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Using plant wax markers to estimate the diet composition of grazing Holstein dairy cows

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ABSTRACT

The objective of this study was to test whether diet selection of dairy cows under grazing conditions could be estimated using plant wax markers. Furthermore, differences between 2 cow strains and the effect of concentrate supplementation on plant species selection were investigated. The experiment was a study with a crossover design performed on an organic farm with 12 Swiss Holstein cows and 12 New Zealand Holstein cows. Both experimental periods consisted of a 21-d adaptation and a 7-d measurement period. All cows grazed full time in a rotational stocking system and received either no concentrate or 6 kg/d of a commercial cereal-grain mix. Representative herbage samples of each grazed paddock were taken and botanical composition of subsamples was manually determined. The average proportions of the plant species were 27.8% *Lolium perenne*, 6.1% *Dactylis glomerata*, 10.4% *Trifolium repens*, and 9.0% *Taraxacum officinale*. Other grass species were merged as "other grass" (38.2%) and other forb species as "other forbs" (8.5%). *n*-Alkanes, long-chain fatty acids, and long-chain alcohols (LCOH) were analyzed in the samples of plant species, concentrate, and feces from each cow. A linear discriminant analysis indicated that diet components were differentiated best with LCOH (96%) and worst with the combination of all marker groups together (12%). For each marker, the fecal marker recovery (FR) relative to dosed ytterbium was determined in 2 ways. Estimation of diet composition was performed with the software "EatWhat," and results were compared with botanical composition with the Aitchison distance. The results indicate that the diet composition of grazing dairy cows can be estimated using plant wax markers. Additionally, the calculation of FR led to mostly reliable results, yet this approach needs further validation. The most accurate estimation was achieved with the marker combination of *n*-alkanes and LCOH with a correction for FR. Less accurate estimations were achieved with long-chain fatty acids alone or in combination with n-alkanes. No difference relating to diet selection between the 2 cow strains was recorded, but supplemented cows apparently ingested higher proportions of *T. repens* than nonsupplemented cows. Awareness that supplementation influences selection behavior of grazing dairy cows may lead to adaptations in botanical composition of the pasture according to the demand of the animals.

Key words: alkane, long-chain fatty acid, long-chain alcohol, concentrate supplementation

INTRODUCTION

The benefits of grassland communities with a higher diversity of species and functional groups, such as higher productivity, increased resources utilization, higher uptake of nitrogen, and increased occupation of available space, are well known (Spehn et al., 2005). Recently, the considerable features of multi-species, legume-based grassland-livestock systems at different stages in the soil-plant-animal-atmosphere system were summarized by Lüscher et al. (2014). They stated that legume-based grassland-livestock systems would constitute one of the pillars for more sustainable and competitive ruminant production systems and will become more important in the future.

Concentrate supplementation of dairy cows in a pasture-based feeding system causes substitution of herbage and grazing time is reduced [McCarthy et al., 2007; C. Heublein, F. Dohme-Meier, K.-H. Südekum, R. M. Bruckmaier (Vetsuisse Faculty, Bern, Switzerland), S. Thanner (Agroscope, Posieux, Switzerland), and F. Schori, unpublished data], but no certainties exist about whether it influences plant species selection in multispecies pastures. According to Villalba et al. (2015), the knowledge of the effects of feed context on preference of grazing animals should pioneer innovative management strategies to enhance forage intake, pro-

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ductivity, and animal welfare. Previous studies examined the suitability of different cow strains or breeds for a pasture-based feeding system [McCarthy et al., 2007; Piccand et al., 2013; C. Heublein, F. Dohme-Meier, K.-H. Südekum, R. M. Bruckmaier (Vetsuisse Faculty, Bern, Switzerland), S. Thanner (Agroscope, Posieux, Switzerland), and F. Schori, unpublished data], but to the authors' knowledge, no studies considered differences in diet selection on pasture. In New Zealand, Holstein cows are bred for an efficient use of pasture and have a higher feeding drive (McCarthy et al., 2007). Therefore, differences might exist in plant species selection between New Zealand and other Holstein cow strains. Such investigations are needed in natural grazing situation with a greater number of plant species, as requested by Villalba et al. (2015).

Plant wax markers, such as *n*-alkanes (hereafter called alkanes), long-chain fatty acids (**LCFA**), and long-chain alcohols (**LCOH**), are used for diet composition estimation of grazing ruminants (Ali et al., 2005; Lin et al., 2012). With the combination of alkanes and LCFA (Ferreira et al., 2009, 2011) or with alkanes and LCOH (Boland et al., 2012; Ferreira et al., 2015), diet composition estimations provided reasonable results for diets with between 2 and 6 components. The combination of all 3 marker groups might be applicable to situations with more complex diets (Ferreira et al., 2015). Supplementary feeds, such as concentrates, can be labeled and considered as an additional component in the diet (Dove and Charmley, 2008; Elwert et al., 2008). However, several studies included shrubs (Ali et al., 2005) or heather-gorse plant species (Ferreira et al., 2015) in the diets, which are not typical plant species occurring on pastures for dairy cows. Various grasses, legumes, and forbs are the main plants growing on pastures grazed by dairy cows, and studies to estimate plant species selection on multispecies pastures with dairy cows are rare. In one of the few studies on this kind of multispecies pasture, using alkanes alone led to erroneous diet composition estimations of dairy cows (Schori et al., 2012). Therefore, we tested whether the approach of estimating diet composition of grazing dairy cows using plant wax markers is applicable under farming conditions and if reasonable results are obtained with different breeds and concentrate supplementation.

The basic precondition for estimating diet selection of ruminants on a multispecies pasture is the sufficient differentiation of marker profiles between plant species. Identification of markers that contribute most to the differentiation between plant species may reduce workload and contribute to a more accurate differentiation as low concentration of markers and large within-species variation may limit their use for diet estimation (Mayes and Dove, 2000). As the recovery of the markers in the feces is incomplete, an important element for gaining accuracy of diet composition estimation is the fecal recovery (**FR**) correction (Ferreira et al., 2015). Corrections are needed for incomplete FR of alkanes (Dove and Mayes, 1991), LCFA (Ferreira et al., 2009), and LCOH (Ferreira et al., 2015), but in the aforementioned studies, FR was determined in indoor feeding experiments with similar diet composition to outdoors, with known amount of DMI, diet composition, and collection of total fecal output. This approach is labor intensive and expensive, so 2 alternative ways for calculating FR were used in the current study. The aim of the study was to test whether the approach using calculated FR to estimate diet selection of dairy cows is applicable under grazing conditions and to investigate which marker group or marker group combination, with or without FR correction, delivers the most accurate estimation. Furthermore, differences between 2 cow strains and the effect of concentrate supplementation on plant species selection were investigated.

MATERIALS AND METHODS

Experimental Design and Animals

All experimental procedures were in accordance with the Swiss guidelines for animal welfare and were approved (no. 2012_51_FR) by the Animal Care Committee of the Canton of Fribourg, Switzerland. Before selecting the cows for the experiment, a medical checkup including vital parameters as well as udder and claw health was performed. The experiment was a 2 × 2 factorial design, which was conducted as a crossover design with 2 concentrate levels and 2 cow strains. It was divided into 2 measurement periods, each consisting of a 21-d adaption period and a 7-d data collection period (Figure 1). For the flow of work and equipment reasons, the cows were equally divided into 2 consecutive data collection periods of 7 d per measurement period, resulting in 4 data collection periods. The experiment took place on the organic farm "Ferme École de Sorens" located 824 m above sea level in Sorens, Switzerland.

A total of 24 Holstein cows, including 12 Swiss Holstein cows (**HCH**) and 12 Holstein cows of New Zealand origin (**HNZ**), were used for the experiment. Sixteen of them were multiparous and 8 were primiparous. Matched pairs of HCH and HNZ cows were formed according to the number of lactation and DIM for multiparous cows. For primiparous cows, age was considered beside DIM. At the start of the first data collection period, HCH cows had an average number of lactations of 2.1 (SD 1.0), had been 101 (SD 23.7) DIM, had an average BW of 580 (SD 56.3) kg, a BCS of 2.6