

Effects of full-time v. part-time grazing on seasonal changes in milk coagulation properties and fatty acid composition

Research Article

Cite this article: Akert FS, Kreuzer M, Kunz C, Reidy B and Berard J (2021). Effects of full-time v. part-time grazing on seasonal changes in milk coagulation properties and fatty acid composition. *Journal of Dairy Research* **88**, 23–28. <https://doi.org/10.1017/S0022029921000169>

Received: 13 June 2020

Revised: 21 December 2020

Accepted: 11 January 2021

First published online: 17 March 2021

Keywords:

Concentrate supplementation; dairy; fatty acid composition; grazing; milk coagulation properties

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Abstract

For this research communication our objective was to investigate to what extent milk coagulation properties and milk fatty acid (FA) composition were affected by different feeding systems, season and their interaction. Eighteen cows in total were subjected to one of three different feeding system treatments: full-time grazing or part-time grazing combined with indoor feeding of fresh grass with low or high concentrate supplementation. Milk was sampled in spring, summer and autumn. Milk coagulation time was 15.0, 19.0 and 17.7 min, coagulation dynamics 1.67, 3.41 and 1.79 min, and curd firmness 52.7, 32.4 and 47.0 mm in spring, summer and autumn, respectively. Thus, milk coagulation properties of the milk were lower during summer. There were strong seasonal effects on milk FA proportions, but there were not always changes with progressing season, or changes were different with respect to the impact of the feeding systems (system × season interaction). The milk fat was favourably rich in oleic acid, conjugated linoleic acid and α -linolenic acid and had a low $n-6/n-3$ fatty acid ratio in all systems. Factors like seasonal variations in grass composition and the energy balance of the cows were considered relevant for the milk FA composition. Overall, seasonal variations in milk quality were less pronounced with part-time grazing with fresh grass indoors as compared to full-time grazing without concentrate.

In mountainous regions, grass-based diets for dairy cows are common. The milk produced is often intended for traditional and regional cheese production, where feeding of concentrate is limited and/or feeding of silage is prohibited. Labelled milk produced from grass-fed cows is also becoming popular e.g. in the Netherlands, Germany and Switzerland. Grass quality and concentrate supplementation have distinct but variable effects on milk fatty acid (FA) composition (Couvreur *et al.*, 2006) and on cheese making properties (Berry *et al.*, 2001; Leiber *et al.*, 2005b). An impairment of cheese making properties found in milk during summer was linked with changes in milk protein fractions in barn-fed cows (Bernabucci *et al.*, 2015). This may also affect grazing cows. Milk produced from cows fed fresh grass is characterized by elevated proportions of polyunsaturated fatty acids (PUFA) such as α -linolenic acid (ALA; C18:3n3), conjugated linoleic acids (CLA), especially rumenic acid (RA; C18:2c9t11) and vaccenic acid (VA; C18:1t11) (Dhiman *et al.*, 1999; Leiber *et al.*, 2005a). Diet is the main factor affecting milk FA composition (Elgersma, 2015) where a strong relationship between the proportion of fresh grass and CLA content in milk exists (Couvreur *et al.*, 2006; Bär *et al.*, 2020). Other factors such as season (Auldist *et al.*, 1998; Rugoho *et al.*, 2014), stage of lactation (Auldist *et al.*, 1998) and grass composition (Collomb *et al.*, 2002; van Dorland *et al.*, 2008) also influence the milk fatty acid composition. Indoor feeding of fresh grass provides an alternative to make use of the advantageous milk FA composition of grass-fed cows under conditions where grazing possibilities are limited. In farm practice, grazing and indoor feeding of fresh grass are often combined. To our knowledge, variations in milk FA composition during the vegetation period with fresh grass feeding have only been studied in comparison to indoor feeding of total mixed rations (e.g. O'Callaghan *et al.*, 2016) or on alpine pastures (Leiber *et al.*, 2005a), and this not at different concentrate levels.

The objective of the present study was, therefore, to investigate differences in composition of major milk constituents, FA and milk coagulation properties (coagulation rate, coagulation time and curd firmness) of full-time grazing v. part-time grazing with indoor feeding of fresh grass at different concentrate levels. The following hypotheses were tested in this study: (1) Seasonal changes are expected in major constituents, FA composition and coagulation properties of the milk. (2) Supplementation of grazing cows at different concentrate levels reduces the proportions of typical grass-associated milk FA such as PUFA, ALA and CLA according to the respective supplementation level. (3) Balancing the diet by supplementation of energy-rich

concentrate has positive consequences for milk coagulation properties. (4) Full-time grazing without concentrate supplementation is more dependent on seasonal variations in grass composition and climate.

Materials and methods

Experimental site and experimental design

The experiment was carried out in 2015 at the experimental pilot farm of the vocational education and training centre Hohenrain (47°10'57"N, 8°19'15"E, 620 m a.s.l.). Data collection was performed in 2015 between 20 April and 4 May (spring), from 29 June and 13 July (summer) and between 28 September and 10 October (autumn). The experiment was approved by the Cantonal Veterinary Office of Lucerne, Switzerland (LU02/15).

Six cows each were selected by lactation stage, milk yield and lactation number in order to form homogenous groups from three larger herds subjected to one of the three grazing systems: One group was full-time grazing with mineral supplementation only (FG), the second and third were part-time grazing and got fresh grass indoors either with low (IF) or high (IFplus) concentrate supplementation. Each group consisted of two Brown Swiss, two Swiss Fleckvieh and either two Holstein Friesian (IF and IFplus) or two Holstein × Jersey crossbreds (FG) cows. The IFplus cows were supplemented when yielding >24 kg/day. This resulted in 235, 169 and 71 g concentrate/kg diet in spring, summer and autumn, respectively. In IF, concentrate was provided only in spring making up 22 g/kg of dietary dry matter (DM). Details on concentrate composition used are given in Akert *et al.* (2020).

Each group grazed separate permanent pastures. Grass for indoor feeding was obtained once a day from leys and offered *ad libitum* in barn to the IF cows. In FG, pasture allowance was 0.38 ha/cow (intensive continuous stocking). In spring and autumn, the FG cows grazed two paddocks in turns from 0700 to 1700 h and from 1800 to 0600 h. In summer, cows were kept in barn from 1200 to 1800 h to minimize heat stress. Cows from IF and IFplus grazed from 0730 h to 1600 h (summer: to 1030 h) in a continuous stocking system alternating between two to four paddocks and a pasture allowance of 0.13 and 0.10 ha/cow, respectively. The remaining time cows received fresh grass in barn. Grazing heights were assessed daily with a rising plate meter and reached an average over all periods of 5.6, 7.7 and 8.1 cm for FG, IF and IFplus, respectively. Details on botanical analysis and composition of pastures and leys are given in Akert *et al.* (2020). Cows were milked twice daily. Evening milk samples were taken on 26 April, 28 April and 1 May (spring), on 5, 7 and 10 July (summer) and on 4, 7 and 9 October (autumn) and on the following mornings. Details on sample treatment and laboratory analysis of milk and feed are provided in the online Supplementary File.

Laboratory analysis

Milk contents of fat, protein, casein, lactose and urea were assessed on a MilkoScan FT6000 (Foss, Hillerød, Denmark). Latest 24 h after milking, cheese making properties were analysed on a Lattodinamografo (Foss, Padova, Italy). Details on this analysis and on cow κ -casein genotype determination are given in the online Supplementary File.

Statistical analysis

Data on milk constituents and milk coagulation properties were averaged per cow per season before statistical analysis with R (R Core Team) using lme4, lmerTest, and lsmeans for the analysis of variance with linear mixed models. The following random intercept model was used for analysing effects on milk gross nutrient and FA composition:

$$\text{Model 1: } Y_{ijk} = F_i + S_j + F_i \times S_j + A_k + e_{ijk}$$

where feeding system (F; FG, IF and IFplus) and season (S; spring, summer, autumn) were considered as fixed effects, and animal (A) was set as a random intercept accounting for unbalanced repeated measurements. For the evaluation of milk coagulation properties, pH and titratable acidity, the κ -casein genotype (C; AA, AB and BB = 1, 2 and 3) was additionally included as fixed effect. Model assumptions for analysis of variance were verified numerically *via* the Shapiro–Wilks test and visually concerning normal distribution and homogeneous variances. Response variables not fulfilling the requirements were log-transformed. Multiple comparisons among means were performed by contrast analysis considering $P < 0.05$ as significant. For more details see supplementary material.

Results and discussion

All major milk constituents, pH and acidity were affected by season (Table 1). The seasonal decrease in milk yield is inevitably linked with the progressing stage of lactation of the dairy cows which were all seasonally calving in spring. The lower protein and casein content found in summer in all grazing systems is in accordance with seasonal patterns found in other studies (Bernabucci *et al.*, 2015; Bär *et al.*, 2020). This phenomenon was attributed to heat stress during the summer months (Bernabucci *et al.*, 2015). Moreover, decreases of α -, β - and κ -casein proportions and an increase of γ -casein proportion were reported in summer (Bernabucci *et al.*, 2015; Bär *et al.*, 2020). There were system × season interactions in contents of milk protein and casein (Table 1). In FG, protein and casein contents were higher in autumn compared to the IF systems, possibly as a consequence of the lower daily milk yield.

Milk coagulation properties were also affected by season (Table 1). For all systems, coagulation rate was higher in summer (3.41 min) compared to spring (1.67 min) and autumn (1.79 min), whereas curd firmness was highest in spring (52.7 mm) and lowest in summer (32.4 mm; data not shown in table). In FG, coagulation time was lowest in spring. In IF, coagulation time showed no seasonal differences and in IFplus the seasonal effect was most pronounced with the highest coagulation time in summer, intermediate time in autumn and shortest in spring. The reduced protein and casein content of the summer milk as described before might have the major cause for the concomitantly impaired milk coagulation properties. Apart from environmental or physiological effects, the κ -casein B variants are also known to have favourable effects on coagulation properties of milk (Mayer *et al.*, 1997). However, in the present study κ -casein genotype did not reach statistical significance when included as covariate in the statistical model.

Milk urea content was highest in FG and increased over the season by 60% compared to spring, whilst in IF urea content increased by 33% compared to spring (Table 1). In IFplus, milk

Table 1. Milk yield and composition as well as milk coagulation properties of the milk from the different feeding systems measured in three seasons

Season (S)	Spring			Summer			Autumn			SEM	P-value			
	FG	IF	IFplus	FG	IF	IFplus	FG	IF	IFplus		S	F	C ¹	S × F
Feeding system (F) ²														
Milk yield (kg/day)	24.1	29.4	30.4	16.8	22.6	24.0	14.7	16.9	18.7	1.76	***	*		NS
Milk composition (g/100 g)														
Fat	3.65	3.40	3.47	3.80	3.43	3.24	4.79	4.36	4.20	0.179	***	NS		NS
Protein	3.42 ^a	3.30 ^a	3.31 ^a	3.17 ^b	2.95 ^b	3.10 ^b	4.12 ^{c,y}	3.61 ^{c,z}	3.63 ^{c,z}	0.108	***	NS		**
Casein	2.78 ^a	2.68 ^a	2.68 ^a	2.60 ^b	2.41 ^b	2.53 ^b	3.29 ^{c,y}	2.88 ^{c,z}	2.91 ^{c,z}	0.079	***	NS		***
Lactose	4.85	4.86	4.91	4.77	4.76	4.86	4.65	4.61	4.66	0.079	***	NS		NS
Urea (mg/dl)	36.1 ^{a,y}	22.9 ^{a,z}	17.6 ^{a,z}	49.8 ^{b,x}	35.5 ^{b,y}	27.8 ^{b,z}	57.8 ^{c,y}	30.5 ^{c,z}	29.0 ^{b,z}	1.90	***	***		***
Somatic cell count (10 ³ cells/ml)	16.9	35.7	44.0	32.5	54.6	64.5	43.4	90.8	51.5	30.64	***	NS		NS
pH	6.83	6.78	6.81	6.94	6.89	6.87	6.84	6.83	6.83	0.028	***	NS	NS	NS
Acidity ³	1.36	1.32	1.45	0.66	0.64	0.70	0.69	0.69	0.71	0.010	***	NS	NS	NS
Milk coagulation properties														
Coagulation time (min)	14.5 ^a	16.0	14.5 ^a	17.2 ^b	18.3	21.4 ^c	18.0 ^b	17.2	18.0 ^b	1.70	***	NS	NS	*
Coagulation rate (k ₂₀ ; min) ⁴	1.76	1.71	1.55	2.85	3.89	3.58	1.64	1.56	2.26	0.606	***	NS	NS	NS
Curd firmness (A ₃₀ ; mm)	53.8	49.7	54.6	37.1	30.8	29.1	52.4	45.7	43.0	5.24	***	NS	NS	NS

^{a,b,c}Least square season means carrying no common differ significantly within feeding system at $P < 0.05$ (tested by contrast comparison).

^{y,z}Least square feeding system means carrying no common differ significantly within season at $P < 0.05$ (tested by contrast comparison).

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS $P \geq 0.05$.

¹Fixed effect of κ -casein genotype as used in model 2 for pH, acidity, and milk coagulation properties.

²FG, full-time grazing; IF/IFplus, part-time grazing with indoor feeding of grass with few/substantial amounts of concentrate.

³Soxhlet-Henkel degrees.

⁴Variables were log transformed for analysis of variance and contrast comparison.

Table 2. Proportions of major individual fatty acids (FA) and groups of fatty acids (g/100 g fatty acid methyl esters) in milk fat as well as *n*-3 FA/*n*-6 FA ratio found in the different feeding systems measured in three seasons (LSmeans)

Season (S)	Spring			Summer			Autumn			SEM	P-value			
	Feeding system (F) ¹	FG	IF	IFplus	FG	IF	IFplus	FG	IF		IFplus	S	F	S × F
C4:0		1.59	1.42	1.73	1.50	1.43	1.58	1.23	1.20	1.41	0.116	***	*	NS
C6:0		1.69	1.82	2.08	1.68	1.65	1.85	1.52	1.44	1.61	0.108	***	*	NS
C8:0		1.23 ^y	1.50 ^{a,yz}	1.56 ^{a,z}	1.18	1.21 ^b	1.30 ^b	1.18	1.11 ^b	1.17 ^b	0.082	***	NS	**
C10:0		2.78 ^y	3.87 ^{a,z}	3.64 ^{a,z}	2.64	2.77 ^b	2.92 ^b	2.77	2.73 ^b	2.70 ^b	0.226	***	NS	***
C12:0		3.22 ^y	4.66 ^{a,z}	4.15 ^{a,z}	3.13	3.20 ^b	3.34 ^b	3.43	3.42 ^b	3.23 ^b	0.275	***	NS	***
C14:0		10.50 ^{a,y}	14.0 ^{a,z}	12.29 ^z	11.27 ^{ab}	11.70 ^b	11.71	11.98 ^b	12.47 ^b	11.32	0.533	*	*	***
C16:0		24.53 ^{a,y}	27.95 ^{a,yz}	29.09 ^z	29.22 ^b	29.70 ^{ab}	31.05	28.92 ^b	30.56 ^b	29.98	1.217	***	NS	**
C18:0 (SA)		10.65	8.88	9.61	10.93	10.66	10.48	10.49	7.91	9.61	0.853	***	NS	NS
C20:0		0.17	0.15	0.16 ^{ab}	0.18	0.19	0.19	0.17	0.17	0.18	0.014	***	NS	NS
C16:1 <i>n</i> 9		1.47	1.12	1.15	1.46	1.32	1.19	1.43	1.54	1.39	0.147	**	NS	NS
C18:1 <i>n</i> 9 (OA)		23.57 ^{a,y}	16.29 ^z	17.38 ^z	21.35 ^{ab}	19.18	17.77	20.55 ^b	16.98	18.84	1.211	NS	**	**
C18:1 t10		0.24	0.36	0.32	0.18	0.24	0.26	0.19	0.26	0.26	0.023	***	***	NS
C18:1 t11 (VA)		4.35 ^a	4.0 ^{ab}	3.32	2.29 ^b	3.20 ^b	2.97	2.59 ^{b,y}	4.60 ^{a,z}	3.68 ^{yz}	0.499	**	NS	*
C18:2 <i>n</i> 6 (LA)		0.87	0.90	1.12	1.01	1.14	1.36	0.86	1.07	1.19	0.055	***	***	NS
C18:2 c9 + t11 (RA)		1.79 ^a	1.54 ^{ab}	1.18	0.98 ^b	1.25 ^a	1.20	1.29 ^{ab,y}	2.36 ^{b,z}	1.72 ^{yz}	0.250	***	NS	**
C18:3 <i>n</i> 3 (ALA)		0.82 ^a	0.71 ^a	0.66 ^a	0.82 ^a	0.95 ^b	0.92 ^b	1.04 ^b	1.08 ^b	1.10 ^c	0.073	***	NS	*
C18:3 <i>n</i> 6		0.096 ^{a,y}	0.067 ^z	0.061 ^z	0.092 ^{a,y}	0.072 ^{yz}	0.060 ^z	0.076 ^b	0.080	0.074	0.007	NS	*	***
C20:5 <i>n</i> 3		0.076 ^{a,y}	0.068 ^{yz}	0.057 ^{a,z}	0.082 ^{ab}	0.077	0.072 ^b	0.093 ^{b,y}	0.068 ^z	0.078 ^{b,yz}	0.006	***	*	*
C22:5 <i>n</i> 3		0.078 ^a	0.076 ^a	0.078 ^a	0.088 ^a	0.096 ^b	0.097 ^b	0.11 ^b	0.089 ^{ab}	0.10 ^b	0.008	***	NS	**
Saturated FA		58.60 ^{a,y}	66.72 ^z	66.90 ^{a,z}	64.07 ^b	64.91	66.74 ^{ab}	63.89 ^b	63.59	63.65 ^b	1.594	NS	NS	***
Monounsaturated FA		34.74 ^{a,y}	26.45 ^z	26.66 ^z	30.13 ^b	28.62	26.81	29.70 ^b	28.38	29.09	1.364	NS	*	***
Polyunsaturated FA		5.12 ^a	5.25 ^a	4.56 ^a	4.27 ^b	5.07 ^a	5.08 ^{ab}	4.98 ^{ab,y}	6.54 ^{b,z}	5.85 ^{b,yz}	0.347	***	*	**
Total CLA ²		3.03	3.32	2.47	2.05	2.60	2.43	2.66	4.04	3.17	0.343	***	*	NS
<i>n</i> 3 FA		1.01 ^a	0.89 ^a	0.82 ^a	1.03 ^a	1.16 ^b	1.13 ^b	1.29 ^b	1.27 ^b	1.32 ^b	0.083	***	NS	*
<i>n</i> 6 FA		1.30	1.45	1.56	1.37	1.56	1.78	1.27	1.54	1.61	0.061	***	***	NS
<i>n</i> 6 FA/ <i>n</i> 3 FA ³		1.30 ^{a,y}	1.66 ^{a,z}	1.94 ^{a,z}	1.34 ^a	1.35 ^b	1.59 ^b	0.98 ^{b,y}	1.22 ^{b,z}	1.23 ^{c,z}	0.105	***	***	*

^{a,b,c}Least square season means carrying no common differ significantly within feeding system at $P < 0.05$ (tested by contrast comparison).

^{y,z}Least square feeding system means carrying no common differ significantly within season at $P < 0.05$ (tested by contrast comparison).

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS $P \geq 0.05$.

¹FG, full-time grazing; IF/IFplus, part-time grazing with indoor feeding of grass with few/substantial amounts of concentrate.

²Conjugated linoleic acids (C18:2 t6, C18:2 c6, C18:2 c9t13/t8t12, C18:2 c9t12, C18:2 t11c15/t9c12, C18:2 c9c15, C18:2 c9t11, C18:2 c9c11, C18:2 t9t11).

³Variables were log transformed for analysis of variance and contrast comparison.

urea content was lowest compared to IF and FG and increased only from spring to summer (by 58% compared to spring). Excessive dietary crude protein is ruminally degraded to ammonia which is disposed of as urea in urine and milk. Energy-rich or balanced concentrates can reduce the dietary protein excess and thus result in lower milk urea contents. The lower milk urea content in the part-time grazing systems also reflects the lower crude protein content in grass fed indoors compared to the pasture grass (<180 v. >220 g/kg DM; reported in Akert *et al.*, 2020).

Total FA content in grass was highest in spring (19.1 and 16.9 g/kg dry matter (DM) in pasture and indoor grass respectively) and lowest in summer (15.1 and 13.3 g/kg DM in pasture and indoor grass respectively; online Supplementary Table S1). The pasture grass was richer (+2.3 g/kg DM) in ALA than the grass fed indoors. With very few exceptions, milk FA proportions changed with season. Variations in the FA composition of the grass, its botanical composition and the progressing lactation stage likely contributed to that. In summer and autumn milk the ALA proportions increased to levels as high as those reported for alpine milk (Leiber *et al.*, 2005a). O'Callaghan *et al.* (2016) found similar changes with progressing lactation in milk from grass-based feeding. For the present case, this is especially interesting as the ALA proportion in grass reached similar levels in spring and autumn but was lower in summer (online Supplementary Table S1). In milk, ALA increased across all systems from 0.73 to 0.90 and 1.10 g/100 g FAME in spring, summer and autumn, respectively (data not shown in table). A reason, therefore, could have been that more constituents affecting biohydrogenation were present in autumn than spring grass, possibly because of herbs rich in secondary compounds (Leiber *et al.*, 2005a). Also, a direct clover effect seems to be small (van Dorland *et al.*, 2008) and seasonal changes of legume proportion and low abundance of herbs make the dietary explanation less likely.

A number of milk FA were influenced by the feeding system, but less than expected (Table 2 and online Supplementary Tables S2 and S3). Milk from grass-fed dairy cows typically has high proportions of PUFA, ALA and CLA (Dhiman *et al.*, 1999; Elgersma, 2015). This was confirmed by the present study. Surprisingly, the level of concentrate supplementation did not cause the expected differences in the FA composition in the part-time grazing systems investigated. Thus, no difference in PUFA, ALA and CLA proportion of the milk was found between full-time grazing without concentrate supplementation and part-time grazing with high concentrate supplementation. This observation might be explained by the very similar daily grass DM intakes of FG and IFplus cows of 13.2 and 13.4 kg DM per day, respectively (data taken from Akert *et al.*, 2020). The effect of concentrate level was comparably weak, this probably due to the relatively high dietary proportion of fresh grass (>700 g/kg) in all systems. However, the higher proportions of total *n*6 FA and LA in milk from IFplus compared to full-time grazing cows are indicative of concentrate feeding (Leiber *et al.*, 2005a). The milk fat obtained from the feeding systems investigated differed in the proportions of OA and total MUFA, which were higher in full-time grazing compared to part-time grazing. This may have been the result of a causal chain related to the ALA intake from grass which is largely ruminally biohydrogenated to intermediary FA and, finally, to stearic acid (SA). Absorbed from the gut, SA and intermediary FA like VA can be desaturated in the mammary gland to OA and RA, respectively, and are thus proportional to

their precursors' supply (Glasser *et al.*, 2008). There were a number of milk FA that showed an interaction of system and season. Another reason for the high milk OA proportion (full-time grazing cows), could be a negative energy balance of the cows which leads to body fat mobilization. The OA is an indicative for intensive mobilization of body fat tissue (Gross *et al.*, 2011). This phenomenon was also found in milk from alpine grazing, where a negative energy balance was discussed as one factor affecting milk FA composition (Leiber *et al.*, 2005a). In spring, for FG, IF and IFplus cows the energy balance calculated from body weight, milk yield and estimated grass intake using the double alkane method (cf. Akert *et al.*, 2020) was -8.4, 6.4 and -15.6 MJ net energy for lactation per day, respectively. Various factors seem to affect milk FA composition of grass-fed cows in addition to grass proportion in diet or concentrate composition.

In conclusion, the major components, the fatty acid composition and the coagulation properties of the milk varied considerably with season, whilst the effects of the feeding systems were less pronounced. However, the frequent interactions still revealed a distinct importance of the feeding system as it showed that indoor feeding of fresh grass balanced seasonal variations in milk coagulation properties and in FA composition independently of the concentrate level. Across systems, summer was the most critical season for milk coagulation properties of the milk. The milk fat was favourably rich in OA, CLA and ALA and had a low *n*-6/*n*-3 ratio in all systems. Factors including seasonal variations in grass composition and the energy balance of the cows are likely to be relevant for the milk FA composition.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029921000169>

Acknowledgements. The authors gratefully acknowledge the support of Dr S. Mellema (HAFL), M. Karpatcheva, M. Mergani and P. Stirnemann (ETH Zurich). This project was supported by Innosuisse, the Federal Office for Agriculture and the Swiss milk producer organizations SMP and ZMP.

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