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Short communication

Effects of plant vegetative stage and field drying time on chemical composition and *in vitro* ruminal degradation of forage soybean silage

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ABSTRACT

The aim of this study was to assess the chemical composition and rumen degradability of ensiled soybean forage harvested at three plant vegetative stages (*i.e.*, R4, R5, R6) and wilted for 20 or 28 h. The wilted forages were chopped from 1 to 2 cm and then manually pressed into 6 kg bags of fresh material (two bags for each combination of maturity stage and wilting duration) prior to storage under anaerobic condition for 90 days. The soybean silages were analysed for fermentative parameters and manually separated into leaves, stalks and pods. Whole forage and separated plant components were chemically analysed and the *in vitro* neutral detergent fibre (NDF) ruminal degradation (NDFD) was determined. Only whole plant silages were assessed for *in vitro* gas production (GP) and *in vitro* crude protein degradability (CPD). The dry matter (DM) content of the ensiled whole plants increased (P=0.005) from 454 to 485 and 518 g/kg from the R4 to R5 and R6 vegetative stages and was higher for forages wilted at 28 h than those at 20 h (528 *vs* 444 g/kg, P<0.001). Advancing plant maturity increased (P<0.001) the crude protein (CP) and ether extract (EE) content from 164 to 199 and from 18 to 53 g/kg DM, respectively, but reduced ash (P=0.011) from 71 to 63 g/kg DM. The NDFD increased with plant maturity from 0.319 to 0.465 (P<0.001). The GP did not differ among maturity stages of forages or wilting lengths, while CPD increased from 0.391 to 0.548 (P<0.001) with advancing maturity and slightly decreased with the longer wilting duration (P<0.008). The DM content of the pods and stalks increased (P<0.01) with the vegetative stage and CP content increased in pods and decreased in leaves. Results indicate that harvesting soybean forage at an advanced maturity stage (*e.g.*, from R4 to R6) greatly increases the protein, the fat and the degradable NDF contents. Moreover, the wilting soybean forage has to be targeted to achieve a DM content of ensiled forage of about 440 g/kg (between about 410 and 480 g/kg, according to the maturity stage), and a further wilting determines no fermentative improvements at ensiling or relevant modification of silage nutritional contents.

Abbreviations: ADF, acid detergent fibre; CP, crude protein; EE, ether extract; DM, dry matter; CPD, crude protein degradability; GP, gas production; LA, lactic acid; NDF, neutral detergent fibre; NDFD, NDF degradability; OM, organic matter.

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1. Introduction

The supply of protein to increase the protein content of rations is one of the most important feeding costs on high yielding ruminant farms. Promoting on-farm availability of forages with a high protein content is a strategy to increase profitability and sustainability of ruminant production systems. Soybean is mainly cultivated for grain, which is used for oil extraction while creating by-products such as expeller and protein cakes with a high protein content. However, in situations of forage shortage, farmers can change soybean cultivation to obtain a whole plant forage with an expected high protein and fat content due to the harvesting of stems and pods with seeds (Casper et al., 2012; Undersander et al., 2007). Furthermore, soybean is a short-season summer annual legume (Creamer and Baldwin, 1999), which generally fits well into cropping systems and can be planted immediately following spring harvest as cover crop to ensure benefits for the soil in accordance with recent 'greening agricultural policy' of the European Community (EC, 2010).

The quality of whole soybean silage has been examined in different papers, where both forage or oilseed-type cultivars of soybean were used as whole plant silage (Mustafa and Seguin, 2003; Mustafa et al., 2007; Kawamoto et al., 2013; Vargas-Bello-Perez et al., 2008). These trials have indicated the need to better define the more adequate wilting of the fresh forage to improve the ensiling process and the more suitable plant vegetative stage to maximize the nutritive value of the forage. In fact, the low sugar content and high buffering capacity of whole soybean forage require long wilting to reduce the moisture in order to facilitate ensiling (Casper et al., 2012; Undersander et al., 2007). Moreover, the plant's vegetative stage at harvesting influences the nutritive value of forage mainly due to progressive development of pods as plant maturity advances (Kawamoto et al., 2013).

In the present study, we examined the effect of three vegetative stages and two wilting times of the whole plant of an oilseed soybean cultivar on some ensiling parameters and on nutritive value of the silage forage.

2. Materials and methods

2.1. Trial organisation

The soybeans were grown in an experimental field of 15 m length and 8.1 m width in Pozzuolo del Friuli (Udine, Italy, 44°59′ N, 13°11′ E) with medium-textured soil and divided into homogeneous sub-fields of 7.5 m length and 2.7 m width. Soybean oilseed cultivar Bahia (*Glycine max*, selected in the North Italy for its high yields) was seeded at 40 plants/m^2 in all the plots on June 12th 2013 and no fertilizers were applied. The whole plant soybean was harvested at three maturation stages on 29 August, 3 September and 9 September (2013) as R4, R5 and R6 respectively (as described by Fehr et al., 1971) at a theoretical cut length of 50 mm using a cutterbar (Haldrup D-45, D-74532, Ilshofen, Germany). After cutting, forages were wilted to a targeted DM content (*e.g.*, range 400–500 g/kg) for 20 h or 28 h and sampled for DM analysis. Immediately after wilting, the forages were chopped to a theoretical cut of 15 mm using a laboratory chopper (Ikra Mogatec ENG 2500) and then packed manually, in duplicate, for each combination of maturity stage and wilting duration into bags with ~6 kg of fresh material/bag, which were introduced into 20 l jars without a headspace and ensiled for 90 days.

2.2. Chemical analysis

At the end of each ensiling period a sample of silage from the bags was immediately measured for pH, analysed for ammonia N (NH_3-N) by direct distillation according to Filya and Sucu (2007) and for lactic acid (LA) by a colorimetric method (Megazyme K-DATE 12/12). Each silage was separated into its component leaves, stalks and pods. The moisture content of the whole plant and its components was determined by drying at 60 °C in a ventilated oven for 48 h. These samples were milled (1 mm screen) before chemical analyses and *in vitro* tests determinations. Samples were assayed in duplicate according to the AOAC (2000) for dry matter (DM), crude protein (CP), organic matter (OM) and ether extract (EE) content by methods 930.15, 976.05, 942.05 and 954.02, respectively. The neutral detergent fibre (aNDF; Van Soest et al., 1991) and acid detergent fibre (ADF; method 973.18; AOAC, 1990) content were determined using Ankom F57 filter bags in an Ankom fibre analyzer (Ankom Technology, Macedon, NY, USA). For aNDF analysis (Mertens, 2002), samples were treated with α-amylase (Sigma A-3306, Sigma–Aldrich® Co., Milan, Italy) and the neutral detergent solution contained sodium sulfite (Carlo Erba 483257, Carlo Erba®Reagents SpA, Rodano, MI, Italy). Residues of aNDF and ADF were not corrected for residual ash. The non-fibre carbohydrates (NFC, g/kg DM) were calculated as: 1000 − (CP + ash + EE + aNDF).

2.3. In vitro rumen fermentation tests

Samples of the whole plant, leaves, stalks and pods were incubated in triplicate in Ankom filter bags (500 mg, 24 bags in three jars filled with ~1600 ml of Ankom buffer plus 400 ml of rumen fluid) for 48 h to measure *in vitro* ruminal aNDF degradability (NDFD) by the Ankom Daisy incubator (Fairport, NY) according to Masoero et al. (2011).

In vitro ruminal CP degradability (CPD) of whole plant forages were assayed in triplicate in 125 ml glass Erlenmeyer flasks containing 500 mg of substrate, 10 ml of rumen fluid and 40 ml of Van Soest buffer, according to the procedure proposed by Ross et al. (2013). Two glasses without samples were added as blanks. After the 16 h of incubation, the contents of the flasks were filtered (Whatman 54 filter paper) and residues were analyzed for CP.