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## Milk fatty acid composition of dairy cows fed green whole-plant buckwheat, phacelia or chicory in their vegetative and reproductive stage



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#### ABSTRACT

The hypothesis was tested that the phenological stage of dicotyledonous forages influences milk fatty acid (FA) composition of dairy cows. For this purpose buckwheat (Fagopyrum esculentum) and phacelia (Phacelia tanacetifolia; both in their first year of cultivation) as well as chicory (Cychorium intybus; second year) were cultivated in mixture with ryegrass (Lolium multiflorum). After 35 days of growth, feeding the green forages to  $3 \times 6$  late lactating dairy cows at ad libitum access started. The realized dietary dry matter proportions of the test plants were (vegetative and reproductive stage): buckwheat, 0.60 and 0.55; chicory, 0.70 and 0.69; phacelia, 0.51 and 0.48. Experimental feeding lasted for 23, 17 and 26 days with buckwheat, chicory and phacelia, respectively. The complete diets additionally contained 2 kg of concentrate and 1 kg of barley straw per day per animal. Milk and feed samples were analyzed from every second day. Feedstuffs were analyzed for proximate contents, FA and fractions of phenolic compounds. In milk samples solids and FA were measured. Contents of FA and of phenol fractions decreased during advancing development stage in all test plants except total extractable phenols and total tannins in phacelia. This decrease was particularly pronounced for  $\alpha$ -linolenic acid (ALA) and linoleic acid (LA) in buckwheat. Phenological stage had no influence on milk yield and gross composition, but ALA and LA proportions of milk fat declined with time in all groups. Proportions of ALA (14.2 g/kg FA) and total poly-unsaturated FA in milk fat were highest with phacelia. Levels of conjugated linoleic acid (CLA) in milk fat were not affected by treatment or phenological stage. Vaccenic acid in milk fat was slightly elevated when feeding buckwheat and phacelia in the reproductive stage whereas it decreased with ongoing development in the chicory group. No systematic influence of phenological stage on the relative recovery of feed FA in milk fat occurred. The study did not confirm the hypothesis that the recovery rate of ALA would increase with maturing of the forages. In terms of generating a desired milk FA profile, phacelia and chicory seem however to be generally promising.

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Abbreviations: ADFom, acid detergent fiber; aNDFom, neutral detergent fiber; ALA,  $\alpha$ -linolenic acid; BCFA, branched-chain fatty acids; CLA, conjugated linoleic acid; CP, crude protein; CT, condensed tannins; DM, dry matter; FA, fatty acid; FAME, FA methyl esters; HT, hydrolyzable tannins; LA, linoleic acid; lignin(sa), lignin determined by solubilization of cellulose with sulfuric acid; NTP, non-tannin phenols; PPO, polyphenol oxidase; RA, rumenic acid; Rep, reproductive stage; TEP, total extractable phenols; TT, total tannins; VA, vaccenic acid; Veg, vegetative stage.

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#### 1. Introduction

Polyunsaturated fatty acids (FA), such as  $\alpha$ -linolenic acid (ALA; 18:3n-3), linoleic acid (LA; 18:2n-6) and rumenic acid (RA; 18:2n-6) as well as the monounsaturated trans-FA vaccenic acid (VA; 18:1n-11), have been linked to several human-health related effects (Barceló-Coblijn and Murphy, 2009; Jutzeler van Wijlen and Colombani, 2010). Although the content of potentially beneficial FA in dairy foods is rather low in comparison to plant oils, they could contribute to improving human health as they are often regularly consumed. Therefore, it is a current goal to find ways how these FA can be enhanced in dairy foods. It is well known that grass-based diets promote the occurrence of these FA in milk (Dewhurst et al., 2006; Elgersma et al., 2006) in comparison with diets based on maize or those containing large proportions of concentrate. Furthermore, diets rich in fresh herbage seem to increase the ruminal bypass of ALA (Petersen et al., 2011). This could be due to an inhibition of ruminal biohydrogenation caused by plant secondary compounds or specific properties of flowering dicots as was hypothesized by Leiber et al. (2005) for cows grazing on alpine swards. Plant phenolic compounds and enzymes like the polyphenol oxidase (PPO) may partly inhibit lipolysis and the first step of biohydrogenation (Buccioni et al., 2012) and thus play a role in protecting dietary polyunsaturated FA from biohydrogenation, which could increase the transfer of feed FA to animal-source foods. However, the effect may largely depend on particular compounds (Lourenço et al., 2008).

In a previous study (Kälber et al., 2011), the apparent transfer rate of ALA from feed to milk was elevated when feeding flowering dicots in comparison to a non-flowering dicotyledoneous plant and to ryegrass. Cabiddu et al. (2005) found that ALA in milk fat was elevated when replacing sulla in its vegetative stage by sulla in its reproductive stage. A likely reason for that is the occurrence of plant compounds distinctive for the reproductive stage. As an example, the flavonoids in buckwheat were described to increase clearly with ongoing development of the plant, especially during the transition from branching to flowering (Kalinova and Vrchotova, 2009). Also in chicory the abundance of phenolic compounds increases with plant development, which is clearly related to the occurrence of certain plant organs (Rees and Harborne, 1985). Besides few investigations (Cabiddu et al., 2005; Buccioni et al., 2012), there are still no systematic studies about effects of the phenological stage of forages on the milk FA profile and the mechanisms behind. Besides increasing phenolic compounds, also other factors like increasing PPO and decreasing ALA (Buccioni et al., 2012) or a decrease of crude protein (CP) content (Gerson et al., 1986) during maturing of plants could play a role.

The main objective of the current study was to investigate the development of plant phenolic compounds and fatty acid profile with ongoing phenological stage of fresh herbage and the relationship of these parameters with the FA profiles in milk from cows having been fed the respective forages. Based on previous research work (Kälber et al., 2011; Kälber et al., 2013) on these plants as feed for dairy cows, fresh buckwheat, phacelia, and chicory were used as experimental model forages. Besides phenolic compounds and FA, also the development of the proximate compounds of the forages was included into analysis and considered to influence the milk FA profile.

The following hypotheses were tested: (i) the concentration of plant phenolic compounds in the forages will increase with ongoing phenological development, (ii) the FA profiles of the plants will change, and (iii) these developments will be associated with an increase of ALA and LA concentrations in the milk fat and with an increase of the apparent recovery rates (transfer rates from feed to milk) of these FA. Such an association of forage properties and the apparent transfer of native plant FA (among them ALA and LA) to milk would provide additional evidence to the hypothesis that the effects of fresh herbage on milk FA composition are at least partly induced by the high concentration and particular composition of phenolic compounds in forages (Jayanegara et al., 2011; Buccioni et al., 2012).

#### 2. Materials and methods

#### 2.1. Experimental forages and concentrates

Two flowering catch crop plants [buckwheat (Fagopyrum esculentum), var. Lileija and phacelia (Phacelia tanacetifolia), var. Phaci] were sown in mixture with ryegrass (Lolium multiflorum ssp. westerwoldicum var. Saproso) in May 2009 at the ETH research station Chamau (Zug, Central Switzerland; 400 m above sea level) similarly as described for a former experiment in Kälber et al. (2011). At the same time a crop of the perennial forage herb chicory [(Cichorium intybus), var. Puna], which is flowering only from the second year on and which had been sown in 2008 also in mixture with ryegrass, was cut and gaps were re-sown. All seeds were obtained from UFA Samen (Fenaco Sämereienzentrum, Winterthur, Switzerland). The intended proportion of ryegrass in the swards was 200 g/kg. From 24 days after sowing or cutting, yield and proportions of test plant, ryegrass and weeds were assessed weekly until the end of the experiment on five patches of a size of  $20 \times 50$  cm per sward. From day 35 after sowing or cutting, treatment feeding with buckwheat and chicory started. As the phacelia sward developed somewhat slower, feeding of this plant started a week later. Buckwheat and phacelia were in the phenological stage of panicle emergence when first fed, whereas chicory just finished the stem elongation stage. Due to a limited biomass development (43 t/ha), which finally restricted the feed available, treatment feeding (after a 4-day period of diet change) lasted only for 17 days for chicory, a time point when proportionately about 25% of the chicory plants were in full blossom. For buckwheat (calculated biomass 49 t/ha) and phacelia (calculated biomass 54 t/ha), 23 and 26 days of treatment feeding were practiced. At the respective ends of treatment feeding, buckwheat was at the end of flowering and phacelia had just reached the stage of full blossom. Two concentrates (producer: UFA AG, Herzogenbuchsee, Switzerland) were fed in amounts

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