The Professional Animal Scientist 24 (2008):675–680

Case Study: A Snapshot in Time of Fatty Acids Composition of Grass Herbage as Affected by Time of Day

P. Gregorini,[1,2] PAS, K. J. Soder, PAS, and M. A. Sanderson
USDA-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, University Park, PA 16802

ABSTRACT

Polyunsaturated fatty acid contents in the products of pasture-fed ruminants depend on the amount and composition of fatty acid ingested and rumen biohydrogenation. The total nonstructural carbohydrates (TNC) and DM concentrations of herbage increase during the day; however, it is not known if fatty acids follow the same pattern. This study aimed to quantify the diurnal variation of herbage fatty acid concentrations. Vegetative micro-swards of orchardgrass (Dactylis glomerata L.) and meadow fescue (Festuca pratensis Hud.) were sampled in July and September, respectively, over 2 consecutive days. Sampling occurred 4 times/d: sunrise (0537 or 0650 h), morning (1037 or 1110 h), afternoon (1537 or 1530 h), and sunset (2037 or 1930 h) for orchardgrass and meadow fescue, respectively. Cut herbage was analyzed for DM, CP, TNC, NDF, ADF, and palmitic, oleic, linoleic and α-linolenic acids. Diurnal variation of temperature, relative humidity, and photosynthetic radiation were recorded every 5 min. Time of day affected ($P < 0.01$) herbage chemical composition. From sunrise to sunset, DM and TNC increased, whereas CP, NDF, and ADF decreased. Time of day did not affect ($P > 0.01$) concentrations of palmitic, linoleic, and α-linolenic acids of herbage. Oleic acid increased ($P < 0.01$) 22 and 12.7% from sunrise to sunset in orchardgrass and meadow fescue, respectively. However, this incremental increase in oleic acid did not affect ($P > 0.01$) time of day effect on total fatty acids. Concentrations of PUFA in grass herbage remain stable during the day, whereas structural and nonstructural carbohydrates, as well as CP, do not.

Key words: time of day, chemical composition of herbage, fatty acid

INTRODUCTION

Increases in the amount of functional fatty acids in foods have positive effects on human health, reducing the severity several chronic diseases, including cancers, atherosclerosis, obesity, and diabetes (McGuire and McGuire, 2000). Manipulation of functional PUFA in ruminant products depends on rumen biohydrogenation rates and on the net amount and composition of fatty acid ingested (Bauman et al., 2003). Despite the relatively low concentration of fatty acids in forage plants, the ability to supply de novo C18:3n-3 (the building block of PUFA; Givens et al., 2001; Dewhurst et al., 2003) makes grazed herbage an excellent source of PUFA precursors for nonsupplemented grazing ruminants. The concentration of PUFA in herbage changes with season (Dewhurst et al., 2001), developmental stage of the plant (Hawke 1963, 1973; Gray et al., 1967; Dewhurst et al., 2001, 2003), and cutting interval (Dewhurst et al., 2001, 2003; Elgersma et al., 2003, 2004). At the plant cellular level, fatty acids are produced in cell plastids (Ohlrogge and Jaworski, 1997), with the greatest concentrations occurring within the thylakoid membranes of the chloroplast (Harwood, 1980). Thus, fatty acids are related to plant photosynthetic activity (Erwin and Bloch, 1963; O'Brien and Benson 1964; Belury, 2002). Under environmentally controlled conditions, plant physiology research has shown that fatty acid synthesis varied diurnally in small discs of spinach (*Spinacia oleracea*) leaves and recently emerged

[1] Corresponding author: Pablo.Gregorini@dairynz.co.nz
[2] Current address: DairyNZ Ltd., Private Bag 3221, Corner Ruakura and Morrisville Roads, Hamilton, New Zealand.

maize (*Zea mays*) leaf blades (Browse et al., 1981). Several field studies have shown significant increases in photosynthate concentrations of herbage during the course of a day (Mayland et al., 2003, 2005; Griggs et al., 2005); however, there is still a lack of information regarding diurnal fluctuation of fatty acids in grass herbage. The knowledge of these potential changes would help improve grazing management aimed at increasing functional fatty acids in ruminant products. From these works emerges the hypothesis that the concentration of fatty acids in herbage varies during the day. The objective of this experiment was to partially test this hypothesis by quantifying herbage fatty acids at different times of day in 2 grass species commonly found in temperate cool-season pastures of the northeastern United States.

MATERIALS AND METHODS

Site and Experimental Setup

The experiment was conducted at the USDA-ARS Pasture Systems and Watershed Management Research Unit, University Park, Pennsylvania. In January 2007, monoculture micro-swards (Orr et al., 2005) of meadow fescue (*Festuca pratensis* Hud.) and orchardgrass (*Dactylic glomerata* L.) were established in 44 plastic boxes (79 × 47 × 11.5 cm). All boxes had 6-mm drainage holes drilled in the bottom and spaced at 10 cm in 4 rows of 7 (28 total holes). Each box was filled with 40 to 45 kg of a potting medium (Scott's Sierra Horticultural Products Co., Marysville, OH). Seeding rate was 500 seeds/m^2, based on a germination rate previously tested. The micro-swards were established and grown in the greenhouse for 14 wk and then were placed outdoors in May 2007. Micro-swards were cut (6-cm stubble height) approximately every 21 d to maintain vegetative (3 fully expanded leaves) tillers. Micro-swards were watered regularly to maintain soil moisture at field capacity and fertilized after each 21-d cutting interval with N, P, and K at 1.79, 3.58, and 1.79 g/m^2, respectively.

Micro-sward Sampling, Chemical Composition Analyses, and Weather Condition Measurements

Sampling was conducted in 2 consecutive days (on July 2 and 3 for orchardgrass and on September 12 and 13 for meadow fescue) using one set of 11 micro-swards per day. Micro-swards were sampled 4 times/d: sunrise (0537 or 0650 h); morning (1037 or 1110 h); afternoon (1537 or 1530 h); and sunset (2037 or 1930 h) for orchardgrass and meadow fescue, respectively. Micro-swards were gridded and samples were taken from randomly selected grid points within each micro-sward. At each time of day, approximately 10 g (DM basis) of herbage was harvested from each micro-sward. Herbage was harvested at a 6-cm stubble height.

Samples were immediately frozen in liquid N for further freeze-drying (Ultra 35 Super ES, Virtis, Gardiner, NY) and chemical analysis. Dry samples were ground to pass a 1-mm screen and analyzed for NDF, ADF, CP (N × 6.25), total nonstructural carbohydrates (**TNC**), and PUFA. The NDF and ADF concentrations were determined using the batch procedures outlined by Ankom Technology Corp. (Ankom Fiber Analyzer, Ankom, Fairport, NY). Concentrations of N were determined by total Kjeldahl N (AOAC, 2000, official method 976.06; using 75-mL calibrated tubes and $CuSO_4$-K_2SO_4 catalyst) and analysis on Quickchem 8000 ion analyzer (Lachat Instruments, Milwaukee, WI). The TNC content was determined following the procedures described by Burns et al. (2006). Fatty acid methyl esters were prepared from the samples by the method outlined in Murrieta et al. (2003). Fatty acids were separated using a Varian CP 3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with an Omegawax 320 capillary column (30 m × 0.32 mm i.d., 0.25-μm film thickness, Supelco, Bellefonte, PA). Injector and detector temperatures were 275°C. The oven temperature was held at 175°C for 40 min and was then increased to 240°C in increments of 10°C/min. Helium was the carrier gas with a split ratio of 50:1 and 0.8 mL/min column flow. A reference standard for peak identification and the calibration standards for quantification of the fatty acids of interest were made from the following fatty acid methyl esters (all obtained from Sigma-Aldrich, St. Louis, MO): methyl palmitate (C16:0), methyl oleate (*cis*-9 C18:1), methyl linoleate (*cis*-9,12 C18:2), and methyl linolenate (*cis*-9,12,15 C18:3). Methyl undecanoate (C11:0, Nu-Check Prep, Elysian, MN) was added to each sample tube as an internal standard.

Photosynthetically active radiation (LI-COR, LI190SB Quantum Sensor, Logan, UT), air temperature, and relative humidity (HMP35C, Vaisala, Logan, UT) were measured every 5 min each day with an automated weather station.

Experimental Design, Treatments, and Statistical Analyses

The data from each grass species were analyzed as a completely randomized design with micro-sward as experimental unit. The 4 herbage sampling times (sunrise, morning, afternoon, and sunset) were considered treatments. Dependent variables were analyzed by ANOVA using PROC MIXED (SAS Inst. Inc., Cary, NC). The model included the effects of day, micro-sward, treatment, and the interaction day × treatment. The random effect was micro-sward within treatment, specified in the RANDOM statement. Least squares means were separated using the PDIFF function of SAS. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

There was no treatment × day interaction ($P > 0.01$) for any variable analyzed. Therefore, averages over both sampling days are presented