



Modeling nutrient availability of alfalfa hay harvested at three stages of maturity and in the afternoon and morning in dairy cows

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ABSTRACT

Nutritive value of alfalfa hay is influenced by stage of maturity and cutting time which affects the potential nutrient supply in dairy cows. The objective of this study was to investigate the effect of stage of maturity and cutting time of alfalfa hay grown under semi-arid climate condition on *in situ* ruminal degradation characteristics and predicted protein availability in dairy cows using two protein models based on different principles (DVE/OEB 1994 and NRC, 2001). Alfalfa was cut at early bud (June 15/16), late bud (June 26/27) and early flower (July 18/19) in the afternoon (18:00 h) and the following morning (06:00 h). Alfalfa hay at early bud and late bud contained higher *in situ* effective degradable nitrogen (ED_N) to effective degradable energy (ED organic matter (ED_{OM}) and ED carbohydrates (ED_{CHO})) ratios compared with alfalfa at early flower ($P < 0.05$) and highest ED_N to ED_{OM} and ED_{CHO} ratios were reached in the first hours of ruminal incubation for all alfalfa hays. Rumen degraded protein balance decreased with advancing maturity ($P < 0.05$). There was a trend towards reduction in metabolizable protein (NRC model; 78, 72 and 66 g/kg dry matter (DM), $P = 0.06$) and truly absorbed protein (DVE value; 57, 51 and 39 g/kg DM, $P = 0.08$) with advancing maturity. Cutting alfalfa in the afternoon increased ED_{CHO} (450 vs. 431 g/kg CHO; $P = 0.08$), ruminal microbial protein synthesis based on total digestible nutrients (NRC model; MCP_E^{NRC}; 62 vs. 59 g/kg DM; $P = 0.03$) and intestinally absorbable MCP_E^{NRC} (40 vs. 38 g/kg DM; $P = 0.03$) compared with cutting alfalfa in the morning. In conclusion, cutting alfalfa hay at early bud stage tended to have the highest metabolizable protein content, but also the highest imbalance between rumen available protein and energy and cutting alfalfa in the afternoon increased potential ruminal microbial protein synthesis in dairy cows.

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Abbreviations: aNDF, neutral detergent fiber; AMCP, truly absorbable microbial crude protein; ARUP, truly absorbable rumen undegraded protein; CT, cutting time; CP, crude protein; CHO, total carbohydrate; D, potentially degradable fraction; DM, dry matter; DVE, truly digested and absorbed protein in small intestine; ED, effective degradability; ECP, endogenous protein; ENDP, endogenous protein losses from the digestive tract; FOM, fermentable organic matter; MCP_E^{NRC}, microbial protein synthesis based on energy available; MCP_{FOM}, microbial crude protein synthesized in the rumen from FOM; MCP_{RDP}^{DVE}, synthesized MCP in the rumen from RDP; MP_{NRC}, metabolizable protein; Kd, fractional rate of degradation; NFC, non fiber carbohydrate; OM, organic matter; OEB and DPB_{NRC}, ruminal degraded protein balance; RDP, rumen degradable protein; RUP, rumen undegradable protein; TPSP, true protein supplied to the small intestine; UDM, completely undegradable DM; SM, stage of maturity; U, undegradable fraction; W, washable fraction.

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1. Introduction

Cultivated alfalfa (*Medicago sativa* L.) is the main forage ingredient for dairy rations in Iran (Kowsar et al., 2008; Yari et al., 2012) with 5.7 million metric tonnes of hay harvested during season 2009–2010 (Iranian Ministry of Agriculture, 2009–2010). Forage alone provides insufficient nutrients for the animal to achieve high milk yields and should therefore be supplemented with concentrated feed ingredients (Oba and Allen, 2005). However, cutting alfalfa hay at the optimum growth stage and time can minimize required supplement inclusion. Nutritive value of alfalfa hay is influenced by cultivar, stage of maturity (SM) (Elizalde et al., 1999; Yu et al., 2003a,b; Coblenz et al., 2008; Yari et al., 2012), climate condition (Van Soest, 1994) and cutting time (CT) (Burns et al., 2007; Brito et al., 2008, 2009; Yari et al., 2012). Nutritive value of feeds can be assessed by predicting nutrient supply of a feed to both the rumen and intestine using sophisticated protein evaluation models (ARC, 1984; Madsen, 1985; NRC, 1985,2001; Tamminga et al., 1994, 2007). Input values for these models are generated by chemical analysis and the *in situ* technique, but differences exist between principles of models (Yu et al., 2003a). Some of these models predict ruminal microbial protein synthesis (MCP) based on rumen fermentable OM (FOM) and consider endogenous protein losses (Tamminga et al., 1994, 2007), while other models predict MCP based on total digestible nutrients and consider absorption of endogenous protein (NRC, 1985,2001).

To date there is no information available on the effect of SM and CT on nutrient supply of alfalfa hay grown under semi-arid condition in dairy cows. The objectives of the current study were to investigate the effect of SM (early and late bud and early flower stage) and CT (afternoon *versus* morning) of alfalfa hay grown under semi-arid condition on *in situ* rumen degradation kinetics and predicted protein supply in dairy cows with two models with different basic principles (Tamminga et al., 1994; NRC, 2001).

2. Materials and methods

2.1. Alfalfa plots management

A second year alfalfa field (20 m × 24 m) seeded with cv. Ranger at the Research Farm of Ferdowsi University of Mashhad (Mashhad, Iran; 36°17'52.8"N, 59°36'20.52"E) was used in this study. The whole field was harvested before the experiment at April 6, 2010 and irrigated every 10 days during experiment. Alfalfa was cut at early bud (June 15/16), late bud (June 26/27) and early flower (July 18/19) both in the afternoon (18:00 h) and the following morning (06:00 h).

Six plots (4 m × 4 m each) within 5 replicate blocks within the field were randomly assigned to 6 treatments in a factorial arrangement (3 SM × 2 CT). The SM was determined according to Kalu and Fick (1981) as described by Yari et al. (2012). At each harvest, an area of 3 m × 3 m was manually clipped using a small scythe at ca. 5 cm above the soil surface.

Fresh alfalfa harvested from each plot was air dried in the shade for 10 to 15 days. After air drying, alfalfa hay samples were chopped using a hay chopper with 20 mm screen (Agri-Equip, Nasr Co., Isfahan, Iran). The hay from the first, second and third blocks were pooled to one sample and hay from the fourth and fifth blocks were pooled to another sample to generate sufficient material for chemical analysis and *in situ* incubations.

2.2. Rumen incubation procedure

For *in situ* incubations, three rumen fistulated non-pregnant dry Holstein Frisian cows were used which had been reviewed and approved by the Animal Care Committee of the University of Saskatchewan (Animal use protocol # 19910012). Cows were individually housed in pens at the experimental farm of the University of Saskatchewan (Saskatoon, SK, Canada) and were cared for according to the Canadian Council on Animal Care guidelines (1993). The cows had free access to water and were fed 15 kg dry matter (DM) per day of a total mixed ration twice daily in equal portions at 08:00 h and 16:00 h. The total mixed ration consisted in g/kg DM of 550 g barley silage, 125 g alfalfa hay, 50 g dehydrated alfalfa and 275 g concentrate as described in more detail by Yu et al. (2009).

Prior to the *in situ* incubations, chopped alfalfa samples were ground to pass through a 2 mm screen using a cyclonic mill (Retsch SM-3000, Brinkmann Instruments, ON, Canada). *In situ* ruminal degradation kinetics were determined as described by Yu et al. (2004), using number-coded nylon bags (10 cm × 20 cm, pore size 40 µm, Nitex 03-41/31 monofilament open mesh fabric, Screen Tech, Mississauga, ON, Canada). Approximately 7 g of alfalfa hay samples was placed into each bag, resulting in a sample-to-bag surface ratio of ~17.5 mg/cm². Filled bags were randomly assigned to the three cows and incubated in the rumen for 72, 36, 12, 8 and 4 h (4, 4, 3, 2 and 2 bag per alfalfa hay sample respectively) by the "all out method". Immediately after retrieval from the rumen, all bags were placed in a bucket with cold tap water and then washed ten times manually followed by oven drying at 55 °C for 48 h. Two bags of zero h alfalfa hay samples were washed in the same way. Rumen incubations were carried out in one run. The two-pooled blocks were used as replicates. Incubation residues from the treatment bags were combined within time per block.

2.3. Degradation characteristics

The rumen degradation characteristics were calculated for organic matter (OM), crude protein (CP), neutral detergent fiber (aNDF), non fiber carbohydrate (NFC) and total carbohydrate (CHO). Three fractions were determined for each component: a

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