



# Fat and fatty acid content and composition of forages: A meta-analysis



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## ABSTRACT

Forages, through the amount and composition of their fatty acids (FA), and because they represent a major part of ruminant diets, can help improve the nutritional quality of milk and meat. However, no comprehensive dataset is available to estimate fat and FA content and composition of forages. This study used the available data on fat and FA content and composition of forages to (i) compute mean composition values for the main forages, and (ii) estimate the influence of forage conservation, cultivation and harvest conditions on fat and FA content and composition. We report mean values for the main forage species in the form of fresh forage, silage or hay. The main factor influencing fat and FA composition was vegetation stage of forage at harvest (estimated by the month of harvest or regrowth interval). Compared with fresh forage at harvest, wilting or drying forages (especially in bad drying conditions) altered their FA, whereas unwilted silage, the use of ensiling additives and N fertilization had only minor effects. The differences between grass (except corn) and legume species were lower than those induced by vegetation stage and wilting or drying. We gave equations to estimate the effects of these factors and thus refine the estimation of the FA content and composition of the forages. Total FA content and proportion of linolenic acid were positively related to crude protein, and negatively related to fiber content of the forages.

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## 1. Introduction

Forages form a major part of dairy cows' diets in most farming systems, and sometimes contain significant amounts of fat and polyunsaturated fatty acids (FA). Diets based on pasture and grass silage can thus improve the nutritional quality of milk and meat by shifting their FA composition toward less saturated FA and more polyunsaturated FA, especially omega-3 FA (Dewhurst et al., 2006). For example, these diets can provide milk that is as rich in linolenic acid (C18:3 *n*-3) as linseed-supplemented diets and lower in *trans*-FA (Dewhurst et al., 2006; Chilliard et al., 2007). Several empirical models have been developed to describe relationships between dietary FA and FA digestion (Glasser et al., 2008b; Schmidely et al., 2008) or milk FA composition (Glasser et al., 2008a). To optimize diet composition for a target milk FA composition, we need to know the FA content and composition of the dietary feedstuffs. Mean values for fat content and FA composition of concentrate feedstuffs (cereals, oilseeds and their products) are available in feed tables (e.g. Sauvant et al., 2004). However, to the best of our knowledge, no quantitative analysis of a comprehensive dataset is available for forages, despite a significant number of publications dedicated to the FA composition of forages, or simply reporting FA composition of some forages used in

**Abbreviations:** ADF, acid detergent fiber; CP, crude protein; DM, dry matter; FA, fatty acid; OM, organic matter; NDF, neutral detergent fiber.

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experiments. The nature and composition of forages influence FA metabolism in the rumen (Buccioni et al., 2012); it is thus of interest to study the factors that modify FA in forages. Fuller knowledge of the factors that influence the FA content and composition of forages could help farmers to optimize cultivation and harvest conditions and thereby improve the quality of their forages (Khan et al., 2012).

This study uses the available data for fat and FA content and composition of forages to (i) compute mean composition values for the main forages and (ii) estimate the influence of forage conservation, cultivation and harvest conditions on fat and FA content and composition.

## 2. Materials and methods

### 2.1. Database building

A database was built from systematic web searches and examination of bibliographic references that included all publications dated post-1970 dealing with the effects of various factors on the FA composition of forages. Publications were included when at least two of the following measurements were reported (or computable from the reported data): FA composition of forages (as g/100 g of total FA or dry matter [DM]), total FA content (in g/kg DM) and fat content (g/kg DM). This focused database comprised a total of 58 publications (Appendix 1) and two unpublished experiments by our laboratory (INRA, UMR1213 Herbivores, Saint-Genès-Champanelle, France).

To estimate the influence of forage conservation and cultivation or harvest conditions on fat and FA composition, the database was split into four sub-databases according to the variation factor studied: 26 publications studied the effect of conservation (fresh forages compared with ensiled, wilted, hay, etc.) (250 forages), 32 studied the effect of vegetation stages (comparing different dates of harvest or regrowth intervals, numbers of cuts, etc.) (281 forages); 9 studied the effect of silage additives (68 forages), and 5 the effect of fertilization (50 forages). Thirteen publications studied other effects and were not included in the four sub-databases.

For the determination of mean composition values for the main forages, this database was completed with various forage analysis data extracted from a non-focused database comprising 136 publications dealing with the digestion of FA in ruminants or relationships between dietary FA and milk FA (Appendix 2).

The study focused on the five main FA, which cover more than 95 g/100 g of total FA: palmitic, stearic, oleic, linoleic and linolenic acids, referred to as 16:0, 18:0, 18:1, 18:2 and 18:3, respectively. The main analytical methods used for fat determination in the publications were ether extraction (53% of total publications, with either petroleum ether or diethyl ether), acid ether extraction (ether extraction preceded by HCl hydrolysis: 16% of total publications), chloroform–methanol extraction (Folch et al., 1957: 10% of total publications) and hexane extraction (6% of total publications). For FA determination, the main methods were one-step methylation (Sukhija and Palmquist, 1988) for 53% of total publications, chloroform–methanol followed by NaOH–methanol (14% of total publications), and chloroform–methanol followed by other procedures (13% of total publications).

### 2.2. Data cleansing

Once the databases had been built, we proceeded to cleanse the data: when the number of data included in a meta-analysis is limited, the atypical data has a high leverage effect on the resulting means, equations or models. To partly overcome this limit, we opted to exclude the statistical outliers: if Q1 is the first quartile and Q3 the third quartile, data below “ $Q1 - 1.5 \times (Q3 - Q1)$ ” or above “ $Q3 + 1.5 \times (Q3 - Q1)$ ” were considered as outliers, a criterion used to identify outliers in boxplots (Tukey, 1977).

Some publications have reported the effect of one particular factor on several cultivars of the same species, leading to an inflation of data for these publications and a high weight in the resulting analysis. In this case, only the means of all the cultivars were used in the analyses, or only one cultivar was selected that exhibited average values for the species. In the publications comparing the composition of a fresh forage and the same forage ensiled with different additives, only the silage with no additive was used in the comparison. The forages that were only wilted without ensiling, were not considered, as they are of no practical use.

### 2.3. Statistical analyses

The publications were very diverse in terms of factor studied, forage species and conservation methods, and of analytical methods used for fat and FA determination. When all these factors were simultaneously taken into account, very small clusters of data resulted, often extracted from only 1 or 2 publications, and so very likely subject to publication bias. To limit this bias, we chose to favor global approaches, pooling several plant species, or several analytical methods, or several modalities, so as to obtain at least 10 data items per pool and thus more robust estimates of the effects studied. Hence some differences are probably disregarded, but the analyses reported are based on a larger dataset, and so less likely to be biased by publication effects.

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