, 2002). By altering the feed intake of animals, it may be possible to reduce the amount of N excreted in urine and dung and hence reduce the amount of N entering the soil system through animal excreta. Consequently, this could reduce denitrification and $N_2O$ emissions. The results from this trial indicated that urine N output per unit of N intake was similar from sheep fed either forage, suggesting that animal N use efficiency for both forage rape and ryegrass was similar.

### Urine

The initial urine N transformation rates (organic N to ammonium-N to nitrate-N) were higher when urine from sheep fed forage rape was applied to the soil, compared with urine from sheep fed ryegrass (Figures 3 and 4). The peak nitrite and nitrate-N level occurred 5 days after the urine had been applied to the soil from the sheep fed forage rape, compared with 26 days for the urine from sheep fed ryegrass. The reason for this difference is unknown. It may be related to urine composition in the two types of urine or different urine-N loading rates used in this trial.

The duration of $N_2O$ flux peaks from the application of urine from sheep fed forage rape was much shorter compared with the urine from sheep fed ryegrass (Figures 3 and 4). The difference in the duration of the $N_2O$ flux peaks was closely associated with the different concentrations of mineral N, particularly $NO_3^-$, in the soil after the application of the two types of urine.

The highest $N_2O$ fluxes recorded were generally associated with rainfall events (Figures 3 and 4). As an increase in the water filled pore space (WFPS) of the soil creates anaerobic conditions and this along with high levels of nitrogen and carbon availability in the soil led to a greater opportunity for $N_2O$ production and emissions. Previously, studies (e.g., Luo et al., 2008a, b, c) have shown that soil WFPS has the strongest influence on $N_2O$ emissions among all measured variables from excretal N input. Generally, $N_2O$ emissions are highest when soil WFPS is above soil field capacity.

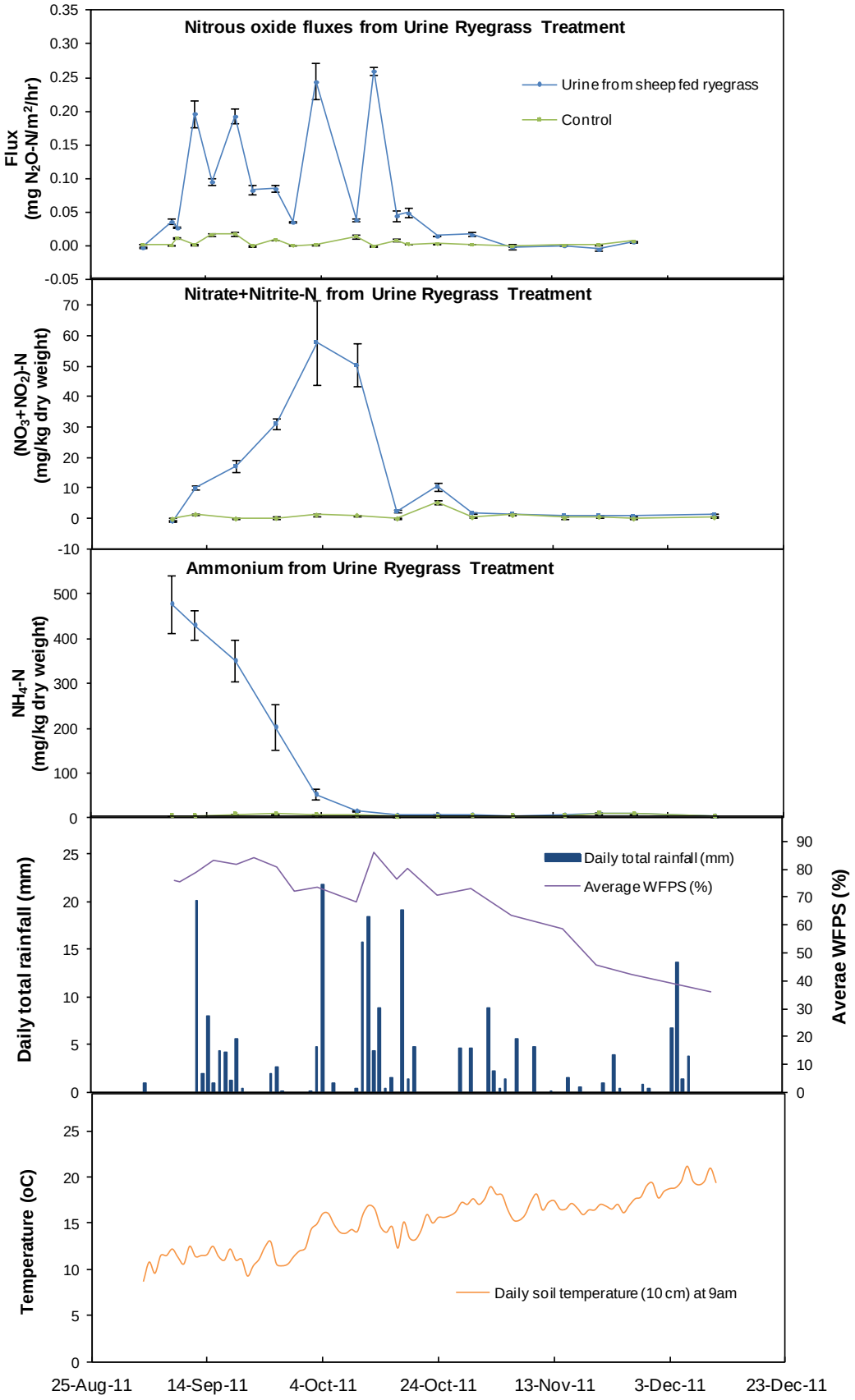


**Figure 3: Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of urine from sheep fed ryegrass.**

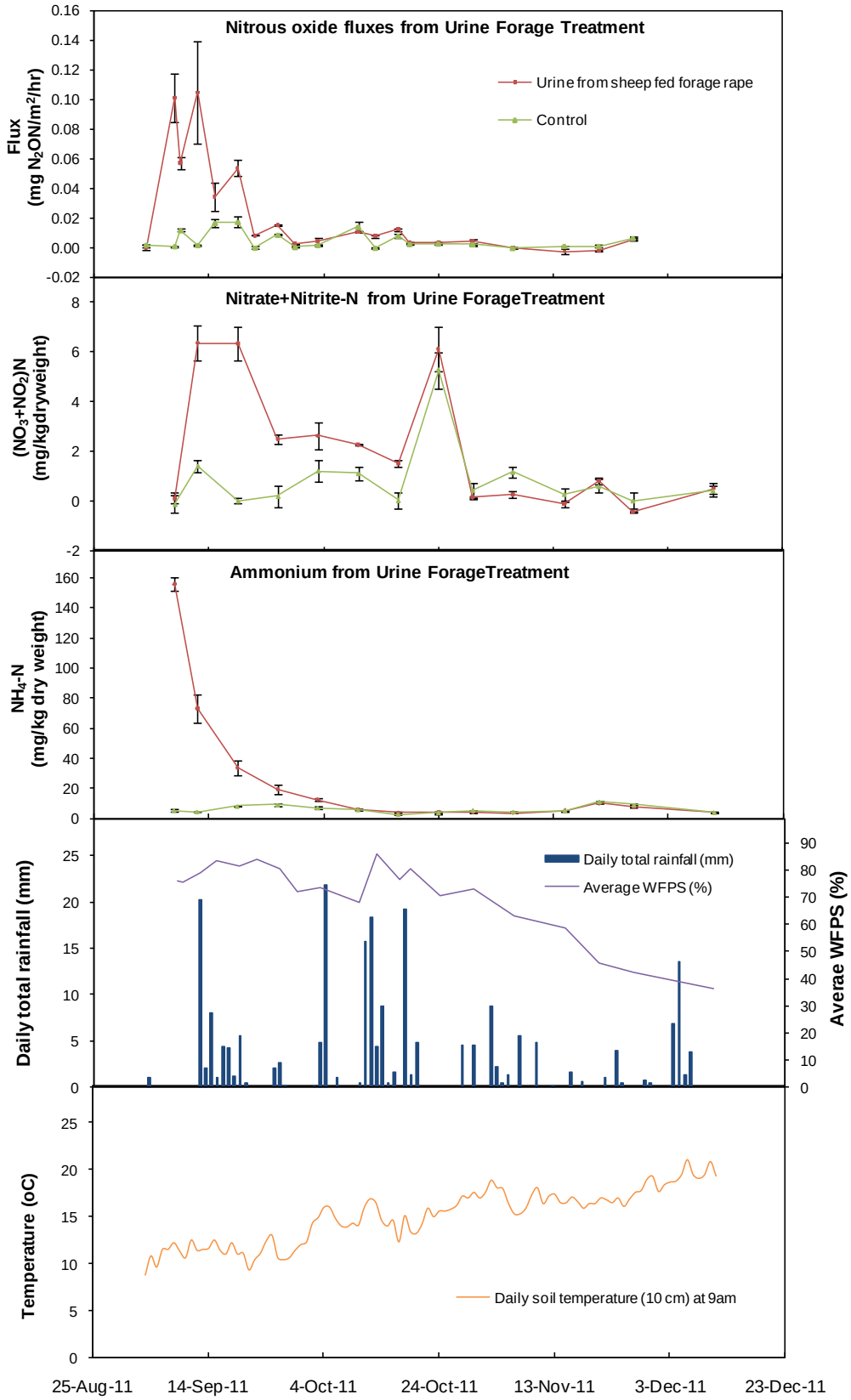


**Figure 4: Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of urine from sheep fed forage rape.**

## Dung

The initial dung N transformation rates (from organic N to soil ammonium-N to nitrate-N) were slower when dung from sheep fed forage rape was applied to the soil, compared with dung from sheep fed ryegrass (Figure 5). A greater soil ammonium-N concentration was

found when dung from sheep fed forage rape was applied to the soil. The N transformation rates from dung applied by the two different types of forages fed were in contrast to what happened when urine N was applied to the soil from the two different types of forages fed.

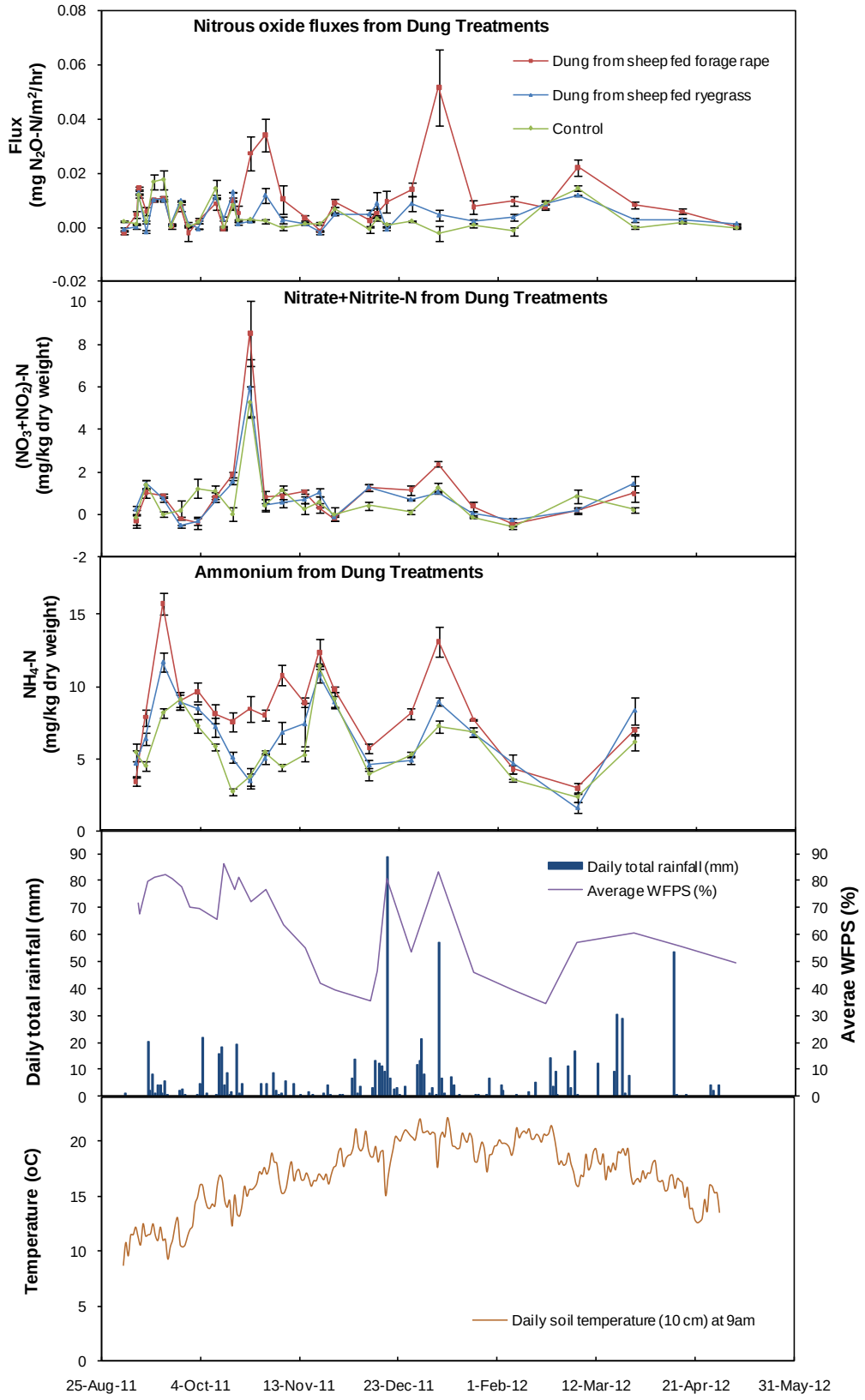


**Figure 5: Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of dung from sheep fed ryegrass or forage rape.**

The duration of $N_2O$ flux peaks from the dung of sheep fed forage rape was longer than of dung from sheep fed ryegrass. This was again in contrast to what was shown when urine from the two different types of forages fed was applied to the soil. However, as in the urine treatments, the difference in the duration of the $N_2O$ flux peaks was closely associated with or probably caused by the different concentrations of mineral N in the soil after the application of the two types of sheep dung (Figure 5).

### Emission factors

The emission factors (EF3) from the urine and dung treatments are the most important factor to consider in terms of the success of the two different types of feed at reducing $N_2O$ emissions. The emission factor for urine from sheep fed rape (0.110%) was lower ($P<0.05$) by about 60% than that from ryegrass fed sheep (0.269%; Figure 6). This finding is significant for developing mitigation measures to reduce EF3 for animal urine. If confirmed in additional trials, the use of forage rape could be promoted as an effective method to reduce $N_2O$ emissions from pastoral animal production systems. The reason for the reduced EF3 for urine from forage rape fed sheep needs to be explored as well.

In this trial urine N loading rates were different between the two types of urine, which may affect the results. However, the effect of urine N loading rate on EF3 is not conclusive (de Klein and Luo, unpublished). In contrast, the emission factor for dung from sheep fed forage rape (0.075%) was 2.68 times higher than that from ryegrass (0.028%), although the difference was not statistically significant ($P<0.05$) and magnitude is much lower for dung than for urine (Figure 6) as observed in other trials (Luo et al., 2012).

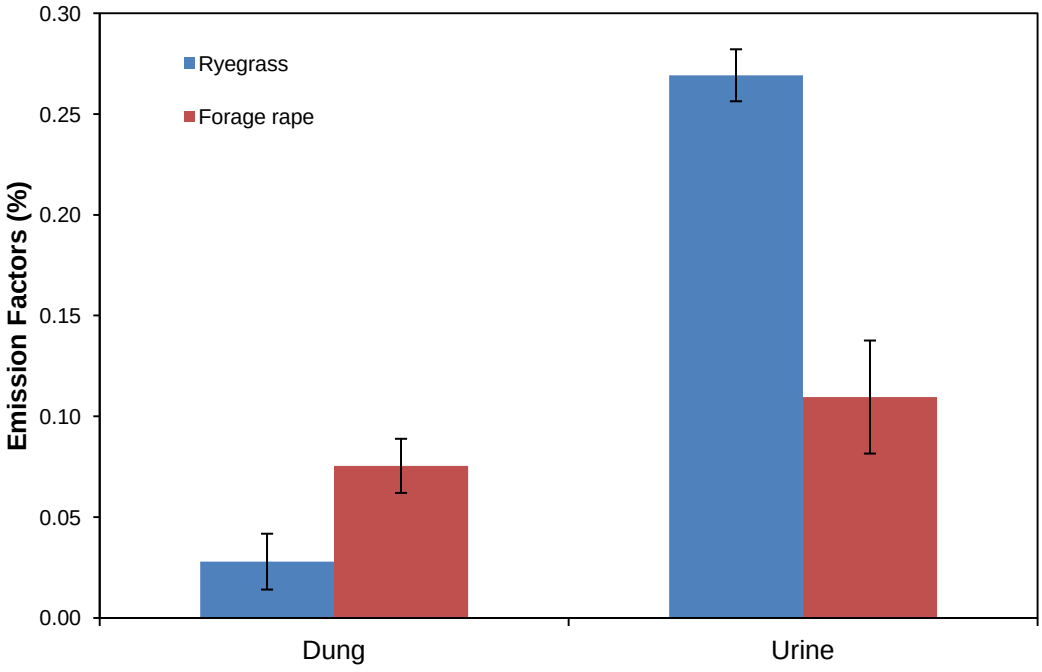


**Figure 6: Emission factors from dung and urine of ryegrass and forage rape fed sheep.**

## CONCLUSION

Urine from sheep fed ryegrass had slower N transformation rates from soil organic N to ammonium-N to nitrate-N, compared with urine from sheep fed forage rape. As a consequence, the duration of $N_2O$ peaks was longer and an emission factor (EF3) was higher for urine from sheep fed ryegrass compared with urine from sheep fed forage rape.

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