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Influence of maturity on alfalfa hay nutritional fractions and indigestible fiber content

A. Palmonari,[1] M. Fustini, G. Canestrari, E. Grilli, and A. Formigoni
Department Of Veterinary Medicine, Università di Bologna, 40084 Bologna, Italy

ABSTRACT

This study focused on changes in fibrous and protein fractions, changes in fiber digestibility and amount of indigestible neutral detergent fiber (NDF) as a consequence of increased maturity in alfalfa. A total area of 720 m^2 was divided in 18 blocks randomly assigned to 3 treatments, differing in cutting intervals. Treatment 1 was harvested with a 21-d cutting schedule, at a prebloom stage; treatment 2 with a 28-d schedule, at about first-bloom stage; whereas a full bloom was observed in treatment 3, harvested with a 35-d cutting schedule. Treatments were replicated 4 times through the spring-summer period for 2 subsequent years, 2011 and 2012. Statistical differences were observed for crude protein [treatment 1: 20.8%, treatment 2: 17.3%, and treatment 3: 17.0%; standard error of the mean (SEM) = 0.83], soluble protein, and nonprotein nitrogen among treatments on a dry matter basis. Similar results were observed for acid detergent lignin (6.3, 6.9, and 7.3%, respectively; SEM = 0.39), lower in treatment 1 compared with others, and in vitro NDF digestibility at 24 or 240 h. Indigestible NDF at 240 h resulted in lower values for treatment 1 compared with treatments 2 and 3 (15.5, 17.2, and 18.3%, respectively; SEM = 1.54). Moreover, the indigestible NDF:acid detergent lignin ratio varied numerically but not statistically among treatments, being as much as 9% greater than the 2.4 fixed value applied for rate of digestion calculation and Cornell Net Carbohydrate Protein System (Cornell University, Ithaca, NY)-based model equations. Assuming the diet composition remained unchanged, treatment 3 (35-d cutting interval) would be expected to yield 1.4 kg less milk per day based on energy supply, and 2.8 kg less milk daily based on protein supply than treatment 1.

Key words: alfalfa, maturity, indigestible neutral detergent fiber

INTRODUCTION

Alfalfa hay, one of the most utilized forages in Italy, is particularly important in the areas of Po Valley involved in production of Parmigiano Reggiano cheese, where silage feeding is prohibited to prevent environmental clostridia contamination (Parmigiano-Reggiano Cheese Consortium, 2011) in milk. Thus, hay is the main source of forages for these dairy rations. In northern Italy, 4 or more cuttings of alfalfa can be harvested annually. Numerous studies have shown that forage quality is affected by growth stage (Nordkvist and Åman, 1986; Yu et al., 2003), species, cultivar (Griffin et al., 1994), and growing conditions (e.g., rainfall, temperature, and soil composition; Minson and McLeod, 1970; Cox et al., 1994; Mathison et al., 1996).

Alfalfa (*Medicago sativa* L.) is a perennial legume with a unique anatomy comprising relatively distinct protein-containing leaves and fibrous stems. Maturity influences both fiber digestibility and protein fractions in alfalfa through increasing the leaf:stem ratio and increasing lignification of stems, which, in turn, alters fiber digestibility (Weir et al., 1960; Yu et al., 2003). Lignin negatively affects forage fiber digestion degree and rate (Albrecht et al., 1987; Van Soest, 1994; Sewalt et al., 1997b), the extent of which depends on its concentration, composition (Buxton and Russell, 1988; Buxton and Brasche, 1991), tissue distribution (Akin, 1989), and phenolic functionality (Sewalt et al., 1997a). Lignin, due to its peculiar phenolic composition, cannot be digested in anaerobic environments, and is able to reduce the proportion of potentially digestible fiber fraction in forages (Jung and Allen, 1995), representing the indigestible NDF (**iNDF**). In Cornell Net Carbohydrate Protein System (**CNCPS**; Cornell University, Ithaca, NY; Sniffen et al., 1992)-based models, such as Cornell-Penn-Miner (**CPM**) Dairy software [version 3.0.10; Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia), The William H. Miner Agricultural Research Institute (Chazy, NY), and the University of Maryland (College Park); Boston et al., 2000], the iNDF is calculated as ADL times a fixed value (2.4), without differentiation among forages, nor between early cut or late cut (Sniffen et al., 1992; Van

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[1]Corresponding author: alberto.palmonari2@unibo.it

Soest et al., 2005). Moreover, iNDF is essential in the rate of digestion (kD) equations, in order to calculate the amount of NDF that could be digested and its rate. The objective of this study was to determine the influence of maturity and cutting treatment on alfalfa chemical composition and, in particular, in vitro fiber digestibility and the iNDF fraction.

MATERIALS AND METHODS

Experimental Design

This study was conducted using a native alfalfa variety, called Romagnola, sown in 2007 in an experimental plot close to the Department of Veterinary Medicine of Bologna University (Bologna, Italy). No insecticides or herbicides were applied, and no soil fertilization or irrigation was conducted. The field had a clay soil with medium porosity, and its relative average humidity and pH were recorded at the beginning of each trial.

A total area of 720 m^2 was divided in 18 plots randomly assigned to 3 treatments, differing in cutting intervals. Treatment 1 (**PreB-21dd**) involved harvesting every 21 d at about prebloom, treatment 2 (**FrsB-28dd**) every 28 d at about first bloom, and 35 d at about full bloom for treatment 3 (**FulB-35dd**). Treatments were replicated 4 times through the spring-summer period in 2 subsequent years (2011 and 2012). All plots were cut at the beginning of the third week of April to obtain a common starting date for all treatments. Plots were harvested approximately at 0900 h, 7 to 10 cm above the soil to aid plants regrowth and avoid soil contamination.

Chemical Analysis, In Vitro Fermentation, and Rationing

Immediately after harvesting, a representative (1.0-kg) sample, randomly obtained by hand from each block of each treatment, was collected and immediately dried in a forced-air oven at 65°C for 48 h to determine DM content. From each block, forage was sampled and dried following the same procedure as above. Once dried, samples were ground through a 1-mm screen in a Cyclone mill (model SM100; Retsch GmbH, Haan, Germany). The samples were analyzed for CP (AOAC, 1990; methods 976.06 and 984.13), NPN, soluble protein, neutral detergent-insoluble protein, acid detergent-insoluble protein (Licitra et al., 1996), amylase-treated NDF (Mertens, 2002) with addition of sodium sulfite, ADF (AOAC, 1990; method 973.18), and ADL (AOAC, 1990; method 973.18). Briefly, the ADF residue was suspended within the crucible in 40 mL of sulfuric acid (72% concentration) for 3 h. Residues were then washed with boiling water and dried overnight at 65°C. Once weighed, crucibles were placed in a muffle furnace for 4 h at 490°C to incinerate the residue. Ash content was then subtracted from the initial weight to calculate the ADL content. In vitro NDF digestibility at 24 and 240 h was determined using the Tilley and Terry modified technique (Tilley and Terry, 1963; Goering and Van Soest, 1970; Robertson and Van Soest, 1981). Rumen fluid was collected by sampling rumens of 2 lactating cows (fed a hay-based diet; Table 1; milk production = 31.8 ± 2.3 kg/d; DIM = 274 ± 2), using an esophageal tube, mixed and placed in a thermostatic bottle. Under insufflation of O_2-free CO_2, liquor was filtered through 4 layers of cheesecloth. A 10-mL volume of rumen fluid was inoculated in each Erlenmeyer flask already placed in a heated water bath under CO_2-positive pressure to ensure anaerobiosis. Flasks contained 40 mL of the solutions described by Goering and Van Soest (1970) and 0.50 g of sample. Each sample was analyzed in triplicate in 2 different in vitro fermentations. For both, sample preparation and diet was the same as for the donor cows. At the end of the fermentation, the content of each flask was analyzed to determine NDF content of the residue. Digestibility was calculated as the difference between 100 and 100 times the residual NDF:initial NDF ratio. Other fermentations were conducted for 240 h to estimate the amount of iNDF. We based our choice about fermentation length on previous studies indicating 240 h as the maximum extent of fiber digestion in an anaerobic environment (Chandler, 1980; Van Soest, 1994). For these fermentations, both rumen fluid and buffer were reinoculated after 120 h to preserve the microbial activity during the whole process. A final volume of 100 mL, containing 0.50 g of sample, was treated for NDF content determination as described above.

To predict RDP, NE_L, and possible milk production due to MP and ME, each hay corresponding to the 3 treatments was used as the main forage source in CPM, a dairy ration software based on the CNCPS. Diet compositions are listed in Table 2. Cows were assumed to weigh 620 kg, have a BCS of 3.00, be in second lactation, be at 120 DIM, and produce 35.00 kg of milk/d with 3.60% fat and 3.30% total protein concentration.

Environmental Conditions

During the trial, the total amounts of rainfall and temperatures were recorded daily and results were summarized for each cutting schedule treatment. The weather station was located within 1,000 m of the experimental area. Data were collected from the official website of the Environmental Protection Regional Agency (ARPA Emilia Romagna, Bologna, Italy).