Causes of variation in fatty acid content and composition in grass silages

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Introduction

Transition of dairy cows from grazing to silage based winter diets lowers the proportions of unsaturated:saturated fatty acids, with a lower concentration of beneficial C18:3 and conjugated linoleic acid in milk fat (Heck et al., 2009). This unfavorable shift in milk fat composition is partly related to the low precursor poly unsaturated fatty acids (PUFA) supply from ensiled grass, due to harvesting more mature swards for ensiling and oxidative losses of PUFA during wilting. A decrease in leaf/stem ratio, maturation of the leaves, as well as initiation of flowering and leaf senescence can all, decrease the content of total fatty acid and C18:3 during maturation of forage plants. The oxidative losses of PUFA during wilting is variable, and depends mainly on the duration of wilting, change in dry matter (DM) content as well as the extent of plant damage at cutting and environment condition during wilting (Van Ranst, 2009). Quantifying these variations in fatty acid content and composition in grass silages can help to design management strategies to increase PUFA content in grass silages. The present study was therefore designed to investigate the variation in fatty acid content and composition in a large number of grass silages and to search for variables that mark these variations using multivariate analysis on data of agronomic practices, sward quality, wilting and ensiling management, as well as data on nutrient contents, feeding value and ensiling quality.

Material and methods

Grass silages (n=101) were randomly sampled from commercial dairy farms in the Netherlands in 2007 and 2008. After collection, each sample was put in a polythene bag, closed, cooled and immediately transported to the laboratory in cool boxes. Individual samples were mixed well, and subsamples were taken for analysis of nutrient composition, feeding value and fatty acids and immediately frozen at -20°C. Data of soil type, N fertilization, sward type and age, date and number of cuttings, DM yield, bruising, weather condition at cutting, duration of the wilting period, additives or acid used to manipulate the fermentation process, silo type, type of silo covering material and heat production during ensiling were recorded for the individual grass silages, following a structured questionnaire. All samples were freeze dried and ground through a 1 mm screen. The DM content of the fresh and freeze dried samples were determined by oven drying at 103°C for 4 h. The content of ash, crude protein (CP), crude fat (Cfat), sugar, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined using near infrared reflectance spectrometry (NIRS). *In vitro* dry matter digestibility (DMD) and cell-walls digestibility (CWD), net energy lactation (NE), digestible protein (DVE), degraded protein balance in the rumen (OEB) and structure index (SI) were also determined using NIRS. The Lipids from ground, freeze dried samples were extracted with chloroform-methanol (2:1 v/v), with some modification as described by Khan et al. (2009). After extractions, fatty acids in the residual fat were (trans) esterified, using both acid and base catalyzed methods as described by Khan et al.(2011).

Data were summarised by descriptive statistics using SAS. To visualize the relationship between the multiple explanatory variables and fatty acid contents, Redundancy Analysis (RDA) were performed. The bi-plot (Fig. 1) displays each of the response and explanatory variables as vectors (arrows point in the direction of increasing variable values). Correlations between variables are shown by the angle between arrows, an angle of less than 90° between two arrows implies a positive correlation (the strength increases as the angle decreases from 90° to zero), whereas an angle of 90° shows no correlation and an angle above 90° shows a negative correlation (the strength increases as the angle increases from 90° to 180°). The length of an arrow depicts the strength of association. A stepwise multiple regression procedure was used to obtain regression equations for the estimation of total and major individual fatty acid contents in grass silages using SAS. Only equations with parameters contributing significantly ($P<0.05$) to the explanation of the dependent variable were considered.

Results

Total fatty acid contents were highly variable (8.10 to 32.47 g/kg DM) in the grass silages. All individual fatty acids also showed high variations. Variation in total fatty acid contents was predominantly associated with variation in C16:0, C18:2 and C18:3. These three fatty acids on average represent 0.93 g/g of the total fatty acids. Notably, the contents of C16:0, C18:2 and C18:3 in grass silages varied linearly with the changes in total fatty acid content (Figure 2). The content of C18:3, however, showed the largest variation ranging from 3.57 to 20.53 g/kg DM. The content of C16:0 varied from 1.83 to 5.55 g/kg DM, while C18:2 varied from 1.74 to 4.69 g/kg DM. The RDA ordination bi-plot visualizes the relationship between the multiple explanatory variables and fatty acid content in grass silages (Figure 1)

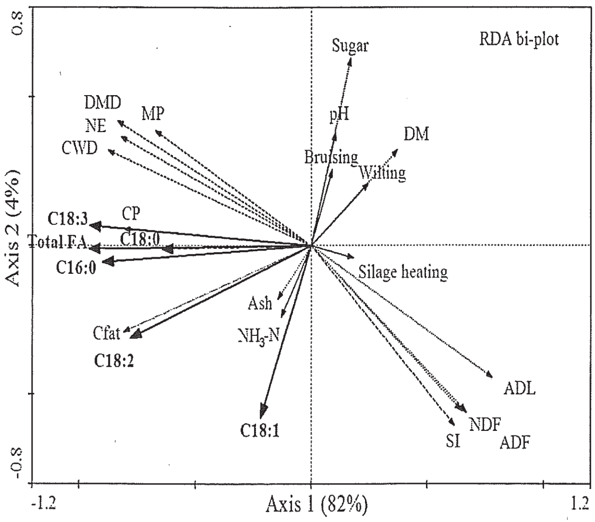

Figure 1

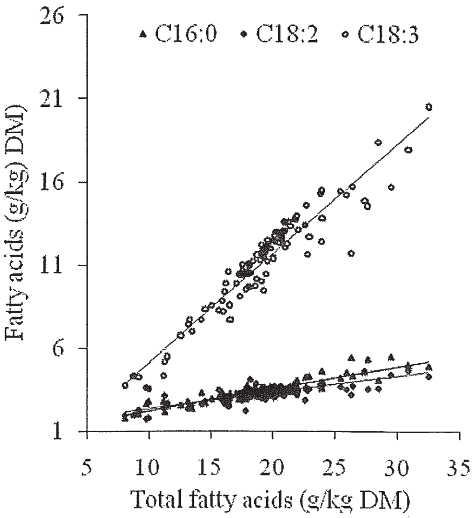

Figure 2

The first axis of RDA explained 82%, while the second axis explained 4% of the total variation in fatty acid content. Axis-1, may be referred to as "plant maturity-axis", as it correlates positively with fibre content and SI of grass silages. Whereas it correlates negatively with CP and Cfat content, digestibility and energy value of the grass silages. Axis-2 may be referred to as "ensiling quality-axis", as it correlates positively with pH, sugar and bruising, whilst it correlates negatively with NH_3-N content. The contents of CP, Cfat as well as DMD, CWD, NE and DVE were positively correlated with the fatty acid content in grass silages. In contrast, DM, NDF, ADF and ADL content, as well as a prolonged wilting period and a high SI were negatively correlated with fatty acid contents in grass silages. Silage heating affected the fatty acid content negatively. This influence was, however, quantitatively much smaller as indicated by the small arrow. Bruising of grass, silage pH and ammonia N (NH_3-N) content did not affect the fatty acid content. The regression analysis gave good estimates for the content of C18:3 ($R^2 = 0.75$) and total fatty acids ($R^2 = 0.65$). Among the nutrient contents and feeding values, variables related to plant maturity were the strongest "predictor" and retained in all the equations.

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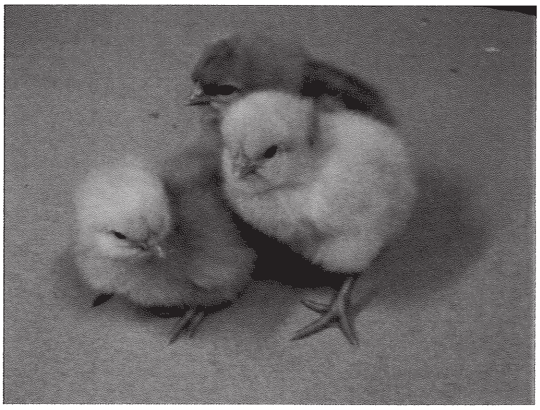

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