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The use of cellulase and filter bag technique to predict digestibility of forages

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ABSTRACT

A study was conducted to determine the reliability of the novel *in vitro* dry matter digestibility (IVDMD) method for predicting the *in vivo* organic matter digestibility (OMD) of forages. The study was carried out on two sets of feeds of known OMD determined on sheep: set A ($n = 35$), consisting of whole crop cereal (corn and barley) herbages and set B ($n = 80$), mostly consisting of grass and clover silages. IVDMD was determined by the CEL48-ND method in which 500 mg of dried and ground feed sample was inserted into filter bag (ANKOM Technology Corporation, Fairport, NY, USA) and then the bags were incubated for 48 h at 39 °C in the cellulase solution (celullase Onozuka R10), in the jars of Daisy[II] Incubator (ANKOM Technology Corporation). After incubation the bags with residues were extracted in a neutral detergent (ND) for 1 h at 100 °C in Ankom[220] Fiber Analyzer (ANKOM Technology Corporation). The data for all sets were used to find the best single and multiple regression equations to predict OMD. For each set the linear regressions were calculated in which OMD was the dependent variable (Y) whereas IVDMD determined by CEL48-ND was the predictor X_1 and contents of nutrients (g/kg DM) were predictors X_2, X_3, X_4 or X_5. The equations were compared by means of coefficient of determination (R^2), standard deviation of differences between observed (Y_i) and predicted value ($\tilde{Y}_i$) (residual standard deviation; RSD), and mean square prediction error (MSPE). Additionally, the verification of equations was also done based on the Akaike information criterion (AIC). Mean R^2 for the regression equations of OMD by CEL48-ND was 0.468 (set A), 0.581 (set B) and 0.539 (set A + B). Including the next predictor (X_2, X_3, X_4 or X_5) increased R^2, and decreased RSD, MSPE and AIC, especially in the equations calculated for whole crop corn herbages, grass silages and clover silages. It can be concluded that the *in vitro* method presented in this study is a simple alternative for existing methods in which buffered rumen fluid is used. Using a standard enzyme available commercially worldwide may decrease variation between laboratories. Further, using filter bags and Daisy Incubator decreases labour costs and use of animals.

Abbreviations: AIC, akaike information criterion; ADF, acid detergent fibre; aNDF, neutral detergent fibre; AFBT, ANKOM filter bag technique; CP, crude protein; CS, subset 'clover silages'; DK, Denmark; DM, dry matter; FIN, Finland; GS, subset 'grass silages'; IVDMD, *in vitro* dry matter digestibility; MSPE, mean square prediction error; ND, neutral detergent solution; OMD, organic matter digestibility; R^2, coefficient of determination; RSD, residual standard deviation; WCB, subset 'whole crop barley'; WCC, subset 'whole crop corn'.

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1. Introduction

High demands for nutrients of modern dairy cows and fast growing beef cattle require a precise diet formulation, based on a reliable evaluation of the nutritive value of feedstuffs, especially forages. While the accuracy of determination of chemical composition is mostly not questionable, digestibility values are still the weakest link in estimation of forage nutritive value. *In vivo* digestibility methods are costly, laborious and inaccessible for most field laboratories, as well as they increasingly raise the concerns about animal welfare (Adesogan, 2002; Huhtanen et al., 2006). On the other hand, majority of the most popular *in vitro* methods (*e.g.* Tilley-Terry or gas-test) requires buffered rumen fluid as an incubation medium (Tilley and Terry, 1963; Goering and Van Soest, 1970; Menke and Steingass, 1988; Hall and Mertens, 2012) which is a serious limitation for laboratories not having an access to cannulated animals to donate the rumen fluid. Such methods are also difficult to standardize due to different feeding conditions of donor animals in different laboratories (Adesogan, 2002; Hall and Mertens, 2012).

Above discussed limitations with an access to rumen fluid encourage the use of enzyme solutions as incubation medium instead of rumen fluid (Jones and Theodorou, 2000; Huhtanen et al., 2006). Among few, the fungal cellulase-based techniques have been the most often tested (De Boever et al., 1988; Aufrère and Graviou, 1996; Adesogan, 2002; Nousiainen et al., 2003). According to Huhtanen et al. (2006), the enzymatic hydrolysis reflects the mechanism of digestibility better than concentrations of components from proximate analysis or detergent fractionation. However, the calibration of enzyme digestion assays has been a major limitation in practical adaptation of them (Broderick and Colombini, 2010). Moreover, enzyme-based predictions of *in vivo* digestibility also vary with forage species, population and season of harvest (Givens et al., 1995; Huhtanen et al., 2006). Thus, forage-specific equations may be needed. Although the *in vitro* digestibility estimated using enzymatic techniques is often lower than *in vivo* digestibility, these differences do not preclude the use of such methods provided that appropriate correction equations are used (Huhtanen et al., 2006).

An important source of variation in the results of *in vitro* gravimetric methods is related to the filtering step (Adesogan, 2002). Enclosing the sample in the filter bag, such as in the method developed by ANKOM Technology Corporation (Fairport, NY, USA), may eliminate this problem. In ANKOM filter bag technique (AFBT), feed samples sealed in the polyester bags are placed in glass jars which are rotated in an insulated chamber, called the Daisy^{II} Incubator. The AFBT also reduces the labour input because it allows batch incubation of several samples in the jar (Adesogan, 2002). The original AFBT is based on the '*in vitro* true digestibility' method described by Goering and Van Soest (1970). It was shown in several studies that digestibility estimated by AFBT correlated well with conventional *in vitro* techniques, *e.g.* Tilley-Terry (Holden, 1999; Mabjeesh et al., 2000; Wilman and Adesogan, 2000; Adesogan, 2005; Ammar et al., 2005). On the other hand, for some forages Vogel et al. (1999) showed higher dry matter (DM) digestibility estimated by AFTB than by the conventional *in vitro* technique. Furthermore, in a study of Damiran et al. (2008) digestibility values estimated by AFBT were correlated (R^2 = 0.58–0.88) with values estimated by conventional *in vitro* techniques, but in most cases AFBT overestimated *in vivo* DM and NDF digestibility. The study of Damiran et al. (2008) is one of the few that attempted to correlate AFBT with *in vivo* observations. According to Wilman and Adesogan (2000), the conventional *in vitro* technique is likely to give more precise results than AFBT, although they postulated that the use of AFBT gave acceptable digestibility estimates for forages when the emphasis was on saving labour.

To our best knowledge only one study has been published in which fibrolytic enzyme mixtures (cellulase and hemicellulase) were used instead of rumen fluid in the Ankom Daisy^{II} Incubator (Colombatto et al., 2000). The study showed that the enzyme mixtures had the potential to describe the DM digestibility of forages.

The present study was conducted to determine the reliability of the novel *in vitro* DM digestibility (IVDMD) method using cellulase and filter bag technique for predicting the *in vivo* organic matter digestibility (OMD).

2. Materials and methods

2.1. Feeds and chemical analyses

The study was carried out on two sets of feeds. Set A (n = 35) consisted of two subsets: "whole crop corn; WCC" (n = 20) consisting of whole crop corn silages (n = 10) and whole crop corn herbages (n = 10) (WCC) as well as "whole crop barley; WCB" (n = 15) consisting of whole crop barley silage (n = 10) and whole crop barley herbages (n = 5). Ensiled and un-ensiled samples were not originated from same source.

Set B (n = 80), consisting of two subsets: "grass silage; GS" (n = 54) consisting of timothy-meadow fescue grass silages as well as "clover silage; CS" (n = 19) consisting of red clover silages. Additionally in set B there were whole crop barley silages (n = 5) and whole crop wheat silages (n = 2).

Samples of feeds of set A originated from Denmark (DK; Department of Animal Science, Aarhus University, AU Foulum), whereas samples of set B originated from Finland (FIN; MTT Agrifood Research Finland, Animal Production Research). Original feeds were dried in force-air oven at 50 (DK) or 60 °C (FIN) for until dry and dried samples were ground to pass 1 mm screen.

Chemical composition was determined by standard methods (AOAC, 2000), using the following procedures: DM–930.15, ash–923.03, crude protein (CP)–990.03, ether extract–920.39. Neutral detergent fibre (aNDF) was determined with heat-stable amylase according to Van Soest et al. (1991). Acid detergent fibre (ADF) and lignin (sa) were determined using the method described by Robertson and Van Soest (1981). Above fibre analysis were performed using Ankom^{220} Fiber Analyzer.