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Influence of formic acid and dry matter on protein degradation in the tanniniferous legume sainfoin

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ABSTRACT

Improved protein utilization due to protein tannin binding has been observed in sainfoin and other tannin containing legumes. Since it is well known that binding of protein to tannins and dissociation of the resulting complexes are pH dependant, effects of low pH after treatment with unusually high amounts of acidic silage additives and different dry down stages on fresh herbage prior to ensiling was investigated. Criteria for tannin binding effects were based on N fractionation in an *in vitro* ruminal digestion system. A mixture of sainfoin varieties were wilted to different dry matter (DM) levels and treated with 0, 4 and 8 ml formic acid per kg fresh matter prior to ensiling for 60 days. Nitrogen fractionation included measurement of total N, buffer soluble N, non-protein N, α-amino acid N and ammonia N. Protein degradation was measured by an *in vitro* ruminal protein digestion system. Results show beneficial effects of moderate and high acidification particularly for buffer soluble N at 180 and 400 g/kg DM ($P<0.0001$) while these effects level off at 500 and 600 g/kg DM ($P<0.852$). However, no negative effect related to impaired tannin protein binding in the silages or the *in vitro* protein degradation occurred.

1. Introduction

Forage conservation as hay or silage is accompanied by decreased nutritive value and loss of dry matter (DM) due to oxidation and fermentation of the forage. These losses occur during harvesting, ensiling and feed-out due to respiration, rain damage, mechanical losses and silage effluents (McDonald et al., 1991). This is an economic loss to farmers and an environmental problem due to increased carbon emissions and pollution of ground water with nitrates (Woolford et al., 1983; Jones and Jones, 1995). To reduce these losses, a variety of ensiling techniques with various types of silage containments and silage additives, have been implemented (Buxton et al., 2003). Wilting and application of acids are two conservation methods widely used in ensiling. Wilting decreases plant protease activity and clostridial growth, lowers the amount of silage effluent and increases the concentration of sugars in the crops thereby improving silage nutritional quality (Gross and Grub, 1968; McDonald et al., 1991; Henderson, 1993). This is particularly important when ensiling legumes, which generally have low sugar and high protein concentrations. Acidifiers, such as formic acid (FA), quickly lower pH at the beginning of ensiling. Depending on the crop ensiled and on the amount of FA used, conditions for bacterial activity and growth are altered (McDonald and Henderson, 1974; Randby, 2000). Trends to lower fermentation acid concentrations, decreased lipolysis and

Abbreviations: AA-N, amino acid N; BSA, buffer soluble N; DM, dry matter; FA, formic acid; FD, fraction degraded protein N; FM, fresh matter; FUD, fraction undegraded protein; NPN, non-protein N; RF, rumen fluid; TCA, trichloroacetic acid.

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higher water soluble carbohydrate concentrations have been reported from FA treatment of legumes (Henderson et al., 1972; Adesogan and Salawu, 2004). Studies by Pursiainen and Tuori (2008) and Lorenz et al. (2010) showed decreased protein degradation in acidified legume silages.

Drawbacks of using FA have been observed in studies where FA was applied to low DM grass silage, and include increased amounts of effluent (Henderson and McDonald, 1971) and higher DM losses due to yeast growth (Henderson et al., 1972). Very low concentrations of FA may also promote growth of clostridia (Beck, 1968). Forages which are difficult to ensile, such as cocksfoot or legumes, benefit from the addition of up to 6 ml FA per kg fresh matter (FM), as shown by Lancaster and Brunswick (1977). This study also showed that higher levels of FA addition, up to 8 ml FA/kg FM increased DM intake, daily weight gain and organic matter digestibility in lambs, although this was not considered to be economically feasible.

Effects of tannins in legumes on protein degradation, resulting from tannin–protein binding and precipitation, has been described in many publications (Karnezos et al., 1994; Koivisto and Lane, 2001) and were also reviewed by Mueller-Harvey (2006) and Rochfort et al. (2008). Tannin–protein binding may increase the nutritive value of the forage for ruminants by an increase in rumen escape proteins which can subsequently be released at the low abomasal pH (Oh and Hoff, 1987) for absorption in the small intestine (Thomson et al., 1970; Jones and Mangan, 1977).

Tannin–protein complexes in silage have been exposed to a low pH before entering the rumen. Hence, if pH is too low, the positive protein sparing effect could be impaired if the complexes have dissociated. However, numerous reports have shown reduced ruminal protein degradation in tannin-rich legume silages such as sainfoin (*Onobrychis viciifolia*; Wang et al. (2007) and Lorenz et al. (2010)), birdsfoot trefoil (*Lotus corniculatus*; Albrecht and Muck (1991)) and tannin supplemented perennial ryegrass (*Lolium perenne*) silage (Salawu et al., 1999).

In light of these known dissociative effects on the protein–tannin complex associated with low pH, coupled with current recommendations for increased FA application to improve silage storage quality, investigations into FA application limits are appropriate. Therefore, our objectives were to assess effects of acidification levels, in combination with different DM contents, on the protein protecting effect of tannins in sainfoin during silage fermentation and digestion on the basis of N-fractionation and *in vitro* ruminal degradation of sainfoin silages.

2. Materials and methods

2.1. Plant material

Thirteen sainfoin varieties, grown at the National Institute of Agricultural Botany, Cambridge, Great Britain (NIAB) were harvested in August 2008, frozen and transported to the Kungsängen Research Center (Uppsala, Sweden). The varieties were: *O. viciifolia SCOP* var. CPI63761, var. CPI63815, var. CPI63826, var. Sparceto, var. CPI63825, var. CPI63752, var. CPI63808, var. Dnepropetrovsk, var. Fizes, var. Palio, var. AR-111 and *Onobrychis antasiatica* var. Sisiani Local and var. Akhurian-107.

2.2. Ensiling

All 13 samples were chopped (~3 cm), pooled and thoroughly mixed together to make one large forage mass representative of all 13 varieties. The mixture was ground in a meat grinder (Bankeryd A90B, Bankeryd, Sweden) and divided into four batches which were either not dried (180 g/kg DM) or dried in a force draft oven at 35 °C to a DM content of 400 g/kg, 500 g/kg or 600 g/kg. Each batch was further divided into three portions and put into separate plastic bags. Formic acid was added at levels of either 0, 4 (*i.e.*, moderate acidification) or 8 ml (*i.e.*, high acidification) FA/kg FM by spraying acid on the chopped plant material followed by thoroughly shaking the bags. The plant material was packed in glass mini-silos (20 cm × 3 cm (inner diameter)) in duplicate. After filling the silos with 80–90 g FM, leaving a 1 cm free headspace, the tubes were closed with a rubber stopper and a water lock. Silos were incubated in a dark 20 °C room for 60 days, after which the content of each silo was divided into two batches, one of which was freeze dried and ground on a cutter mill (Brabender, Type 880803, Brabender OHG, Duisburg, Germany) to pass a 1 mm screen awaiting further analysis and the other batch was frozen for analysis of α-amino acid N (AA-N) and ammonia (NH_3) N.

2.3. Analytical methods

Representative 5 g subsamples of each silo were collected immediately after application of FA and after opening the silos after 60 days and mixed with 10 ml of de-ionized water for pH measurement.

Dry matter of fresh samples was determined by drying at 105 °C to constant weight in a forced draught oven and for ensiled material by freeze drying. Dry matter contents were corrected for remaining water and volatile losses by multiplying by a correction factor of 0.94. Nitrogen analyses were in duplicate on the freeze dried material by a Kjeldahl procedure using a Kjeltec Analyzer 2400 and a 2020 Digestor (Foss, Hillrød, Denmark) with Cu as a catalyst. Buffer soluble N (BSN) was determined by extracting freeze dried samples with a borate phosphate buffer, pH 6.75, at 39 °C for 1 h according to a modified method by Licitra et al. (1996): 1.5 g of the freeze dried plant material was weighed into a 50 ml tube (Sarstedt, Nümbrecht, Germany) and mixed with 50 ml of borate phosphate buffer. Tubes were sealed, shaken and incubated for 1 h in a 39 °C water bath with an additional thorough shaking every 15 min. Thereafter, tubes were centrifuged at 3000 × *g* for 10 min at 20 °C on a swing-out rotor centrifuge (G4.11, Jouan, Saint Herbain, France). Twenty ml of the supernatant was