Animal Feed Science and Technology 245 (2018) 48–53

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Short communication: A survey of grass-clover ley management and creation of a near infra-red reflectance spectroscopy equation to predict clover concentration

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ARTICLE INFO

Keywords:
Grass-clover
Agronomy
Silages
Clover concentration
Management
NIRS

ABSTRACT

The purpose of the present study was, firstly, to examine current practice for the agronomy of grass-clover mixed swards used for silage-making in the UK, and secondly, to develop and validate a Near Infra-Red Reflectance Spectroscopy (NIRS) equation capable of predicting clover concentration (CC) in undried and unmilled grass-clover silage samples. A calibration set of 94 grass-clover (white, *trifolium repens*, and red, *trifolium pratense*) mixture silage samples were sourced from UK farms and an accompanying questionnaire was used to obtain information on the sward agronomy used to produce each sample. Questionnaire data highlighted that (i) reducing the use of fertiliser inputs (ii) increasing uptake of new varieties, and (iii) increasing the farmer's ability to measure botanical composition as potential strategies for improving the utilisation of clover in grass swards. Botanical composition was measured by hand separation for each sample and a new NIRS equation to predict CC was created and assessed using blind validation with an independent set of 30 grass-clover samples. The relative standard error of cross validation (SECV, as a percentage of the measured mean) of the optimised equation produced was 36.8%, and, in an independent validation test, the ratio of standard error of prediction to the standard deviation of the reference data set (RPD) was 1.56. The equation could be improved by increasing accuracy at high CCs but showed promise as a simple tool to assist growers in sward management decisions.

1. Introduction

The use of mixed grass-clover swards for both grazing and silage production is now relatively wide-spread across temperate European agricultural systems and particularly in the UK where 70% of grass swards on dairy farms are thought to contain clover (DEFRA, 2015). Grass-legume swards offer a sustainable approach to reduce fertiliser input into grasslands, as the atmospheric nitrogen (**N**) fixed by clover can be utilised by grass, an example of niche complementarity between two species (Nyfeler et al., 2011; Phelan et al., 2015). Utilising niche complementarity in this way is becoming an area of increasing interest for both binary and complex (3+ species) sward mixtures. Combining species of different functional groups, can not only increase productivity and minimise the need for inputs, but may also supply different beneficial nutrients, minerals, and secondary plant compounds to livestock (Provenza et al., 2007). A key determinant of the success of mixed swards is determining and maintaining the correct

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https://doi.org/10.1016/j.anifeedsci.2018.09.003
Received 29 April 2018; Received in revised form 6 September 2018; Accepted 10 September 2018

concentration ratio of species or functional groups so that they work in harmony. Previous research has shown that, in general, the best results can be achieved by an even distribution of species within a sward, with no single species becoming dominant (Finn et al., 2013). Where one species is over-dominant the others may not reach their production potential or fulfil their niche functionalities, and the productivity of the whole sward could be reduced (Kirwan et al., 2007; Lüscher et al., 2014).

To date, we are not aware of any published surveys that document the management strategies farmers utilise for grass-clover leys, and to what extent these conform to best practice guidelines for maintaining species evenness. Additionally, there is a need for increased development of practical methods by which growers can manage species evenness within a sward, beginning with simple binary mixtures. The first step to improved management is the ability to measure the botanical composition of a sward with ease. Near infra-red reflectance spectroscopy (**NIRS**) analysis offers a quick and inexpensive method, already routinely used for silage analysis, by which the composition of a mixed sample might be determined. Prediction equations for NIRS analysis of clover in a mixed grass-clover silage sample have been successfully reported previously using dried samples for calibration (Wachendorf et al., 1999; Cougnon et al., 2014) however no prediction equations currently exist which are appropriate for the UK where silage analysis is performed on undried (fresh) and unmilled samples. Once the botanical composition of a sward is known, management may be adjusted to suit one species or another by varying cutting height, cutting frequency, fertilisation or grazing intensity (Yarrow and Penning, 1994; Phelan et al., 2014).

The objectives of the present study were therefore to develop an NIRS equation to measure the botanical composition of fresh grass-clover silages appropriate for uptake by laboratories in the UK, and secondly, to assess current management practices of grass-clover swards to better understand where further research into management of botanical composition is required.

2. Material and methods

2.1. Experimental design

Ninety-four grass-clover silages (58 baled and 36 clamped) were sourced from 50 commercial farms spread throughout England, Scotland, and Wales, and brought to the University of Reading's Centre for Dairy Research (**CEDAR**; Arborfield, UK) for processing. A further 95th sample was created by combining one of the original 94 samples with additional grass silage to create a new sample, this was done to create a greater quantity of material for other *in vivo* analyses. The samples were obtained to evaluate the use of NIRS analysis for nutrient concentrations as described previously (Thomson et al., 2018) over three consecutive years (2012/13, 2013/14, and 2014/15). The quantity of each silage collected was approximately 500 kg. Where the clover species was known ($n = 65$) 66% of samples were red clover (*trifolium repens*), 20% were white clover (*trifolium pratense*) and 14% were a mixture of both. The number of samples that were first, second, third and fourth cuts were 36, 20, 16, and 4 respectively (22 samples unknown). Sample processing is described in detail by Thomson et al. (2018), however, in brief, samples were mixed either in a feeder wagon containing knives (Hi-Spec Mix Max, Hi Spec Engineering, Co. Carlow, Ireland for 45 min), or in a DataRanger feed mixer without knives (American Calan, Northwood, NH, USA) for unchopped and pre-chopped samples respectively. After mixing, representative subsamples of each silage sample were stored separately at -20 °C for future analysis by manual separation and NIRS.

2.2. Silage questionnaire

A questionnaire was given to each farmer who donated a silage sample to the study. The questionnaire comprised 17 questions (Supplementary Table 1) relating to the timing of establishment, fertiliser applications, and harvesting, the composition of seed mixtures used, and ensiling practices. For the botanical composition of the seed mixture, the variety sown was recorded for ryegrass and clover whereas any other components were simply recorded at the species level as variety was rarely provided. In addition farmers were asked to retrospectively estimate the percentage of clover in the sward at the time of harvest (Question 9, Supplementary Table 1). Farms were permitted to contribute more than one silage sample to the study provided the samples originated from differing cuts, years, or swards. Separate questionnaires were completed for each of the samples. Questionnaire forms were returned for 64 of the 94 samples however not all questions were answered on all returned questionnaires and in some instances answers were insufficiently detailed to be included. These 64 completed questionaires originated from 36 individual farms, reflecting that a number of farms returned more than one questionnaire, each relating to a different crop of silage.

2.3. Sample analysis and NIRS scanning

Approximately 200 g of silage was manually separated into clover, grass and other species as a means of determining the clover concentration (**CC**) in the silage. Resulting fractions were then oven dried at 60 °C for 72 h to determine CC on a DM basis. A second 2 kg subsample of frozen material was sent to the Agri-Food and Biosciences Institute (**AFBI**; Hillsborough, Northern Ireland) where all samples were hand-chopped to 2.5 cm length. Two separate sub-samples were created from each sample, each containing 100 g of undried and unmilled silage wrapped in non-PVC cling film which were placed in coarse transport cells for scanning (Park et al., 1999). NIRS spectra for each scan, recorded as Log 1/Reflectance over a 400–2498 nm range (2 nm gaps), were obtained using a Foss NIRSystems 6500 machine (Foss, Hillerød, Denmark) and ISI v.3.10 software (Infrasoft International, Port Matilda, PA, USA).