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In vitro method for determining the ruminal degradation rate of rapeseed meal protein using ^{15}N isotope labelled ammonia nitrogen

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ABSTRACT

An *in vitro* method based on observations of ^{14}N and ^{15}N isotope fluxes between ammonia N and non-ammonia (NAN) pools was established to study the ruminal degradation rate of rapeseed meal protein. Feed protein equal to 125 mg of N/l was incubated in the presence of rumen fluid, mineral buffer, and a carbohydrate mixture formulated to provide a constant supply of fermentable energy over the entire incubation period. The ammonia N was labelled with the ^{15}N isotope, and the incubations were carried out for 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 10 h. A model with six pools was used to estimate the rate of protein degradation to ammonia N and the rate of microbial N synthesis from ammonia N. The parameter values were adjusted based on the sizes of the ammonia ^{14}N, ammonia ^{15}N, ^{14}NAN, and ^{15}NAN pools observed at different time points over the incubation period. The rate of rapeseed meal N degradation was 0.06/h (0.028 standard deviation between runs), and the predicted effective protein degradability was 0.38 (0.122 standard deviation between runs). The current approach seemed appropriate for determining microbial N synthesis from ammonia N, but measurement of the direct incorporation of amino acids into microbial N may be required to adequately characterize the metabolic events involved in ruminal protein degradation.

Abbreviations: CNCPS, Cornell Net Carbohydrate and Protein System; CP, crude protein; DM, dry matter; OM, organic matter; NAN, non-ammonia nitrogen; RSM, rapeseed meal.

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1. Introduction

Quantitative information on the metabolism of common protein feedstuffs is essential for models used for optimizing dairy cow diets in terms of cost and efficient use of nutrients. Dietary protein provides peptides, amino acids, and ammonia for rumen microbial N synthesis and supplies undegradable feed protein to the animal beyond the limits of microbial N yield. The rate of protein degradation and passage determine the relative proportions of substances either metabolized in the rumen or escaping ruminal degradation. The *in situ* method has been widely used to determine the rate of ruminal protein degradability (Ørskov and McDonald, 1979), but this approach has the inherent limitation that the rate of degradation of soluble N fractions cannot be measured and must be assumed to be infinite. This assumption is inconsistent with the observations of substantial quantities of soluble feed N fractions leaving the rumen (Choi et al., 2002). As an alternative, a number of *in vitro* methods have been introduced to determine the susceptibility of different protein supplements to ruminal degradation and to assess the effects of protective treatments on the rate of microbial metabolism (Raab et al., 1983; Broderick, 1987; Mahadevan et al., 1987; Hristov and Broderick, 1994).

In vitro methods that are based on ammonia release but do not account for ammonia uptake by microbes have been recognized to be inadequate (Broderick, 1978). Several approaches have been introduced to quantify the utilization of protein degradation end products by microbial N synthesis. Broderick (1978, 1987) investigated the utilization of various inhibitors to completely prevent microbial uptake of ammonia N and amino acids. Hristov and Broderick (1994) introduced a system using ^{15}N labelled ammonium N to determine the microbial N synthesis from degraded protein. These approaches have involved a limited number of time points, which do not allow a detailed time course analysis of ammonia N fluxes *in vitro*. The objective of the current study was to establish an *in vitro* method for determining the rate of protein degradation based on a time course analysis of ^{15}N and ^{14}N fluxes between the ammonia N and non-ammonia N pools. Rapeseed meal was chosen as the source of model protein because it is one of the most important protein supplements in European dairy cow diets.

2. Materials and methods

The *in vitro* system introduced in the current study was based on the principles outlined by Hristov and Broderick (1994) but included two major modifications: (i) the *in vitro* incubations were carried out for an extended period of up to 10 h, and (ii) the composition of the carbohydrate mixture was formulated to provide a constant energy supply over the course of incubation.

2.1. Rapeseed meal

Rapeseed meal (RSM) was prepared from whole rapeseed using the diethyl ether extraction procedure to obtain a product with well-defined process conditions. To remove the oil fraction, whole rapeseed was crushed with a mortar and pestle, and then suspended in diethyl ether with one part rapeseed and three parts diethyl ether in a 5-l glass bottle. After 6 h, particulate matter had sedimented, and the top layer of ether and oil was removed by aspiration. Rapeseed particles were re-suspended in fresh diethyl ether, and the extraction procedure was repeated a total of 10 times. The mixture of ether and oil removed from the glass bottle was mixed with water (10:1) to facilitate the recovery of small particles from the suspension. After 2 h, the top layer of the ether and oil mixture was removed by aspiration to collect small rapeseed particles suspended in water at the bottom. Small particulate matter was pooled with ether-extracted RSM, which had been allowed to stand at room temperature on a tray overnight to evaporate excess ether. The next day, the RSM was dried at 60 °C for 24 h and homogenized using a mortar and pestle. The dry matter (DM) concentration of the RSM was 923 g/kg, and the chemical composition was the following: organic matter 916, N 63.1, neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash 271, and ether extract 45 g/kg DM. The rapeseed meal protein composition characterized according to the Cornell Net Carbohydrate and Protein System (CNCPS) (Licitra et al., 1996) was 250, 208, 440, 40, and 62 g/kg N of CNCPS N-fractions A, B_1, B_2, B_3, and C, respectively. The composition is rather similar to commercial