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

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Supplementary Material

Further Details on Material & Methods

Concentrate composition, fertilisation, growth state at harvest and milking protocol

In spring, summer and autumn, the concentrates fed were composed of 390, 421 and 452 g/kg of a maize-wheat mixture complemented with 140, 70 and 0 g/kg of rapeseed-meal corn gluten mixture, respectively. Additionally the concentrates contained (g/kg) molasses, 20; palm oil, 10; magnesium oxide, 20, 30 and 40 in spring, summer and autumn, respectively; calcium carbonate, 10; sodium chloride, 5; calcium phosphate (10, 8 and 5 in spring, summer and autumn respectively); and vitaminised trace element mix, 5.

Animal manure and mineral fertiliser were applied on pastures and leys in amounts of 165 and 190 kg N/ha, respectively. Following farm practice, the growth stage of the leys at use (beginning of panicle emergence) for indoor feeding was more advanced than that of the pastures used for grazing.

The experimental cows were milked together with their herd fellows in the order of IFplus, IF and FG in a herringbone milking parlour equipped with an automatic milk recording system (Lemmer-Fullwood, Lohmar, Germany).

Treatment of milk samples and laboratory analysis of feed and milk

Milk samples from evening and consecutive morning milking were pooled per cow according to milk yield. Part of the milk was preserved with Bronopol® (for MilkoScan analyses), the other part without conservative. Samples were either stored at 5 °C or -20 °C (for fatty acid (FA) analysis).

For FA analysis, the grass samples were pooled per season and group. Lipid extraction was performed in duplicate by accelerated solvent extraction (ASE 200; Dionex Corp., Sunnyvale CA). Transformation to FA methyl esters (FAME) and purification through silica gel columns followed Wettstein et al. (2001). The FAME were analysed as described by Ineichen et al.

(2019). Concerning FA in milk, 0.5 ml of one composited sample per cow per season was added to 5 ml *n*-heptane containing triundecanoin, methyl pelargonate and 1-tetradecene as internal standards. For cold transesterification to FAME, Na-methylate was added (Suter et al. 1997). The FA composition of the milk fat was determined by the same GC and column as the feeds, and under conditions described by Ineichen et al. (2019). Identification of FAME in feed and milk was performed using a 37 component standard (Supelco, Bellefonte PA, USA), a Cis-Trans FAME Isomer Standard Mixture and a PUFA-3 (Matreya, LLC, Sate College PA, USA). For milk, peak identification was further confirmed using chromatograms from Collomb and Bühler (2000).

For the analysis of the cheese-making properties 10 ml of milk per cow were warmed to 35 °C during 30 min, before adding 200 µl of diluted (1:50) chymosin (Clerici Standard liquid, 1:19’000, Caglificio Clerici, Cadorago, Italy). In addition, pH (pH meter model 744, Metrohm, Herisau, Switzerland) and titratable acidity of the milk (volumetric method using NaOH 0.25 M and phenolphthalein as the colour indicator) were determined. The cows’ κ-casein genotypes were determined by PCR restriction analysis from DNA extracted from tail hairs. After amplification of the DNA sequence, enzymatic restriction was performed (Medrano and Aquilar-Cordova 1990), and fragments were separated via gel electrophoresis during 60 min at 100 V.

Detailed statistical analysis

Fatty acids with concentrations of < 0.02 g/100 g FAME were considered as traces and only considered in calculating sums of groups or total FA. Milk samples with somatic cell counts > 250’000 were considered as indicators of mastitis and excluded from the dataset. This concerned one IFplus sample in spring and summer and one FG sample in autumn. In spring, three samples of one IF cow were excluded due to erroneously high concentrate allocation

Further Description of the Results

The main FA found in grass were ALA, LA and C16:0, resulting in grass lipids rich in polyunsaturated FA (PUFA), medium in saturated FA (SFA) and low in monounsaturated FA (MUFA) (Supplementary Table 1). For ALA, the difference between grass grazed and fed indoors was particularly high (10.1 vs. 7.8 g/kg DM).

Milk yield significantly decreased from spring to autumn (Table 1, main manuscript) and was significantly lower in FG cows compared to IFplus cows. Only season significantly affected contents of fat and lactose, where fat increased, and lactose decreased from spring to autumn. Significantly higher protein and casein content were observed in FG compared with IF and IFplus in autumn (significant interaction). The milk urea content differed among seasons and among feeding systems, and there was a significant interaction. In detail, the urea content of the FG milk significantly increased from spring to autumn and was always significantly greater with FG compared with the part-time grazing systems. The urea content of IF milk was lowest in spring and highest in summer, whereas in IFplus milk it was highest in autumn (all significant). The somatic cell count (10^3 cells/ml) was significantly lower in spring (30) compared to summer (49) and autumn (59), while the non-adjusted pH of the milk was significantly higher in summer compared to spring and autumn. The titratable acidity of the milk was significantly higher in spring compared to summer and autumn. In FG milk, an increasing coagulation time was recorded from spring to summer and autumn, whereas in IF milk in coagulation time no season effect was found. In IFplus milk coagulation time increased from spring to autumn (significant interaction). Independent of the feeding system, the k_{20} value was significantly higher in summer (3.41 min) compared to spring (1.67 min) and autumn (1.79 min). The A_{30} value was significantly highest across all feeding systems in spring (53.7 mm), lowest in summer (32.4 mm) and intermediate in autumn (47.0 mm).

Findings on the minor FA are specified in the Supplementary Tables 2 and 3. A significant season effect was observed in all identified saturated short, medium and long-chain FA. In addition, there was a significant feeding system × season interaction in the medium-chain FA. The proportions of C4:0, C6:0 and C14:0 significantly differed between feeding systems. In spring, the proportion of C14:0 was significantly highest in IF, lowest in FG and intermediate in IFplus, whereas no feeding system differences were found in summer and autumn (significant interaction). The proportion of the most abundant SFA, C16:0, was unaffected by season in IF and IFplus, but significantly increased by 19% in FG from spring to summer (significant interaction). Independent from feeding system and season, the SFA accounted for more than half of all FA. In FG, the proportion of SFA was significantly lower by 9% in spring than in summer, whereas in IF the proportion of SFA remained unaffected by season and it was significantly reduced by 5% in IFplus in autumn compared to spring (significant interaction).

The proportion of the dominant C18:1 isomer, C18:1n9 (OA), was significantly higher by 15% in spring compared to autumn in FG, but remained unaffected by season in IF and IFplus at on average 17.5 and 18.0 g/100g FAME, respectively (significant interaction). In FG, the proportion of the most prevalent C18:1 *trans* isomer, VA decreased by 47% from spring to summer and autumn. In FG and IF, the lowest VA proportion was found in summer, unlike to IFplus where there was no season effect (significant interaction). In autumn, the VA proportion was significantly lower in FG than in IF, and intermediate in IFplus. The proportion of C18:1t10 (g/100 g FAME) was significantly lower in FG (0.20) compared to IF (0.29) and IFplus (0.28). The C18:1t10 proportion was significantly highest in spring with 0.31 g/100 g FAME. Complementary to the SFA, MUFA proportion was significantly highest in spring in FG (significant interaction).

The LA proportion of the milk fat was significantly highest in IFplus and significantly differed between seasons (0.963, 1.170 and 1.040 g/100 g FAME in spring, summer and autumn, respectively). The proportion of the dominant CLA isomer, RA was significantly lower in FG compared to IF in autumn, and intermediate with IFplus (significant interaction). Different from FG and IFplus, the RA proportion significantly increased by 89% from summer to autumn in IF. The proportion of total CLA was significantly lower by at least 19% in FG and IFplus compared to IF. In summer, the proportion of total CLA was significantly reduced compared to spring and autumn. Within feeding systems, There was no season effect in FG and IFplus in CLA proportion, whereas it was lower in IF in summer compared to autumn, with spring being intermediate (significant interaction). The proportion of ALA significantly increased from spring to autumn by 26, 52 and 67% in FG, IF and IFplus (significant interaction). The proportion of C18:3 n6 (γ-linolenic acid) significantly decreased from spring to autumn in FG, and remained unaffected by season in IF and IFplus (significant interaction). The proportions of C20:5n3 and C22:5n3 significantly increased from spring to autumn in IFplus and FG, but not in IF (significant interaction). Total PUFA proportion significantly increased from summer to autumn in IF and from spring to autumn in IFplus, but decreased from spring to summer by 17% in FG (significant interaction). The proportion of total n3 FA was significantly higher in autumn compared to spring in all feeding systems; its proportion significantly increased from summer to autumn by 25% in FG milk and from spring to autumn by 43% and 61% in IF and IFplus, respectively (significant interaction). Total n6 FA proportion was significantly lowest in FG and highest in summer (significant interaction). The n6/n3 FA ratio significantly decreased with progressing season, with differences between feeding systems found in spring and in autumn (lowest ratio in FG milk) (significant interaction).

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Supplementary Table 1. Content of total, groups (saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) as well as individual fatty acids (FA; g/kg dry matter) in fresh grass from pasture and fed indoors during the three seasons, and in concentrate.

Season	Spring		Summer		Autumn		Concentrate	
Feed	Pasture	Indoor	Pasture	Indoor	Pasture	Indoor	Balanced	Energy rich
Total FA	19.1	16.9	15.1	13.3	18.5	14.1	11.3	11.3
C8:0	0.000	0.006	0.002	0.000	0.000	0.000	0.051	0.033
C10:0	0.035	0.042	0.005	0.006	0.013	0.066	0.036	0.006
C12:0	0.029	0.033	0.040	0.031	0.028	0.027	0.308	0.011
C12:1	0.015	0.016	0.031	0.016	0.024	0.014	0.000	0.004
C14:0	0.065	0.070	0.082	0.069	0.072	0.087	0.178	0.025
C14:1	0.003	0.006	0.006	0.002	0.020	0.000	0.000	0.000
C15:0	0.014	0.022	0.019	0.025	0.019	0.029	0.018	0.013
C15:1	0.048	0.067	0.165	0.057	0.104	0.039	0.000	0.002
C16:0	2.777	2.707	2.723	2.513	2.649	2.321	3.639	3.721
C16:0 iso	0.575	0.392	0.420	0.289	0.593	0.347	0.009	0.011
C16:0 aiso	0.006	0.012	0.008	0.008	0.013	0.022	0.013	0.010
C16:1	0.042	0.021	0.046	0.031	0.079	0.039	0.035	0.022
C17:0	0.028	0.026	0.033	0.031	0.027	0.028	0.023	0.026
C17:1	0.021	0.005	0.013	0.013	0.013	0.014	0.000	0.004
C18:0	0.295	0.274	0.287	0.310	0.288	0.230	0.718	0.769
C18:1 c9	0.412	0.497	0.550	0.497	0.305	0.293	3.865	4.349
C18:1 c11	0.058	0.051	0.056	0.055	0.052	0.044	0.252	0.171
C18:1 c15	0.019	0.012	0.009	0.022	0.011	0.011	0.004	0.000
C18:2 c6	2.576	2.685	2.504	2.161	2.305	2.150	1.609	1.555
C18:3 n6	0.057	0.014	0.050	0.040	0.056	0.041	0.000	0.000
C18:3 n3	11.419	9.196	7.549	6.458	11.239	7.789	0.103	0.074
C20:0	0.076	0.099	0.061	0.090	0.067	0.078	0.103	0.115
C20:1 n7	0.053	0.050	0.017	0.023	0.015	0.028	0.000	0.000
C20:1 n9	0.027	0.021	0.005	0.018	0.042	0.023	0.058	0.064
C20:1	0.000	0.000	0.000	0.000	0.013	0.006	0.000	0.004
C20:2	0.005	0.013	0.007	0.013	0.011	0.011	0.008	0.009
C20:3 n3	0.063	0.114	0.057	0.140	0.052	0.070	0.005	0.047
C20:4 n3	0.004	0.012	0.010	0.009	0.012	0.006	0.009	0.011
C20:5 n3	0.015	0.019	0.011	0.014	0.012	0.017	0.007	0.010
C21:0	0.004	0.011	0.009	0.010	0.008	0.013	0.012	0.011
C22:0	0.138	0.128	0.106	0.107	0.132	0.101	0.085	0.046
C22:1	0.020	0.015	0.010	0.007	0.000	0.000	0.000	0.008
C22:2	0.022	0.056	0.031	0.058	0.040	0.047	0.015	0.019
C23:0	0.040	0.017	0.031	0.022	0.024	0.016	0.013	0.021
C24:0	0.107	0.127	0.085	0.109	0.113	0.121	0.069	0.064
C24:1 n9	0.012	0.013	0.012	0.007	0.008	0.009	0.009	0.006
Total SFA	4.191	3.968	3.911	3.620	4.045	3.484	5.275	4.883
Total MUFA	0.729	0.773	0.920	0.749	0.688	0.520	4.224	4.634
Total PUFA	14.161	12.110	10.219	8.892	13.726	10.131	1.755	1.725

Supplementary Table 2. Proportions of minor individual chain fatty acids (FA) < C18 (g/100 g fatty acid methyl esters).

Season (S)	Spring			Summer			Autumn				*P*-value		
Feeding system (F)[1]	FG	IF	IFplus	FG	IF	IFplus	FG	IF	IFplus	S.E.M.	S	F	S × F
C10:1	0.27^{a}	0.32	0.31	0.31^{ab}	0.26	0.30	0.36^{b}	0.30	0.32	0.034	*	NS	*
C12:0 iso	0.067^{a}	0.087^{ab}	0.076	0.076^{a}	0.060^{a}	0.067	0.102^{b}	0.091^{b}	0.081	0.012	***	NS	*
C12:1[2]	0.069^{y}	$0.101^{a,z}$	$0.092^{a,z}$	0.063	0.050^{b}	0.053^{b}	0.059	0.047^{b}	0.048^{b}	0.009	***	NS	***
C13:0	0.11^{y}	$0.18^{a,z}$	$0.17^{a,z}$	0.092^{a}	0.088^{b}	0.094^{b}	0.11^{a}	0.12^{c}	0.10^{b}	0.011	***	*	***
C13:0 iso	0.043	0.036	0.035	0.053	0.043	0.044	0.056	0.053	0.046	0.009	**	NS	NS
C14:0 iso[2]	0.015^{y}	0.016^{y}	$0.092^{a,z}$	0.017	0.011	0.016^{b}	0.014	0.015	0.013^{b}	0.032	*	NS	*
C14:0 aiso	0.21	0.21	0.20	0.22	0.17	0.17	0.23	0.23	0.20	0.014	**	NS	NS
C14:1	1.26	1.31	0.74	1.45	1.18	1.23	1.67	1.55	1.37	0.176	***	NS	NS
C15:0	1.30^{y}	$1.66^{a,z}$	$1.57^{ab,z}$	1.38	1.43^{b}	1.37^{b}	1.36^{y}	$1.66^{a,z}$	$1.49^{b,yz}$	0.069	**	**	*
C15:0 iso	0.026^{a}	0.038^{a}	0.031^{a}	0.052^{b}	0.058^{b}	0.072^{b}	0.057^{c}	0.066^{c}	0.076^{b}	0.047	***	**	*
C15:1	0.31^{a}	0.28	0.28	0.30^{ab}	0.30	0.31	0.26^{b}	0.30	0.30	0.018	NS	NS	*
C16:0 iso	0.27	0.26	0.25	0.26	0.23	0.20	0.22	0.19	0.21	0.012	***	NS	NS
C16:0 aiso	0.040	0.053	0.043	0.044	0.037	0.038	0.070	0.064	0.050	0.008	**	NS	NS
C16:1[3]	0.56^{a}	0.50	0.54^{a}	0.48^{b}	0.48	0.45^{ab}	$0.39^{c,y}$	$0.46^{y,z}$	$0.48^{b,z}$	0.024	***	NS	***
C17:0	0.81^{b}	0.72	0.75	0.83^{b}	0.85	0.75	0.67^{a}	0.78	0.79	0.042	*	NS	**
C17:0 iso	$0.033^{a,y}$	$0.028^{a,yz}$	$0.020^{a,z}$	$0.028^{b,y}$	$0.021^{b,z}$	$0.016^{b,z}$	$0.035^{a,y}$	$0.021^{b,z}$	$0.020^{ab,z}$	0.002	***	***	*
C17:0 aiso	$0.023^{a,y}$	0.013^{z}	0.019^{yz}	0.020^{ab}	0.018	0.017	0.015^{b}	0.015	0.016	0.006	***	NS	**
C17:1	$0.35^{a,y}$	0.21^{z}	0.26^{z}	0.30^{a}	0.27	0.22	0.21^{b}	0.26	0.26	0.028	NS	NS	***

[a,b,c] Least square means carrying no common differ significantly within system at P < 0.05 (tested by contrast comparison).

[y,z] Least square means carrying no common differ significantly within period at P < 0.05 (tested by contrast comparison).

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

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

[2] Variables were log transformed for analysis of variance.

[3] C16:1 exclusive C16:1 n7

Supplementary Table 3. Proportions of minor individual chain fatty acids (FA) > C17 (g/100 g fatty acid methyl esters).

Season (S)	Spring			Summer			Autumn				P-value		
Feeding system (F)[1]	FG	IF	IFplus	FG	IF	IFplus	FG	IF	IFplus	S.E.M.	S	F	S × F
C18:1 c10	0.11	0.12	0.10	0.09	0.09	0.09	0.08	0.07	0.09	0.011	***	NS	NS
C18:1 c11	0.72	0.68	0.77	0.64	0.69	0.65	0.59	0.60	0.64	0.052	***	NS	NS
C18:1 c12	0.067[a]	0.065	0.078[a]	0.078[a,y]	0.085[y]	0.12[b,z]	0.096[b]	0.070	0.085[a]	0.011	**	NS	**
C18:1 c13	0.13	0.18	0.15	0.088	0.12	0.11	0.10	0.15	0.13	0.014	***	*	NS
C18:1 c14, t16	0.17	0.16	0.16	0.18	0.17	0.15	0.19	0.16	0.19	0.015	NS	NS	NS
C18:1 t6–8	0.28	0.20	0.23	0.22	0.22	0.21	0.24	0.26	0.25	0.025	*	NS	NS
C18:1 t9	0.17	0.21	0.19	0.14	0.18	0.19	0.15	0.18	0.16	0.016	*	**	NS
C18:1 t12	0.16[a]	0.13	0.12	0.094	0.098	0.096	0.064[b]	0.087	0.093	0.014	***	NS	*
C18:1 t13–14, c6–8	0.21[y]	0.29[a,z]	0.27[a,z]	0.20	0.23[b]	0.25[ab]	0.23	0.22[b]	0.23[b]	0.018	***	NS	**
C18:2 t6	0.22	0.36	0.26	0.17	0.25	0.23	0.24	0.31	0.26	0.030	***	**	NS
C18:2 c9+t13 +t8+c12	0.30[z]	0.44[a,y]	0.27[z]	0.27	0.27[b]	0.23	0.34	0.30[b]	0.29	0.038	**	NS	*
C18:2 c9 + t12	0.059	0.074	0.050	0.060	0.057	0.051	0.072	0.066	0.060	0.008	NS	NS	NS
C18:2 t11+c15 +t9+c12	0.48	0.54	0.55	0.41	0.61	0.57	0.56	0.84	0.66	0.094	**	*	NS
C18:2 c9+c15	0.14[z]	0.36[a,y]	0.13[z]	0.14	0.14[b]	0.13	0.14	0.13[b]	0.14	0.047	*	NS	*
C18:2 c9+c11	0.030	0.038	0.031	0.019	0.031	0.026	0.026	0.033	0.032	0.006	NS	NS	NS
C20:1 c5	0.044	0.078	0.041	0.028	0.044	0.038	0.041	0.044	0.047	0.013	**	*	NS
C20:1 c9	0.078	0.089	0.077	0.062	0.087	0.078	0.071	0.11	0.096	0.009	**	**	NS
C20:1 c11	0.104	0.086	0.081	0.11	0.091	0.104	0.11	0.11	0.11	0.007	**	NS	NS
C20:4 n6	0.029[a,y]	0.038[yz]	0.049[a,z]	0.039[b,y]	0.044[y]	0.061[b,z]	0.044[b,yz]	0.036[y]	0.049[a,z]	0.004	***	**	**
C22:0[2]	0.033[a,x]	0.041[ab,y]	0.056[z]	0.042[b,y]	0.049[b,y]	0.061[z]	0.046[b,yz]	0.042[a,y]	0.053[z]	0.003	***	***	***
C22:1[2]	0.009[y]	0.013[y]	0.034[a,z]	0.010	0.012	0.012[b]	0.011	0.010	0.013[b]	0.035	***	***	***
C22:4 n6	0.070[a,y]	0.074[a,y]	0.047[z]	0.043[b]	0.040[b]	0.039	0.040[b]	0.031[b]	0.038	0.005	***	**	**

[a,b] Least square means carrying no common differ significantly within system at $P < 0.05$ (tested by contrast comparison).
[x,y,z] Least square means carrying no common differ significantly within period at $P < 0.05$ (tested by contrast comparison).
[1] FG, full-time grazing; IF/IFplus, part-time grazing with indoor feeding of grass with few/substantial amounts of concentrate.
[2] Variables were log transformed for analysis of variance.