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Original Research Article

Sheep numbers required for dry matter digestibility evaluations when fed fresh perennial ryegrass or forage rape

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ABSTRACT

Research trials with fresh forages often require accurate and precise measurement of digestibility and variation in digestion between individuals, and the duration of measurement periods needs to be established to ensure reliable data are obtained. The variation is likely to be greater when freshly harvested feeds are given, such as perennial ryegrass (*Lolium perenne* L.) and forage rape (*Brassica napus* L.), because the nutrient composition changes over time and in response to weather conditions. Daily feed intake and faeces output data from a digestibility trial with these forages were used to calculate the effects of differing lengths of the measurement period and differing numbers of sheep, on the precision of digestibility, with a view towards development of a protocol. Sixteen lambs aged 8 months and weighing 33 kg at the commencement of the trial were fed either perennial ryegrass or forage rape (8/treatment group) over 2 periods with 35 d between measurements. They had been acclimatised to the diets, having grazed them for 42 d prior to 11 days of indoor measurements. The sheep numbers required for a digestibility trial with different combinations of acclimatisation and measurement period lengths were subsequently calculated for 3 levels of imposed precision upon the estimate of mean dry matter (DM) digestibility. It is recommended that if the standard error of the mean for digestibility is equal to or higher than 5 g/kg DM, and if sheep are already used to a fresh perennial ryegrass or forage rape diet, then a minimum of 6 animals are needed and 4 acclimatisation days being fed individually in metabolic crates followed by 7 days of measurement.

1. Introduction

Apparent total tract digestibility (feed digestibility) is commonly used to indicate the nutritive value of a feed. It reflects the availability of nutrients to the animal, by estimating the proportion of feed that is not excreted in the faeces but is assumed to be digested nutrients being absorbed from the digestive tract (McDonald et al., 2011). A very small proportion of feed dry matter (DM) is lost to carbon dioxide and methane, but this may be ignored for our purposes. The conventional method to determine digestibility is *in vivo* total faecal collection technique by recording the amount of feed eaten and faeces excreted. Other approaches, such as *in vitro* and *in sacco* techniques and chemical and physical measurements, offer a quicker and cheaper alternative (Kitessa et al., 1999; Minson, 1990), but these approaches rely on *in vivo* measurement for validation. Therefore, the total faecal collection technique is the standard method and still widely used.

The measurement of digestibility in feed evaluation and animal nutrition studies is important, yet researchers have been aware for more than 100 years of errors associated with digestibility measurements. For example, Grindley et al. (1917), cited by Schneider and Flatt (1975), recommended that "not less than three animals

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should be used in each lot". They also suggested if feasible five or even more animals should be used and commented that results obtained with more than 4 animals are much more reliable than those obtained with 1 or 2. The number of digestibility trials has grown over the years, and protocols for measuring digestibility have developed, incorporating such factors as animal choice, equipment, experimental procedure which affect digestibility. These have been summarised and reviewed (Cochran and Galyean, 1994; Grassland-Research-Institute, 1961; McDonald et al., 2011; Minson, 1990; Schneider and Flatt, 1975). Most of these recommended protocols were based on experience rather than experimental data, and sometimes recommendations were contradictory. However, some studies did provide experimental data for comparing digestibility protocols (Forbes et al., 1946; Raymond et al., 1953).

Animal husbandry in New Zealand relies on animals eating fresh forage. Fresh forage differs from dry or other conserved diets in many respects. Dry matter content varies from day to day, and nutritive value varies because the chemical composition of pasture changes continuously over time – between and within days. An animal's individual preference for and selection of particular forage species and plant parts further increases the variation in material eaten and nutrient intake (McDonald et al., 2011). Previous attempts to reduce feed intake variation have included harvesting and drying or freezing (Grassland-Research-Institute, 1961; Heaney et al., 1969), however, this approach might not be appropriate, either because drying or freezing facilities are unavailable or because it is imperative to conduct experiments with fresh forages where objectives relate to grazing. In addition, drying or freezing causes physical changes that result in quite different plant characteristics to pasture. For these reasons daily cut-and-carry protocols are required.

Perennial ryegrass (*Lolium perenne* L.) is the predominant forage species in New Zealand. Forage rape (*Brassica napus* L.) has both a high yield and nutritional value and is increasingly used by farmers (Barry, 2013). Forage rape differs from grass because it is a dicotyledonous plant with morphological and chemical differences, and may contain compounds that are deleterious to ruminants (Barry, 2013). Protocols which are designed for evaluating and measuring digestibility of grasses may be less applicable when applied to forage rape. Accurate determination of digestibility, as well as of its variation between and within individual animals, is required to enable proper comparison between cultivars, and also to develop industry recommendations for their use.

Many trials involving measures of digestibility include 2 phases: 1) A few days' acclimatisation or adaptation to the diet and facilities followed immediately by, 2) A number of days of measurement of feed eaten and faeces excreted. The feeding and measurements in these 2 phases, together with the number of animals used and the duration of each phase, are key factors associated with errors in digestibility measurements, but they could be standardised to develop a digestibility protocol for sheep (Schneider and Flatt, 1975). The objective of this study was to determine the minimum number of days of these 2 phases, and the number of animals required to estimate digestibility for fresh perennial ryegrass and forage rape.

2. Materials and methods

2.1. Animals, housing and experimental design

The management of animals described here was approved (No. 12645) by the Grasslands Animal Ethics Committee (AgResearch Ltd., Palmerston North, New Zealand). This study was conducted near Palmerston North, New Zealand, from May to September 2012 with 8-month-old Romney cryptorchid lambs: 112 individuals having a mean live weight of 33 ± 2.3 kg (mean ± SD). The lambs were randomly allocated to a diet of either perennial ryegrass (*L. perenne* L. variety *Ceres One* 50 containing endophyte AR1) or forage rape (*B. napus* L. variety *Titan*), with 56 sheep in each group. The forage rape diet was introduced gradually over 1 week, and the lambs were grazed on it for another 5 weeks prior to measuring digestibility indoors. Over the same time interval, the lambs in the ryegrass group were grazed on pasture. Eight animals from each forage treatment were selected for the indoor trial; all were selected to have similar live weight (44 ± 2.2 kg).

The indoor measurements comprised total faecal collection over 2 experimental periods of 11 days each (9–22 July, and 27 August – 9 September, 2012). Between these 2 periods, the animals were grazed on their respective pastures for 35 days.

During each indoor period the sheep were held in pens (8/pen) for 3 days acclimatisation, and then transferred to metabolic crates where harnesses were attached for total faecal collections over a 10-day period. Fresh forages were provided twice a day and there was free access to water. Forages were harvested daily between 09:00 and 12:00 and stored at 4°C prior to feeding at 16:30 and 09:30. An allowance of cut forage was given to all sheep, with a target amount of approximately 2.3 times the metabolisable energy (ME) requirement for maintenance (MEm), calculated at the beginning of the experiment. The actual amount provided depended on the dry matter content, which was 14.5% and 15.9% for ryegrass and 11.0% and 12.2% for forage rape in the first and second indoor feeding periods, respectively. Maintenance requirements were based on the Australian-Agricultural-Council (1990) feeding standards, with ME of forage DM predicted by infrared reflectance spectroscopy (NIRS; Bruker Optics, model MPA, Ettlingen, Germany) and described by Sun et al. (2010).

2.2. Sample collection and processing

Faeces were collected from faecal bags attached to the harnesses at 08:00 each morning, the fresh weight of faeces recorded, and 10% of the total faecal output subsampled for each sheep each day and stored at −20°C. The subsamples were freeze-dried, followed by oven drying at 65°C to a constant weight for DM estimation.

Four sub-samples of approximately 200 g were taken from each fresh forage every day during the two experimental periods. One of the four daily sub-samples was dried at 65°C for 48 h and these were then pooled for each forage over each experimental period and the pooled sample sent for nutrient profile analysis by the Nutrition Laboratory of Massey University (Palmerston North, New Zealand), as described by Sun et al. (2012). The remaining three daily sub-samples were individually dried at 105°C for 24 h to determine DM content of the forage (AOAC, 1990; method 930.15). The dietary chemical composition is presented in Table 1. Feed refusals collected at 08:00 were weighed each day, subsampled and dried at 65°C for 48 h to estimate forage DM not eaten.

2.3. Sample size calculations

The aim of this trial was to obtain measurements of digestibility for all possible lengths of consecutive acclimatisation and measurement phase during the two 11-day indoor periods, in order to determine the standard deviations associated with various durations of measurement and to calculate the numbers of sheep required for treatment comparisons. The digestibility for a measurement phase of more than 1 consecutive day was calculated from the sum of DM intakes and faecal DM outputs.

The required sample size (i.e., the number of sheep) for any given length of measurement phase was calculated using the following equation:

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