Animal Feed Science and Technology 205 (2015) 31–41

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

New recommendations for the ruminal *in situ* determination of indigestible neutral detergent fibre

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ARTICLE INFO

Article history:
Received 28 February 2014
Received in revised form 8 April 2015
Accepted 9 April 2015

Keywords:
Forage evaluation
Indigestible fibre
In situ incubation
Methodology
Near infrared reflectance spectroscopy
Ruminants

ABSTRACT

Three different experiments were conducted aiming at evaluating different aspects of the methodology for ruminal *in situ* determination of ash-free indigestible neutral detergent fibre (iNDFom) with reference to a near infrared reflectance spectroscopy (NIRS) application. Sample grind size, *in situ* bag pore size and cloth characteristics were compared for different feed samples in the first trial. In the second trial, the concentration of iNDFom in silage was compared with the value of the parental grass crop, and finally in a third trial four different steps in the methodology of iNDFom determination were evaluated between two different laboratories. The determinations of the concentration of iNDFom using 12-μm Saatifil PES bags were generally greater compared to iNDFom determined in the Sefar Petex bags. To avoid potential errors from particle losses or impaired microbial activity we recommend a grind size of 2.0 mm and bags made from Sefar Petex 07-6/5 or 07-11/5 to be used in ruminal *in situ* determination of iNDFom. When the iNDFom concentration was measured from grass and subsequent silage samples, the values were similar revealing that iNDFom concentration of the plant material was not altered during the ensiling process. Further, the NIRS spectra generated from the grass and subsequent silage samples were very similar. Based on the results of the current study, one common calibration dataset is enough for NIRS predictions of iNDFom in grass silages despite feed value determination from the herbage material. Last, of the four different methodological steps that were tested, bag type and cow within site affected the determinations, while washing procedures and the analysis of ash-free neutral detergent fibre concentration in the incubated residues did not contribute to any differences between the two laboratories. When iNDFom values from multiple laboratories are used, it is recommended to choose one biologically validated reference laboratory, and apply laboratory specific corrections for the others.

Abbreviations: aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; iNDFom, indigestible neutral detergent fibre expressed exclusive of residual ash; k_d, digestion rate; NIRS, near infrared reflectance spectroscopy; OMD, organic matter digestibility; pdNDFD, potentially digestible neutral detergent fibre digestibility; RMSE, root mean square error; TMR, total mixed ration.

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http://dx.doi.org/10.1016/j.anifeedsci.2015.04.008
0377-8401/

1. Introduction

Ruminants have a unique potential to convert fibrous plant material into high quality human edible food. Forages comprise a large part of total diet dry matter (DM) in livestock production systems. The nutritive value of forages is much more variable than that of concentrate feeds. Further, variation in nutrient supply of ruminants is mostly related to forage characteristics and intake potential (Mertens, 1994). This emphasizes the importance of accurate predictions of their feeding value to be able to support a sustainable forage-based livestock production.

Digestibility is the most important trait of forages in feed value determinations (Huhtanen et al., 2006b). Indigestible neutral detergent fibre (iNDFom) determined by a 288-h ruminal *in situ* incubation in dairy cows has been demonstrated to be a good predictor of forage digestibility (Huhtanen et al., 2006b; Krizsan et al., 2012) and therefore standardization of the methodology is important. Near-infrared reflectance spectroscopy (NIRS) has successfully been calibrated for iNDFom (the *in situ* method; Nousiainen et al., 2004) and is applied in routine analysis of farm samples in all Nordic countries. A precise calibration of NIRS requires a continuously updated and representative reference data set of well-known quality; *i.e.* new forage types (and mixtures and varieties within forage types) combined with the results of original reference samples and determined with least possible error using a standardized method. New reference samples are usually collected by the farm service laboratory. A NIRS application for dried feed samples usually requires a finer grind size of the samples because particle size can have large effect on the light reflectance measure, while a coarser grind size generally have been preferred to avoid losses of particles in ruminal *in situ* determination of iNDFom.

The Nordic feed evaluation system NorFor introduced a standardized protocol for *in situ* determination of iNDFom in 2007 aiming to decrease the variation observed between laboratories in the Nordic countries (Eriksson et al., 2007). This standardized protocol was later presented by Åkerlind et al. (2011). The procedure mainly introduced recommendations of bag cloth type, grind size, numbers of animals and length of incubation (Åkerlind et al., 2011). However, many factors still seem to contribute to variation in analytical precision of the iNDFom procedure between laboratories as indicated by the most recent Nordic ring test by Eriksson et al. (2012). A decomposition of the observed variance has not been conducted, *i.e.* the greatest methodological source of variation between laboratories has not been properly defined.

In this study we aimed at comparing the incubation results of samples displaying a range in fibre concentration and composition for two different grind sizes and incubated in polyester bags of four different qualities. Further, due to potential DM losses and fibre hydrolysis during ensiling the project examined whether predictions of iNDFom by NIRS could be similarly determined from grasses and silages. This was accomplished by analysing a set of cut herbage and corresponding silage with known *in vivo* digestibility. In the final experiment the contribution of different methodological steps in the 288-h ruminal *in situ* procedure to the total variation in estimates of iNDFom from two different laboratories was evaluated.

2. Materials and methods

2.1. Experiment 1

Samples of oat (*Avena sativa*) and rapeseed (*Brassica napus*) meal were provided by the feed manufacturer AB Västerbottens Fodercentral in Umeå, Sweden. A sample of dried lucerne (*Medicago sativa*) was taken from a bag of a commercial feed Krafft Lusern (Krafft AB, Falkenberg, Sweden). Samples taken from two grass silages that were harvested on June 10 (first cut) and on July 19 (second cut) in 2012 from a timothy (*Phleum pratense*) dominated sward at Röbäcksdalen research farm in Umeå (63°45′ N, 20°17′ E), Sweden were included in this experiment. Samples of barley (*Hordeum vulgare*) straw and crimped barley grain were also obtained from feeds harvested at Röbäcksdalen research farm. A hay sample from a timothy dominated sward harvested in July 2010 in Umeå was included in the study. Additionally, faecal samples from a dry and a lactating cow were collected at Röbäcksdalen research farm. All experimental samples were dried at 60 °C for 48 h and were ground through a 2.0-mm screen for ruminal *in situ* incubations or a 1.0-mm screen for chemical analysis, using the same cutting mill (Retsch SM 2000; Retsch GmbH, Haan, Germany). The faecal samples were ground with mortar and pestle to pass screen sizes of both 1.0 and 2.0 mm. A fine grind size representative of that needed for scanning in a NIRS system was achieved by grinding the samples at the research laboratory at Valio Ltd. in Helsinki, Finland to pass a 0.8 mm screen with a Laboratory Mill 3100 (Danfoss AB, Linköping, Sweden).

The iNDFom concentrations of the 0.8- and the 2-mm grind sizes of each sample were determined following *in situ* incubations of 288 h in the rumen (Huhtanen et al., 1994; Krizsan and Huhtanen, 2013) using three cows. One lactating and two dry Nordic Red cows were used for the ruminal *in situ* incubations. The lactating cow had an average production of 28.2 kg of milk/day, and was fed total mixed ration (TMR) of 60% grass silage and 40% concentrate on DM basis *ad libitum* during the incubation. The dry cows were kept in a pen that housed 11 cows in total, and were group-fed to provide 5–6 kg of grass silage DM per animal and day. Additionally, the dry cows were fed 1 kg of the same commercial concentrate as the lactating cow on an air-dry basis in separate concentrate feeders.

Samples of 2 g were weighed into polyester bags with a pore size of 6 μm, 11 μm, 12 μm and 17 μm and pore areas equal to 5%, 5%, 6% and 9%, respectively, of the total surface area. The 12-μm bags were made of the cloth Saatifil PES 12/6 from Saatitech S.p.A., Veniano, Como, Italy, which is classified as the Nordic standard per today (Åkerlind et al., 2011). The other bags were made of 07-6/5, 07-11/5 and 07-17/9 Sefar Petex from Sefar AG, Heiden, Switzerland. The internal dimensions of the polyester bags and the sample size were adjusted to give a sample size to surface area ratio of 10 mg/cm^2. All samples