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Protein value and degradation characteristics of pulp fibre fractions from screw pressed grass, clover, and lucerne

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ABSTRACT

Biorefinery of grassland plants may enable the production of feed for both ruminants and monogastrics at the same time. The pulp fibre fraction from twin-screw processed white clover, red clover, lucerne, and perennial ryegrass, is a major side-stream commonly considered of low value. However, it maintains a crude protein (CP) fraction of which a high proportion is expected to be fibre-associated. The pulp is expected to be suitable as forage for ruminants but knowledge of the feed value is limited.

The objectives of this study were to assess the protein degradation in the rumen and the protein value of pulp compared to the original plant material, and to study the protein distribution among fibre fractions in order to evaluate the pulp as a source of forage for dairy cows.

The ash concentration in the pulp was 51.1–72.3 g/kg DM compared to 86.3–104 g/kg DM in the original plant, while the *in vitro* digestible organic matter (DOM) in dry matter (DM) did not decrease significantly in pulp (532–689 g/kg DM) compared to plant (564–694 g/kg DM). Neutral detergent fibre (aNDF) concentration was higher in pulp (529–694 g/kg DM) than in original plant (342–503 g/kg DM), while the CP concentration of the aNDF from pulp (77.8–186 g/kg aNDF) did not differ from the concentration found in the plant aNDF (74.7–137 g/kg aNDF). Furthermore, the total amino acid (AA) concentration increased in pulp (825 g/kg CP) compared to original plant (768 g/kg CP), and the individual concentration of all essential AA in DM increased.

The *in situ* rumen degradation of CP showed that the potentially degradable fraction was not different between plant (889–953 g/kg) and pulp (817–939 g/kg), although the fractional rate of degradation was lower in the pulp (0.093–0.109 h^{-1}) than in the plant (0.113–0.192 h^{-1}). Thus effective degradability (ED) of CP was lower in pulp (579–647 g/kg) than in plant (657–786 g/kg) increasing rumen escape protein (REP). The intestinal digestibility of REP was similar, thus a higher proportion of the pulp CP (225–256 g/kg) was digested in the intestine compared to plant CP (125–190 g/kg). The indigestible neutral detergent fibre (INDF) proportion of aNDF was not different between plant (88.1–422 g/kg aNDF) and pulp (85.3–460 g/kg aNDF).

Abbreviations: AA, amino acid; ADF, acid detergent fibre; ADIP, acid detergent insoluble protein; aNDF, neutral detergent fibre; CNSPS, Cornell net carbohydrate and protein system; CP, crude protein; DM, dry matter; DOM, digestible organic matter in DM; ED, effective degradability; INDF, insoluble neutral detergent fibre; NDS, neutral detergent solubles; NPN, non-protein nitrogen; OM, organic matter; REP, rumen escape protein

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This study demonstrated that pulp from twin-screw pressing of white clover, red clover, lucerne, and perennial ryegrass has a protein concentration and value for ruminants, which is similar to the original plant material.

1. Introduction

Grassland plants such as legumes and grasses have the potential to become a locally grown and sustainable source of protein and energy for livestock in order to comply with the increasing demand for animal protein worldwide (Chadd et al., 2002; Boland et al., 2013). Soluble protein from white clover, red clover, lucerne, and perennial ryegrass can be extracted and used as a protein supplement for monogastrics leaving a substantial quantity of fibre-rich pulp, which may be used for ruminant feeding (Wieringa, 1982; Bruins and Sanders, 2012). The soluble protein concentrate has already been examined thoroughly in terms of chemical composition, quality, and nutritional value (Pirie, 1978; Chiesa and Gnansounou, 2011; Stødkilde et al., 2018), while studies on pulp as a source of forage for ruminants have been limited until now (Jones, 1982; Broderick et al., 1999). The fibre-rich pulp has often been considered a low value side-stream. However, pulp produced in a twin screw press has recently shown to have a CP concentration similar to original plant (Damborg et al., 2016). A large proportion of this protein is cell wall-bound and/or –retained, and less accessible for monogastric consumption (Edwards et al., 1975) but could be valuable as forage for ruminants thereby increasing the utilisation of the nutrients in the grassland plants used for biorefining (Wieringa, 1982; Bruins and Sanders, 2012). A proportion of soluble nutrients are removed during processing, though it is also suggested that this physical treatment enhances the digestibility of DM left in the pulp (Koegel et al., 1992).

The objectives of this study were to evaluate the potential of pulp as forage source for dairy cows compared to original plant regarding chemical composition, *in vitro* digestibility, and protein degradability and digestibility, as well as protein distribution among fibre fractions and changes in AA composition between plant, pulp and aNDF fractions from a diverse sample set of common Danish grassland plants. The hypothesis was that rumen escape protein (REP) increased in pulp compared to original plant.

2. Materials and methods

2.1. Plant material and processing

White clover (*Trifolium repens* L., variety; Klondike and Silvester), red clover (*Trifolium pratense* L., variety; Rajah and Suez), lucerne (*Medicago sativa* L., variety; Creno), and perennial ryegrass (*Lolium perenne*, variety; Trocadero and Calvano 1), grown at the experimental farm at Aarhus University, Foulum were harvested at a 7–10 cm stubble height in November 2013 (white clover and red clover 2nd regrowth, perennial ryegrass 3rd regrowth), June (lucerne, July) 2014 (1st regrowth), and September 2014 (3rd regrowth) and frozen directly at −20 °C until processing. The frozen plant material was thawed at 5 °C over-night prior to extraction. Plant material of varying chemical composition and stage of maturity was used in order to observe the overall effects of the processing. Individual values for each plant species were included to show the effects of processing on a broader variation of plant types. Plant materials were sorted by hand to discard foreign plant fragments, wilted fragments *etc.* and fed to the twin screw press (Angelia 8500S Angel slow-juicer, Angel Co. ltd., Korea). The processing was conducted at room temperature, and the speed of the screws was 82 rpm.

To be able to use more harvests simultaneously and take representative samples pulp and plant samples were freeze-dried (Scanvac Coolsafe type 55-4) and ground to 1.5 mm for *in situ* analyses using a cutter mill (Pulverisette 15; Fritsch GmbH, Idar-Oberstein, Germany). Subsamples were further ground to 1.0 mm for fibre analyses and to 0.5 mm for AA analyses.

2.2. Fibre and fibre-associated protein characterisation

A slightly modified version of the Van Soest fibre analysis (Van Soest et al., 1991) was used to produce fibre fractions to determine fibre-associated nitrogen (N) and AA in plant and pulp. In short, for each plant and pulp, three bags (one for aNDF, one for ADF, and one for lignin (sa)) (Dacron bags, 10 x 9 cm, and 12 µm pore size), were filled with 10 g of sample (1.0 mm). The bags were washed for 1 h at 60 °C to eliminate immediate soluble material. All bags were then boiled in neutral detergent solution plus Na_2SO_3 and α-amylase for 1 h, and washed in demineralised water according to the standard Van Soest method (Van Soest et al., 1991). The samples were dried for 48 h at 60 °C and weighed. The aNDF-residues were used for further analysis. The remaining bags were boiled in acid detergent solution for 1 h and washed in demineralised water, dried 48 h at 60 °C and weighed. The ADF-residues were used for further analysis. The remaining bags were immersed in 72% sulphuric acid solution for 3 h, washed and dried at 60 °C for 24 h and weighed, and the ADL-residues were used for further analysis. All samples were removed from the bags and ground to 0.5 mm in a centrifugal mill before further analysis. The aNDF and ADF fractions in the present study were estimated without correction for residual ash.

The concentration of neutral detergent solubles (NDS) was calculated as the difference between OM and aNDF. The residues were dried, N was measured in all samples, and AA were measured in aNDF samples.