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Effects of fertiliser nitrogen rate to spring grass on apparent digestibility, nitrogen balance, ruminal fermentation and microbial nitrogen production in beef cattle and *in vitro* rumen fermentation and methane output

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Highlights

- Reducing fertiliser nitrogen (N) application rate reduced total and urinary N excretion.
- Intake, rumen fermentation and diet digestibility were unaffected by N application.
- Fertiliser N application rate did not affect *in vitro* methane and total gas output.

ABSTRACT

The effects of two fertiliser nitrogen (N) application rates - 15 (LN) or 80 (HN) kg N/ha - to *Lolium perenne* dominant swards in spring, on grass dry matter (DM) intake, digestion, rumen fermentation, microbial N production and N-balance in beef cattle, and *in vitro* fermentation and methane production were studied. Sixteen Charolais steers with a mean live weight (s.d.) of 475 (18.4) kg, were used in a completely randomised block design experiment and offered zero-grazed grass harvested 21-d post N application. The same grass was incubated in an eight-

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vessel RUSITEC in a completely randomised block design experiment. The HN treatment had a 540 kg/ha higher grass DM yield, and a 20 g/kg DM higher crude protein (CP) concentration compared to LN. There was no difference ($P > 0.05$) in DM intake, or *in vivo* DM, organic matter (OM) and N digestibility between treatments. Rumen fermentation variables pH, lactic acid, ammonia (NH_3) and total volatile fatty acid (VFA) concentration were similar ($P > 0.05$) for both treatments. Nitrogen intake was 19 g/d higher ($P < 0.05$) for HN compared to LN. Total and urine N loss was 16 and 14 g/d greater ($P < 0.05$), respectively, for HN compared to LN, but faecal N loss did not differ ($P > 0.05$) between treatments. The quantity of N retained and N-use efficiency did not differ ($P > 0.05$) between LN and HN. Plasma urea concentration was 1 mmol/L greater ($P < 0.05$) for HN compared to LN. Estimated microbial N production was greater ($P < 0.05$) for HN compared to LN. *In vitro* NH_3 concentrations were higher ($P < 0.05$) for HN compared to LN, whereas *in vitro* rumen pH, lactic acid and VFA concentrations and molar proportions did not differ ($P > 0.05$) between HN and LN. *In vitro* methane and total gas output were not different ($P > 0.05$) between treatments. Reducing fertiliser N application rate to grass in spring reduced total and urinary N excretion, which has environmental benefits, with no effects on *in vitro* methane output.

Nitrogen (N)

Dry matter (DM)

Crude protein (CP)

Organic matter (OM)

Ammonia (NH_3)

Total volatile fatty acid (VFA)

Greenhouse gas (GHG)

Carbon dioxide (CO_2)

Methane (CH_4)

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Nitrous oxide (N_2O)

Megatonne CO_2 equivalents (Mt CO_2-e)

Health Products Regulatory Authority (HPRA)

DM intake (DMI)

DM digestibility (DMD)

Organic matter digestibility (OMD)

Neutral detergent fibre (NDF)

Acid detergent fibre (ADF)

Water soluble carbohydrate (WSC)

Organic matter intake (OMI)

Nitrogen use efficiency (NUE)

Rumen degradability of N (RDN)

Keywords: beef cattle, nitrogen-balance, zero-grazed grass, rumen, methane, RUSITEC.

1. Introduction

Grass-based ruminant production systems are operated in many countries with temperate climates such as those in Northern and Western Europe. Ruminants play an important role in converting human inedible materials such as grass, into human-edible meat and milk products (Van Zanten et al., 2016). As a result of the increasing global human population the production of meat and milk is projected to double by 2050 compared with 2000 (Hume et al., 2011). To achieve this target, intensification of grass-based ruminant production systems are likely, with the additional challenge of reducing any negative impact on the environment (Myers et al., 2017).

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Ireland operates grass-based beef production systems with the majority of animal lifetime live weight gain achieved from a diet consisting of grazed pasture only (Drennan and McGee, 2009; McGee et al., 2014), a feedstuff which is considerably cheaper than conserved forage or concentrates (Finneran et al., 2010). Profitable grass-based beef production systems entail production and efficient utilisation of high yields of low-cost, high-nutritive value grass throughout the growing season, operating at relatively high stocking rates, optimising the proportion of grazed grass in the annual feed budget and maximising animal performance from pasture (Crosson and McGee, 2011). Fertiliser N is the primary (anthropogenic) driver of grass production (Peyraud and Astigarraga, 1998) and for moderately-intensive grass-based beef production systems total annual inorganic fertiliser N use ranges from 200 to 250 kg N/ha with 50 to 100 kg N/ha typically applied in spring and early-summer to the grazing area (Drennan and McGee, 2009). Such high rates of fertiliser N application will elevate temperate grass crude protein (CP) concentration (N ×6.25) (Hennessy et al., 2008) to a magnitude that, particularly for beef cattle, can be in excess of dietary N requirements (Gregorini et al., 2008).

Livestock are identified as a major contributor to greenhouse gas (GHG) emissions and N pollution of water (Hoekstra et al., 2007). Ruminant production contributes to GHG emissions in the form of methane (CH_4) from enteric fermentation in the animal and manure storage, nitrous oxide (N_2O) from animal excretions and the use of organic and inorganic nitrogenous fertilisers (O'Mara, 2011). In addition, ruminant production contributes to water pollution through the leaching and run-off of inorganic nitrate into water systems (Nitrates Directive 91/676/EEC). Both CH_4 and N_2O are potent GHG with global warming potentials of 25 and 298, respectively, times that of CO_2 (Eckerd et al., 2010). In Ireland, agriculture contributes to circa 33% of national GHG emissions, and of that CH_4 from enteric fermentation accounts for circa 64% and N_2O from fertiliser N applications and animal N excretion accounts for circa 31% (Lanigan et al., 2018). Under the European Union Climate and Energy

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Framework and subsequent Effort Sharing Proposals (COM/2016/482) member states have committed to reducing agriculture GHG emissions; and for Ireland these reductions are from 20.45 to 14.95 megatonne CO_2 equivalents (Mt CO_2-e) per annum between 2021 and 2030 (Lanigan et al., 2018). Therefore, in order to increase the output of ruminant meat and milk products whilst maintaining environmental sustainability, GHG emissions - CH_4 and N_2O - must be mitigated (Lanigan et al., 2018) and additionally the risk to water pollution from N reduced (Nitrates Directive 91/676/EEC).

Fermentation and microbial degradation of grass in the rumen yields energy mainly in the form of the VFA acetate (Peyraud and Astigarraga 1998; Owens et al., 2008). In addition, the majority of the dietary N is degraded in the rumen, yielding NH_3 (Peyraud et al., 1997; Warner et al., 2015) which, in the present of sufficient energy, is converted into microbial N by the rumen microbes. In beef cattle, dietary N is partitioned into tissues or excreted mainly in faeces or urine. Compared to monogastrics, N-use efficiency by ruminants is low and variable (10-40%) (Calsamiglia et al., 2010). Nitrogen excretion increases linearly with N intake with proportionally more N excreted in urine than faeces when N intake increases (Salah et al., 2015). Previous studies of N utilisation by beef cattle used diets of various chemical composition, in particular N concentration, mainly based on conserved forages and/or concentrates (Yan et al., 2007; Waldrip et al., 2013; Jiao et al., 2014; Dong et al., 2014); there is very little published research quantifying N losses in beef cattle consuming fresh temperate grass, especially spring grass receiving divergent application rates of fertiliser N and its effects on N excretion from beef steers.

Manipulating the diet to cause a reduction in one pollutant, may have the opposite effect on another (Dijkstra et al., 2011). For example, reducing fertiliser N application rate to grass can increase *in vitro* methane output (Lovett et al., 2004; Navarro-Villa et al., 2011; O'Connor et al., 2019) with limited published research on the effects of altering fertiliser N application

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rate to spring grass on N excretion and methane output measured simultaneously. Furthermore, *in vitro* rumen fermentation techniques have been successfully used as an alternative to *in vivo* methods to assess the potential methane emission from diets fed to ruminants (Danielsson et al., 2017).

Therefore, the objectives of this study were to determine the effects of fertiliser N application rate to spring grass on feed intake, apparent digestibility, rumen fermentation, microbial N production and nitrogen balance in beef cattle and *in vitro* rumen fermentation and methane output.

2. Material and methods

2.1 Geographical site, experimental area and treatments

This study was carried out at Teagasc, Animal & Grassland, Research and Innovation Centre, Grange (longitude 6°40 W; latitude 53°30 N; elevation 92 m above sea level) between 25 April and 26 May 2016. Meteorological data were recorded by a weather station, centrally located, at Grange; the 10-year (2006–2015) mean annual rainfall and daily ground (5 cm) temperature were 906 mm and 9.6°C, respectively. The mean annual rainfall and daily ground temperature in 2016 were 794 mm and 10.5°C, respectively. The 5-year (2011–2015) mean annual grass production at Grange determined according to O'Riordan (1997) was 11,701 kg dry matter (DM)/ha and mean yield in 2016 was 13,130 kg DM/ha.

The experimental area (5 ha) consisted of a *Lolium perenne* dominant sward. It was rotationally grazed to a targeted grass height of 4 cm by weanling beef cattle the previous autumn, before being 'closed' (end of October 2015) for the winter. In 2016, prior to the start of this experiment, the area was not grazed or mown. The soil type was imperfectly to

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moderately well drained Brown Earth of high base content and of loam to clay loam texture. The soil pH, P and K status, measured on 24 February 2016 according to the guidelines reported by Wall and Plunkett (2016), was 5.9, 2.8 mg/L and 53 mg/L, respectively. The experimental area received applications of 24.5 and 7 kg P/ha, and 105 and 30 kg K/ha, during the months of March and May 2016 (prior to commencing and during the experiment), respectively; it was applied as 350 and 100 kg/ha of 0:7:30 (N: P: K) compound fertiliser.

Treatments comprised of two inorganic fertiliser N application rates -15 (LN) and 80 (HN) kg N/ha per cut - in the form of calcium ammonium nitrate (270 g N/kg). The area was divided into 12 plots of equal size, with 6 subplots per treatment. It was designed so that each subplot would provide sufficient herbage for 3 to 4 d zero-grazing during the *in vivo* feeding study. For preparation of the experimental herbage, fertiliser N was applied 21 d prior to harvesting each of the respective subplots, such that duration between fertiliser N application and zero-grazing was 21-24 d for both the LN and HN treatments. Fertiliser N was applied to the first subplot in each treatment on 16 March 2016 and thereafter to the remaining subplots at 3-4 d intervals. On 15 March the average grass height and DM yield on the first subplots in each treatment was 6.1 cm and 640 kg/ha, respectively. Mean grass height of the remaining subplots was less than 5 cm. During the experiment, grass was harvested un-chopped daily at 08.00 h to a stubble height of 4 cm using a zero-grazer machine (chopping knives disengaged) (AB60, capacity 24 m^3, cutting width 1.85 m, Zero Grazer, Dromone, Oldcastle, Co. Meath, Ireland). Pasture sward height above 4 cm was measured weekly using a rising plate meter (Jenquip, Fielding, New Zealand) with a metal plate (0.1m^2 and 4.97kg/m^2) and the pre-grazing herbage mass (kg DM/ha) of the experimental pastures were estimated weekly based on sward height using the equation of O'Riordan (1997): Yield (kg DM/ha) = [242 × sward height (cm)] – 804.

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2.2 In vivo study: Animal management

All animal procedures used in this study were carried out under experimental license from the Irish Health Products Regulatory Authority (HPRA), licence number AE19132/P04, in accordance with the European Communities Regulations 2002 and 2005. Sixteen Charolais steers with an average live weight (s.d.) of 475 (18.4) kg, at the start of the experiment were blocked by live weight and used in a completely randomised block design experiment. Prior to commencing the experiment all animals were offered moderate nutritive grass silage *ad libitum* and 2.5 kg of concentrate per head daily for a 120 d indoor winter 'store' period (McGee et al., 2014), following which they were turned out to pasture and grazed on non-experimental, but similar, pasture for 35 days before re-housed indoors again. Animals had free access to water at all times and were treated for the control of ecto- and endo-parasites.

Experimental measurements were carried out over 20 d. This comprised 10 d of dietary adaptation and 10 d (2 for acclimatisation and 8 for sampling) in individual purpose-built 'metabolism' stalls, which permitted the total collection and measurement of separated urine and faeces. During dietary adaptation steers were accommodated indoors, individually, in slatted floor pens. As only 8 metabolism stalls were available for use, the experiment comprised of two sequential 'phases', using 8 steers each time; the 4 heaviest blocks (4 steers per treatment) followed by the remaining 4 (lightest) blocks.

2.3 Animal measurements and sampling

Steers were weighed at the beginning and end of the experiment. During dietary adaptation each animal was individually offered its respective grass *ad libitum* (circa proportionately 1.1 of the previous days intake). Feed refusals were weighed daily to estimate DM intake (DMI). After dietary adaptation animals were moved to the metabolism stalls whence grass was offered at 0.9 of the average intake of d 2-10, in two equal daily meals at

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09.00 and 15.00 h. Grass for the second feed was stored at 4°C to restrict heating until required. Intake was restricted to minimise between- and within-day variations in intake during intensive sampling.

Duplicate representative samples of grass offered were obtained daily. The grass DM allowance offered daily was estimated by rapid DM determination of 3×50 g representative samples per treatment using microwave drying for a period of 12 minutes (O'Kiely, 1997) and a sub-sample was used for DM determination using oven drying. Samples were stored at −18°C until processing, which consisted of homogenising each individual sample using a bowl chopper (Muller, Type MKT 204 Special, Saarbrucken, Germany). Samples were composited on an equal DM weight basis for d 13-20 of the *in vivo* study yielding four samples (duplicate) per treatment for chemical analyses.

After 2 d in the metabolism stalls, total faeces and urine (with 100 mL 9 M sulphuric acid per 10 L urine) were collected on a 48 and 24 h basis, respectively, for 8 d. On each d of collection total urine (urine + acid) was weighed and a 1% v/w sub-sample was taken for N determination, and a 0.25% v/w sub-sample, diluted with distilled water in the ratio 1 in 4, was obtained for purine derivative (allantoin and uric acid) determination; urine samples were stored at -18°C prior to analysis. Faeces samples were weighed, mixed and duplicate subsamples were taken. Two representative samples of faeces (circa 500 g) for DM determination, and another (10% by weight) for chemical analysis – were obtained and stored at -18°C.

To characterise rumen fermentation variables, a rumen fluid sample was obtained once from each steer on d 10 and 20, at 3 h after offering the morning feed. Approximately 20 mL of rumen fluid were collected using a trans-oesophageal sampler (Flora Rumen Scoop, Profs-Products, Guelph, Canada) (Fitzsimons et al., 2014). Ruminal fluid pH was measured

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immediately using a digital pH meter with electrode (HI98127; Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, UK). Samples were then preserved using 0.5 mL of 9 M sulphuric acid and stored at -18 °C.

Total N loss was determined by the sum of N loss from urine and faeces. Retained N (or N balance) was calculated by subtracting total N loss from N intake. The apparent digestibility of N was estimated by subtracting faeces N excretion from N intake and dividing the answer by N intake. Nitrogen use efficiency (NUE) was calculated as retained N divided by N intake.

Blood samples were collected from each animal immediately before, and 3 and 6 hours after the morning feeding on the same days as rumen fluid sampling was performed. Samples were taken via jugular venepuncture using a 9 mL tube containing sodium fluoride-EDTA K3 as an anticoagulant (Greiner Vacuette, Cruinn Diagnostics, Dublin, Ireland). Samples were centrifuged at 2,500 × g for 20 minutes at 4^{o}C. The plasma was then collected in borosilicate glass scintillation vials and stored at -18^{o}C until laboratory analyses.

2.4 In vitro study

This experiment consisted of a single eight-vessel RUSITEC apparatus (Czerkawski and Breckenridge, 1977) to simulate the rumen environment *in vitro* during a single 18 d period. The same LN and HN grass was used as offered during the entire *in vivo* study (described above). Representative samples were obtained daily and stored at -18°C until prepared for incubation.

2.5 Grass preparation for incubation

Grass for each treatment was removed from storage (-18°C) and immediately cut using a guillotine into 2-4 cm lengths. For each treatment the daily grass samples were pooled on an

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equal DM weight basis and mixed to provide one composited sample per treatment. Seventeen nylon bags (10 cm × 20 cm; circa 53 μm pore size; Ankom Technology Corporation, New York, USA) were prepared for each vessel. The corresponding fresh matter weight of 10 g DM was put into each feedbag. All feedbags were then re-frozen (-18°C). Prior to incubation in the RUSITEC they were allowed to thaw gradually at 4°C for 24 h.

2.6 Rumen inoculum

Rumen fluid and digesta solid was obtained from six ruminally-fistulated sheep offered a diet of hay *ad libitum* and 300 g fresh weight per day of a barley-based concentrate. A total of 4 L of rumen fluid were collected, before daily feeding, into pre-heated vacuum flasks. The fluid was immediately transported to the laboratory where it was thoroughly mixed and strained through a double-layer of cheese cloth. Digesta solid was also taken concurrently with the fluid from each donor sheep, pooled on an equal weight basis and mixed as a separate entity to provide one homogenous sample.

2.7 In vitro RUSITEC procedure

The RUSITEC system consisted of eight airtight fermentation vessels immersed in a water bath maintained at a constant 39°C. Each 900 mL fermentation vessel was charged with 500 mL of the strained rumen fluid and 200 mL of buffer (McDougall, 1948). A nylon bag containing circa 80 g of rumen digesta solids and another containing either LN or HN grass were added to each vessel in a perforated feed container. This container was then submerged in the vessel liquid, topped up with buffer, the vessel closed immediately and returned to the water bath. The vessel was connected to a plunger and the motor engaged. Buffer was pumped into each vessel at 0.5 mL/minute using a peristaltic pump (Watson Marlow 205CA, Watson-Marlow Fluid Technology Group, 37 Upton Technology Park Wilmington, MA 01887, USA.) from 10 L stock solutions. After 24 h the fermentation vessels were opened. The initial bag

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containing rumen digesta was removed, the contents washed in buffer with the liquid washings returned to the fermentation vessel, and replaced with a new nylon bag containing the appropriate experimental grass. On subsequent days the feed bag that resided in the vessel for 48 h was replaced with a new bag containing LN or HN herbage.

2.8 In vitro sampling

Days 0-7 of the experiment were allowed for microbial adaption and fermentation stabilisation (Jaurena et al., 2005). On d 8-18 vessel liquid and effluent were sampled for rumen fermentation variables, and on d 12 and 15 vessel liquid was sampled at 0 (at grass incubation), 1, 2, 3, 4, 6, 14 and 24 h. Immediately before opening the fermentation vessel a 15 mL liquid sample was extracted via aspiration from a port in the top of the vessel cap using a tube attached to a sterile/disposable syringe. The pH of this liquid was measured immediately using a digital pH meter with electrode (as described previously). The liquid sample was then preserved using 0.5 mL of 9 M sulphuric acid frozen (-18°C) prior to analysis at the end of the experiment. The grass residues (digested grass) were collected daily from d 8-16 and stored at -18°C for determining DM disappearance.

Total gas and methane output were measured and sampled daily on d 8-18 using a, reusable, hermetic gas collection bag attached to the effluent bottle. Following collection, the gas was manually expelled through a gas flow meter (DC^{-1} dry gas test meter Sinagawa Corp.; Tokyo, Japan) and methane concentration was determined using an infra-red gas analyser (SB2000; ADC wall mount analyser, ADC gas analysis Ltd, Herts, UK).

2.9 Chemical analysis

The DM content of the grass was determined by drying at 98°C for 16 h in an oven with forced-air circulation. Composited grass samples were dried at 60°C for 48 h, ground through

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a mill (Retsch SM100, Retsch-Allee, 1-5, 42781, Haan, Germany) (1 mm aperture screen) and analysed for DM digestibility (DMD) and organic matter digestibility (OMD) (Tilley and Terry, 1963), neutral detergent fibre (NDF) and acid detergent fibre (ADF) (ANKOM), ash (muffle furnace at 550^{o}C), CP (N x 6.25 – Leco FP 528 N analyser) and water soluble carbohydrate (WSC) (anthrone method) concentrations as described by Purcell et al. (2014). At the end of the experiment urine and faecal samples for chemical composition were thawed and pooled on an equal weight basis, per steer, prior to laboratory analysis. Urine N concentration was determined using a Leco FP 528 N analyser. Faeces samples for chemical analysis were mixed thoroughly and a circa 500 g sub-sample dried to a constant weigh at 60°C, then ground through a mill (Retsch SM100, Retsch-Allee, 1-5, 42781, Haan, Germany) (1 mm pore/aperture screen) and analysed for N, ash, NDF and ADF as described above. Faeces DM content were determined by drying in an oven with forced air circulation for 48 h at 98°C. Rumen fluid samples were analysed for concentration of VFA - acetate, propionate and butyrate - (gas chromatography), lactic acid (chemical analyser and a UV-method test kit) and NH_3 (chemical analyser and NH_3 reagent kinetic method) as described by Purcell et al. (2014). Plasma urea concentration was determined using a chemistry analyser (Olympus AU400, Beckman Coulter). Concentrations of allantoin and uric acid were determined using HPLC (Czauderna1 and Kowalczyk, 2004) and the sum of the values for each referred to as total purine derivatives, and from which microbial N production was estimated as outlined by Chen and Gomes (1992).

In the *in vitro* experiment vessel liquid samples were analysed for concentration of VFA, lactic acid and NH_3 as described above. After the experiment was completed the grass residues were allowed to gradually thaw at 4°C for 24 h, then washed (in the absence of detergent) using a domestic washing machine set to cold rinse for 20 minutes to remove any

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loosely adherent bacteria, dried at 60°C for 48 h and then weighed to calculate DM disappearance.

2.10 Statistical analysis

Animal data were analysed as a completely randomised block experiment using the GLM procedure of SAS (SAS, 2003. Statistical Analysis System. SAS Inc., Cary, NC). The statistical model had fixed effects for treatment and block. Time-related plasma urea concentration data was statistically analysed using the MIXED procedure in SAS. The model included the effects of treatment, block, animal and repeated measurement time (i.e. 0, 3 and 6 h) and their interaction (treatment × time).

In vitro rumen fermentation, total gas and methane data were analysed as a completely randomised block experiment using the MIXED procedure in SAS. The model included the fixed effects of treatment and vessel. The time-related rumen fermentation data model included the fixed effect of treatment, subject vessel and repeated measurement time (0, 1, 3, 5, 8, 12 and 24 h) and their interaction. Data were considered statistically significant when $P < 0.05$.

3. Results

3.1 Weather and grass production

Total rainfall for the months of April and May 2016 was 80 and 72 mm, respectively, compared with the corresponding 10-year (2006-2015) average for these months of 45 and 71 mm. Corresponding mean daily ground (5 cm) temperatures were 8.2 and 14.2°C, and 10-year (2006-2015) averages were 8.8 and 12°C. Grass DM production for the months of April and May recorded at Grange was 861 and 2135 kg DM/ha, respectively, compared with the 5-year (2011-2015) averages of 1261 and 2073 kg DM/ha.

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3.2 Grass yield and chemical composition

The pre-cutting DM yield, chemical composition and *in vitro* digestibility of grass used is presented in Table 1. The mean April-May pre-cutting grass DM yield and sward height was 540 kg DM/ha higher and 2.9 cm higher, respectively, for the HN than the LN treatment. The *in vitro* digestibility and chemical composition of the grass was similar for both treatments except for herbage DM, WSC and CP concentrations, which were 17 g/kg DM and 23 g/kg DM lower, and 20 g/kg DM higher, respectively, for HN compared to LN.

3.3 In vivo study: Intake, rumen fermentation, digestion and N balance

Dry matter intake, digestion, rumen fermentation, N metabolism and plasma urea concentration results are presented in Table 2. There was no effect of treatment ($P > 0.05$) on DM intake, or on the apparent digestibility of DM, OM, NDF, ADF and N. There was an effect ($P < 0.05$) of time (d), but no treatment × time interactions ($P > 0.05$) for rumen fermentation variables. Rumen pH, lactic acid, NH_3 and total VFA concentrations, and the molar proportions of VFA, did not differ ($P > 0.05$) between treatments, except for butyrate proportion which was 18 mmol/mol greater ($P < 0.05$) for LN compared to HN.

Nitrogen intake was 19 g/d greater ($P < 0.05$) for HN compared to LN (Table 2). In addition, total and urine N loss were 16 and 14 g/d greater ($P < 0.05$) for HN, with no difference ($P > 0.05$) between treatments for faeces N loss. The quantity of N retained (g/d) on an absolute basis and as a proportion of N intake, N absorbed and animal live weight (g/kg) did not differ ($P > 0.05$) between treatments. Concentrations of plasma urea increased over time post-feeding, but there was no treatment × time interactions ($P > 0.05$). The mean plasma urea concentration (across the three time points) was higher ($P < 0.05$) for HN compared to LN.

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The excretion of urine purine derivatives and estimated microbial N production is presented in Table 3. Allantoin excretion (mmol/d) was greater ($P < 0.05$) for HN compared to LN with no effect ($P > 0.05$) of treatment on uric acid (mmol/d) excretion. Total purine derivatives (mmol/d) excreted increased ($P < 0.05$) with increasing fertiliser N application rate and, thus, estimated microbial N production was greater ($P < 0.05$) on a daily (+72 g) and per kg DMI (+10 g/kg) and OMI (+12 g/kg) basis for HN compared to LN.

3.4 In vitro study – RUSITEC

Results pertaining to grass digestion in the RUSITEC are presented in Table 4. Dry matter disappearance values were similar ($P > 0.05$) for both treatments. There were no ($P > 0.05$) treatment × time interactions, but there was an effect of time ($P < 0.001$) for pH, lactic acid and NH_3 concentrations. There was no difference ($P > 0.05$) between treatments for pH and lactic acid concentration, whereas NH_3 concentrations were greater ($P < 0.05$) for HN compared to LN (+38 mg/L). Total gas and methane output did not differ ($P > 0.05$) between treatments.

4. Discussion

The current study was carried out to assess the effects of inorganic fertiliser N application on temperate spring grass DM intake, digestibility, rumen fermentation, nitrogen utilisation and microbial N production in beef cattle, and *in vitro* digestion and methane output.

4.1 Grass yield and chemical composition

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The pre-harvesting grass DM yield for LN and HN were within the range reported by Humphreys et al. (2001) for optimal performance of beef cattle grazing pasture in spring. In the present study the increase in grass DM yield with increasing fertiliser N application rate is equivalent to a growth response difference of 8 kg DM/kg N and 540 kg DM/ha in absolute terms. This grass growth response to N is intermediate to the range of values of 5 to 15 kg DM/kg N applied reported in the review of Peyraud and Astigarraga (1998). The response obtained is also similar to the 9 kg DM/kg N reported in the study of Black et al. (2009) but greater than the 3 kg DM/kg N reported by O'Donovan et al. (2004) who measured temperate grass production between March and May, 3 weeks post fertiliser N applications of 29 and 86, and 0 and 90 kg/ha, respectively.

In the present study increasing fertiliser N application rate reduced herbage DM concentration (-17 g/kg DM), which is in accord with the review findings of Peyraud and Astigarraga (1998) and comparable to the 26 g/kg DM absolute reduction reported in the recent study by O'Connor et al. (2019) for autumn grass that received 15 or 80 kg N/ha per rotation. The magnitude of the reduction is much lower in absolute terms than the 45 and 50 g/kg DM reported by Peyraud et al. (1997) (0 vs. 80 kg N/ha/cut) and by Warner et al. (2015) (0 vs. 90 kg N/ha), respectively, and may be partly explained by the local environmental conditions.

The increase in grass CP concentration (+20 g/kg DM) due to increased fertiliser N application rate is lower in absolute terms compared with the review findings of Peyraud and Astigarraga (1998) (50 to 90 g/kg DM for every 100 kg N/ha). Additionally, the unit change in grass CP concentration is less when compared to previous studies by Hennessy et al. (2008) (41 g/kg DM), Warner et al. (2015) (61 g/kg DM) and O'Connor et al. (2019) (35 g/kg DM) using divergent fertiliser N application rates to grass of 10 or 50 kg N/ha in 'spring', 20 or 90 kg N/ha in 'summer' and 15 or 80 kg N/ha per rotation in 'autumn', respectively. The effect of increasing fertiliser N application rate on grass CP concentration is less responsive when soil

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organic N mineralisation is high (Keating and O'Kiely, 2000) with high rates of 328 kg N/ha/year removed in grazed grass receiving 0 kg N/ha previously documented adjacent to the site of the current experiment (Humphreys et al., 2001). Furthermore, as grass CP concentration decreases during March and April, and is lowest in the months of May and June (Hennessy et al., 2008), season may also partly explain the limited effect of increasing fertiliser N application rate on grass CP concentration. In the present study increasing fertiliser N application rate decreased WSC concentration in absolute terms by 23 g/kg DM which is equivalent to 11.5 g/kg DM per 10 g/kg DM increase in CP concentration. Similarly, the review by Peyraud and Astigarraga (1998) reported a reduction in grass WSC concentration by 10 g/kg DM per 10 g/kg CP concentration increase.

A number of previous studies have reported an absence of effect of fertiliser N applied to grass on the *in vitro* digestibility of DM or OM (Delagarde et al., 1997; Humphreys et al., 2001; Hennessy et al., 2008). Likewise, *in vitro* (Tilly and Terry, 1963) digestibility of grass DM and OM was similar for both LN and HN treatments. Additionally, there was no effect of fertiliser N rate applied to grass on NDF and ADF concentrations in the present study which is in accord with the recent studies of Warner et al. (2015) applying fertiliser N application rates of 20 or 90 kg N/ha

4.2 In vivo intake, rumen fermentation, digestion and N balance

In the present study, unrestricted DMI expressed relative to live weight (g/kg LW) was 17.6. This DMI scaled for weight is similar to values obtained in previous studies where temperate grass of high digestibility was offered to beef cattle; Clarke et al. (2009) (15.7 g/kg LW) for grazing late-maturing breed steers, and Hart et al. (2009) (16.9 g/kg DM) and Coyle et al. (2017) (16.2 g/kg LW) for zero-grazed Charolais heifers and steers. An absence of an effect of fertiliser N application rate on DMI in the present study is in agreement with previous

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research with zero-grazed lactating dairy cows (Peyraud et al., 1997) and beef cattle (O'Connor et al., 2019). As the aforementioned grass fibre (i.e. NDF and ADF) concentrations and apparent *in vivo* digestibility of DM and OM were alike for both LN and HN, the similar DMI values for both treatments is not surprising considering the influence that these plant characteristics have on herbage ingestion (Gregorini et al., 2008).

Absolute values for *in vivo* digestibility of N were high for LN and HN treatments, with equivalent values for temperate grass reported in the studies of Peyraud and Astigarraga (1998), Warner et al. (2015) and O'Connor et al. (2019). Although not statistically significant the *in vivo* digestibility of N was greater for HN (23 g/kg DM) compared to LN, which is in accord with previous research findings (Peyraud and Astigarraga, 1998; Warner et al., 2015; O'Connor et al., 2019).

In the present study rumen fermentation variables pH, and lactic acid, NH_3 and VFA concentrations and proportions were comparable to other studies where beef cattle were offered fresh temperate grass of high digestibility and rumen fermentation variables determined via rumen fistula (Owens et al., 2008) and using a trans-oesophageal sampler (Lawrence et al., 2013). Rumen pH values were like those reported for beef cattle offered fresh grass in the experiments of Owens et al. (2008) (6.65-6.78), Noviandi et al. (2012) (6.85-7.21) and Lawrence et al. (2013) (6.94). Similar to the present findings, Noviandi et al. (2012) and Warner et al. (2015) also found no effect of fertiliser N application rate to grass on beef and dairy cattle rumen pH. As the rumen degradability of N (RDN) for temperate grass is relatively high (Olsson et al., 2005) (62-70%), particularly spring grass (Owens et al., 2008) (72%), the rumen NH_3 concentrations for both LN and HN were greater than the 22 mg/L (Slyter et al., 1979) required for microbial synthesis and in excess of the required range (25-50 mg/L) for optimal fibre digestion (Kennedy et al., 1992). Although not statistically significant, rumen NH_3 concentrations were greater (17 mg/L) for HN compared LN, reflecting the greater N

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intake, and is consistent with previous findings with zero-grazed lactating dairy cows (Peyraud et al., 1997; Warner et al., 2015) and grazing beef cattle (Noviandi et al., 2012). The absence of an effect of increasing fertiliser N application rate on rumen total VFA concentrations and proportions in the present study is in agreement with the review findings of Peyraud and Astigarraga (1998). Low nitrogen grass had greater molar proportions of butyrate compared to HN which is in accord with the studies of Noviandi et al. (2012) and Warner et al. (2015), possibly reflecting the numerically higher WSC concentration of the LN grass (Warner et al., 2015).

The quantity of N retained in the current study was similar for both treatments and absolute values were intermediate to those reported for growing-finishing beef cattle of various breeds consuming grass silage-based diets (-6 to 101 g/d; Yan et al., 2007), and growing cattle offered various diets (-9 to 89 g/d; Salah et al., 2015). Nitrogen retained values can be used to estimate animal average daily live weight gain (kg) (Sanchez Chopa et al., 2016). Considering that circa 750 and 250 g/kg of meat is water and protein, respectively, and a N to CP coefficient of 6.25, this implies that estimated average daily live weight gain for steers on both treatments in the current study was circa 1250 g. Daily live weight gains in excess of 1000 g are readily achievable by late-maturing breed yearling cattle, especially during the first part of the grazing season (Drennan and McGee, 2009). Furthermore, all cattle in this experiment were most likely experiencing some compensatory growth, which is usually most evident in the early part of the grazing season following turn-out to pasture in spring (McGee et al., 2014). Assuming a RDN for spring grass of 72% (Owens et al., 2008) and an average daily live weight gain of 1250 g, the predicted CP requirements for maintenance and growth (NRC, 2016) of the cattle in the present study was circa 770 g/d. Therefore, the dietary CP supply of LN and HN treatments was 942 and 1065 g/d of CP, respectively, which is 22 and 38 % greater than predicted CP requirements. As a consequence of excessive CP intake the quantity of N excreted via urine is

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usually much greater than faeces (Fanchone et al., 2013) and, in agreement, quantities of N excreted via urine compared to faeces were 10 and 34 % greater for LN and HN. Based on this the dietary CP supply could be reduced to circa 107 g CP/kg DM without affecting animal growth but would substantially reduce the quantity of N excretion particularly from urine.

Sanchez Chopa et al. (2016) summarised published NUE data for beef cattle studies and reported values ranging from 11 to 42 %; our values (32 and 31 %) were relatively high within that range but similar to the value (35 %) reported by Jiao et al. (2014) for growing beef cattle consuming temperate grass silage. Although not statistically significant, there was a decrease in NUE for HN compared to LN reflecting the greater N intake and similar quantities of retained N. Similarly, Archibeque et al. (2001) reported a decrease in NUE, from 33 to 25%, when N intake increased as a result of applying fertiliser N to switchgrass at rates of 56.2 or 168.5 kg N/ha.

The higher plasma urea concentration in HN steers reflected the increase in N intake and is consistent with the findings of Kenny et al. (2001) whereby plasma urea concentration increased from 2.8 to 6.6 mmol/L in grazing beef heifers associated with an increase in fertiliser N application from 0 to 85 kg ha per 14-18 d rotation.

Allantoin and uric acid are the only purine derivatives of rumen microbial origin excreted in cattle urine, and can be used as an estimate of microbial N synthesis (Chen and Gomes, 1992; Sanchez Chopa et al., 2016). Allantoin accounted for 99 % of the total purine derivatives excreted for both HN and LN treatments, which is greater than the figure reported by Chen and Gomes (1992) of 80-85 % but similar to that (99 %) found by Barchiesi-Ferrari and Anrique (2012) for steers offered grass silage-based diets. In the present study allantoin excretion (mmol/kg OMI) was similar to values reported for zero-grazed yearling beef cattle (36 to 41 mmol/ kg OMI - Howard, 2004) and grazing lactating dairy cows (34 mmol/ kg OMI

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- Schöbitz et al., 2013) but greater than other values found for steers offered grass silage (19 mmol/ kg OMI - Südekum et al., 2006) and lactating dairy cows offered temperate grass silage and zero-grazed grass (22.5 mmol/ kg OMI - Dewhurst et al., 2010; 20.3 mmol/ kg OMI - Waghorn et al., 2018).

The greater allantoin excretion (and thus greater estimated microbial N production) associated with HN compared to LN is in accord with Sanchez Chopa et al. (2016) who reported a tendency for greater (2.91 vs. 3.18 g/d) allantoin excretion in beef cattle with increasing fertiliser N application rate to winter oats. In the present study, estimated (Chen and Gomes, 1992) microbial N production (g/kg OMI) was much greater than values reported using rumen-fistulated cattle consuming fresh grass (11.7 to 12.7 g/kg OMI - Lee et al., 2002; 12.5 to 13.8 g/kg OMI - Owens et al., 2008); this over-estimation is likely associated with the difference in methodologies used.

Equations to predict N excretion from cattle have been developed by a number of authors (Table 5). The majority of the equations are based on diets consisting of either conserved forages only or conserved forages and concentrates with the exception of one recent study (Stergiadis et al., 2015), using non-lactating dairy cows, that reported equations based on solely fresh grass. These prediction equations used dietary CP concentration or N intake to predict faeces, urine N and (or) total N excretion from cattle. Using the grass CP concentration and N intake values from LN and HN and the respective equations (Table 5), faeces, urine and (or) total N excretion of the steers in the current study were estimated. The two equations that used CP concentration as the predictor variable (Waldrip et al., 2013; Dong et al., 2014) estimated faeces, urine and total N excretion values for LN and HN within 10% of the values obtained in the present study. Using N intake as the 'predictor' variable, the equation of Jiao et al. (2014) predicted total N excretion within 5% and faeces and urine N excretion within 15% of the values obtained for both LN and HN in the present study. In contrast, the other equations,

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including Stergiadis et al. (2015) (albeit based on dry dairy cows) were substantially poorer at predicting, with under and over-predictions of urine faeces and (or) total in excess of 20 % of the values obtained for LN and HN in the present study. Interestingly, compared to N intake, CP concentration was a relatively good predictor for N excretion from beef cattle offered fresh spring grass of high nutritive value and from a practical perspective, can be measured quite easily on commercial grassland beef farms. This should allow for more informative data collection for the quantification of animal contributions to the N-balance within whole-farm beef systems (Crosson et al., 2007).

4.3 In vitro rumen fermentation and methane production

In the present study the absence of an effect of fertiliser N application rate on the disappearance of DM using RUSITEC is consistent with the present *in vitro* (Tilly and Terry, 1963) and *in vivo* digestibility of grass DM results. Similar findings were reported by Hennessy et al. (2008) using the *in vitro* method of Tilly and Terry (1963) and fertiliser N application rate of 10 or 50 kg N/ha applied to temperate grass in spring. Absolute values for LN and HN DM disappearance were comparable to the absolute values reported in the study of O'Connor et al. (2019) (840 vs. 870 g/kg) using similar fertiliser N rates applied to autumn grass.

Like in the present *in vivo* study, increasing fertiliser N application rate had no effect on the *in vitro* rumen pH, which is in contrast to the *in vitro* studies of Navarro-Villa et al. (2011) and O'Connor et al. (2019) who reported lower and higher pH values, respectively, in the *in vitro* rumen fluid for grass that received divergent fertiliser N application rates. Lactic acid and VFA concentrations and proportions were similar for both LN and HN treatments, which is consistent with the findings from the present *in vivo* study and the *in vitro* studies of Navarro-Villa et al. (2011) and O'Connor et al. (2019). In the present study, increasing fertiliser N application rate increased NH_3 concentration (38 mg/L), which is consistent with the

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numerical increase in NH_3 concentration obtained *in vivo*. Similarly, *in vitro* nitrate and NH_3 concentration increased with fertiliser N application rate to grass in the studies of Navarro-Villa et al. (2011) and O'Connor et al. (2019). Furthermore, when compared to the *in vivo* results, the absolute values obtained for all aforementioned rumen fermentation variables were similar, indicating that, for these variables, the *in vitro* RUSITEC method represents *in vivo* relatively well, and supports the conclusions of Martinez et al. (2010).

Nutritional strategies to reduce N excretion in ruminants can potentially increase methane output (Dijkstra et al., 2011), which implies antagonistic effects or 'pollution swapping' (Gerber et al., 2013). However, this is not always clearly evident. From their meta-analysis of ruminants fed forage-only Savant et al. (2014) found a positive relationship between CH_4 and urinary N production per kg DMI, but the opposite occurred with faecal N. Although not statistically significant, the numerical increase in *in vitro* total gas and methane output from LN grass compared to HN is consistent with previous *in vitro* studies were temperate grass received divergent fertiliser N application rates (Lovett et al., 2004; Navarro-Villa et al., 2011; O'Connor et al., 2019). In addition, a reduction in methane output was observed in lactating dairy cows, offered grass silage which had 150 compared to 65 kg N/ha applied 28 days before harvesting (Warner et al., 2016) and when feeding nitrate to finishing cattle (Duthie et al., 2018) and to lactating dairy cows (Olijhoek et al., 2016). The CP and nitrate concentration of herbage can be increased by increasing fertiliser N application rate (Lovett et al., 2004). The fermentation of protein relative to carbohydrates in the rumen will yield less gas and methane (Navarro-Villa et al., 2011) and more NH_3, which is a process that consumes hydrogen (Janssen, 2010), and thus less hydrogen is available for methane formation. This potentially explains why methane was reduced with increasing fertiliser N application rate to grass in the present study.

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5. Additional considerations

Annual total (inorganic and organic) N application rates in excess of 160 and up to 250 kg N/ha are required for moderately-intensive (stocking rate > 170 kg organic N/ha) temperate pasture-based beef farms (Drennan and McGee, 2009; Wall and Plunkett, 2016), with 100-150 kg of the total N applied in spring and early summer. In Ireland, annual total N rates applied to the grazing area on grassland farms increases with stocking rate (24 to 219 kg N/ha for stocking rates of <85 to >210 kg organic N/ha) (Dillon et al., 2018). The current study was designed using two contrasting fertiliser N rates applied during the months of March, April and May that represent 'extensive' and 'intensive' pasture-based beef production systems. As evident in the present study, reducing N application rate to rotationally-grazed spring grass has the potential to reduce total N excretion, in particular urine N excretion by 21 % without any negative effects on grass nutritive value, animal intake and ultimately animal performance. However, farm carrying capacity (maximum stocking rate that will achieve a target level of animal performance) needs additional consideration as reducing fertiliser N application rate, resulted in a reduction in grass DM production by 23%. Therefore, stocking rate would also need to be reduced proportionately by an equivalent amount in order to produce a sufficient quantity of high nutritive grazed grass for optimum individual animal performance and conserved grass to meet the animals winter feed requirements.

6. Conclusion

The findings from this study indicate that, under the prevailing conditions, there was no effect of reducing fertiliser N application rate to spring grass on (zero-grazed) intake, rumen fermentation, *in vivo* digestibility and NUE but resulted in a reduced grass DM yield, CP concentration, rumen microbial N production, and total and urinary N excretion from beef

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cattle. Reducing fertiliser N application rate to spring grass had no effect on *in vitro* disappearance of DM, rumen fermentation and *in vitro* methane and total gas output; however, NH_3 concentration was reduced. In this regard reducing fertiliser N application rate to grass can be one strategy to reduce N excretion to the atmosphere and water systems from beef cattle consuming spring grass.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Table 1

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Herbage yield and chemical composition (s.d.) with low (LN) and high (HN) rates of fertiliser nitrogen applied

	LN	HN
Herbage yield (kg DM/ha)	1777 (14.2)	2317 (326.5)
Herbage height (cm)	13.5 (0.71)	16.4 (1.74)
DM (g/kg)	190 (3.1)	173 (11.7)
Crude protein (g/kgDM)	124 (13.4)	144 (5.1)
DMD (g/kg)	832 (1.6)	808 (3.3)
OMD (g/kg)	825 (1.7)	800 (3.5)
DOMD (g/kg)	766 (1.3)	740 (9.4)
NDF (g/kgDM)	446 (5.9)	460 (8.6)
ADF (g/kgDM)	293 (16.4)	268 (38.4)
WSC (g/kg-DM)	182 (21.2)	159 (13.3)
Ash (g/kgDM)	72 (3.5)	75 (7.6)

DM, dry matter.
DMD, dry matter digestibility.
OMD, organic matter digestibility.
DOMD, digestibility of organic matter in the dry matter.
NDF, neutral detergent fibre.
ADF, acid detergent fibre.
WSC, water soluble carbohydrate.

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Table 2
Intake, apparent digestibility, rumen fermentation, nitrogen balance and plasma urea concentration in beef cattle consuming spring grass fertilised with low (LN) and high (HN) rates of fertiliser N.

	LN	HN	SEM	P-value
DM intake (kg/d)	7.6	7.4	0.20	0.349
Digestibility (g/kg)				
DM	797	784	8.5	0.288
OM	777	794	8.7	0.195
NDF	834	837	7.6	0.758
ADF	850	861	7.2	0.306
Nitrogen	678	701	16.6	0.329
Rumen fermentation				
pH	6.85	6.69	0.07	0.130
Lactic acid (mg/L)	225	127	72.5	0.355
Ammonia-N concentration (mg/L)	90	107	12.5	0.356
Total volatile fatty acids (mmol/L)	51	49	2.9	0.723
Molar proportions (mmol/mol)				
Acetate	536	563	12.7	0.164
Propionate	234	228	6.1	0.480
Butyrate	168	150	5.0	0.025
Valerate	60	58	6.2	0.772
Acetate: Propionate	2.3	2.4	0.11	0.266
Nitrogen intake (g/d)	151	170	4.2	0.007
Nitrogen loss (g/d)	101	117	3.8	0.009
Urine nitrogen loss (g/d)	53	67	3.2	0.010
Faecal nitrogen loss (g/d)	49	50	2.1	0.382
Retained nitrogen				
g/d	50	53	5.6	0.752
g/kg Nitrogen intake (NUE)	323	309	27.2	0.737
g/kg absorbed	467	441	36.0	0.613
g/kg live weight	110	110	11.1	0.752

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Plasma urea concentration (mmol/L)	3.9	4.9	0.19	0.003

NUE: Nitrogen use efficiency

Nitrogen loss (g/d) = urine + faecal N loss (g/d)

Table 3

Urinary purine derivative (PD) excretion and estimated microbial N supply in beef cattle consuming spring grass with low (LN) and high (HN) rates of fertiliser N applied.

	LN	HN	SEM	P-value
PD excretion (mmol/d)				
Allantoin	194.4	278.4	11.52	0.002
Uric acid	1.4	1.3	0.23	0.880
Total	196	280	11.5	0.002
Microbial N (g/d)	134	206	9.8	0.002
Microbial N (g/kg DMI)	18	28	1.6	0.005
Microbial N (g/kg OMI)	22	35	2.3	0.005

DMI = Dry matter intake

OMI = Organic matter intake

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Table 4

Apparent digestibility, rumen fermentation, gas and methane output of spring grass fertilised with low (LN) and high (HN) rates of fertiliser N using *in vitro* rumen simulation technique (RUSITEC)

				P-value		
	LN	HN	SEM	Trt	Time	Trt × Time
DM disappearance (g/kg)	885	887	10.2	0.909	-	-
Rumen fermentation						
pH	6.8	6.8	0.04	0.521	<0.001	0.844
Lactic acid concentration (mg/L)	149	147	8.0	0.857	<0.001	0.993
Ammonia-N concentration (mg/L)	66	104	5.4	0.003	<0.001	0.313
Total volatile fatty acid (mmol/L)	64	69	3.5	0.343	<0.001	0.805
Molar proportions (mmol/mol)						
Acetate	511	531	12.2	0.291	0.082	0.653
Propionate	212	226	3.9	0.428	0.043	0.741
Butyrate	170	155	6.8	0.159	<0.001	0.801
Valeric	97	88	7.9	0.440	0.419	0.883
Acetate: Propionate	2.3	2.3	0.03	0.514	0.081	0.591
Gas production						
Total gas (mL/d)	1730	1510	207.2	0.462	-	-
Methane (CH_4i)	0.55	0.42	0.086	0.311	-	-
Methane (mmol/d)	5.7	4.3	0.90	0.308	-	-

Methane (CH_4i) = mmol of methane output per gram of total DM incubated

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Table 5

Comparison of prediction equations using CP concentration or N intake from the present study to predict total N (TN), faeces N (FN) and urinary N (UN) excretion (g/d) from beef cattle consuming spring grass with low (LN) and high (HN) rates of fertiliser nitrogen applied

Reference	Animal type	Diet	Equations	Faeces		Urine		Total	
				LN	HN	LN	HN	LN	HN
Present study				49	50	53	67	101	117
Reed et al. 2015	Beef cattle – steer	Various	FN = 0.506 + 0.352 × NI UN = 6.80 + 0.405 × NI TN = 6.91 + 0.759 × NI	56	64	71	79	128	143
Stergiadis et al. 2015	Cow - non-lactating	Grass	FN= 28.6 + 0.044 × NI UN=7.907 + 0.613 × NI TN= 37.9+ 0.640 × NI	36	36	105	118	140	152
Dong et al. 2014	Beef cattle	Various	FN = 1.81 × CP + 19.68 UN = 6.04 × CP −22.00	42	46	53	65	95	111
Dong et al. 2014	Beef cattle	Various	FN = 0.20 × NI + 15.82 UN = 0.51 × NI −14.12	48	52	67	77	115	129
Jiao et al. 2014	Beef cattle - steer & heifer	Grass silage	FN = 0.294 × NI + 0.9 UN = 0.483 ×NI – 15.7 TN = 0.733 × NI – 7.4	48	54	61	71	109	125
Waldrip et al. 2013	Beef cattle	Various	FN = 1.165 × CP + 30.91 UN = 5.91 × CP − 21.52	45	48	52	64	97	112
Waldrip et al. 2013	Beef cattle	Various	FN = 0.154 × NI + 24.28 UN = 0.56 × NI − 21.18	49	52	68	79	117	131
Yan et al. 2007	Beef cattle	Grass silage	TN =1.392 × $BW^{0.75}$ + 7.7 TN = 0.661 × NI + 20.1 TN = 0.567 × NI + 0.651 $BW^{0.75}$ – 22.3					149 125 134	 138 145