



Milk composition, but not cheese properties, are impaired the day after transhumance to alpine pastures

Madeline Koczura ^a, Bruno Martin ^b, Germano Turille ^c, Massimo De Marchi ^d, Michael Kreuzer ^a, Joël Berard ^{e,*}

^a ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland

^b Université Clermont Auvergne, INRA, VetAgro Sup, UMR1213 Herbivores, 63122, Saint-Genès-Champagnelle, France

^c Institut Agricole Régional, Regione La Rochere 1/A, 11100 Aosta, Italy

^d Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente (DAFNAE), Università degli Studi di Padova, Viale dell'Università 16, 35020, Legnaro, Italy

^e ETH Zurich, AgroVet-Strickhof, Eschikon 27, 8315 Lindau, Switzerland

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ABSTRACT

Short-term effects of transhumance to high altitude were investigated in Valdostana bovine milk and Fontina cheeses. The study included samples taken at each milking on days 1, 2 and 5 after transhumance compared with 5 days before; individual cow milk was collected and bulk milk was manufactured to cheese. Somatic cell count and milk fat content increased the day immediately after transhumance. In bulk milk, transhumance effects were less pronounced. Except for sour taste, no clear trend for impaired composition or sensory properties was observed in the cheeses produced on the day after transhumance and ripened for 115 days. Milking time and, with that, manufacturing time influenced milk and cheese quality, leading to higher fat and more melting cheeses in the evening. These results allow better characterisation of the changes during this transition and will help farmers to tackle the difficulties in producing cheese during this period.

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1. Introduction

In mountainous regions, cows graze on pastures of increasing altitude with progressing season. For this, they are seasonally moved by truck or walk, or both, between fixed summer pastures and winter lowland sites (transhumance). This practice allows an optimal valorisation of highland pastures. Transhumant dairy systems are often associated with the production of farmhouse cheeses, providing an appreciable added value to systems that have to deal with harsh conditions and low productivity (Sturaro et al., 2013). In Europe, 18.5% of the land consists of mountains and a prospering agricultural sector in these areas supports other sectors such as tourism in economy (Santini, Guri, & Gomez y Paloma, 2013). Moreover, the agricultural and food production linked with tradition and local know-how (terroir) are of great importance to the local culture. Such products are labelled and protected by the European Union (Santini et al., 2013). In Aosta Valley (Italy), a semi-

hard cheese is traditionally produced with raw milk from autochthonous cows grazing high alpine pastures in summer, the Fontina PDO (Protected Designation of Origin). Farmers manufacturing the cheese directly in the buildings at the site of the alpine pastures often report facing challenges in cheese production shortly after the transhumance. These difficulties and defects noted in the cheeses seem to appear specifically in the first few days after animals have been moved.

Environmental changes such as the modification of the diet of the animals are known to affect milk composition and especially fat content (Chilliard et al., 2007; Elgersma, Tamminga, & Ellen, 2006), which is playing an important role in cheese manufacturing. These effects are even more pronounced under high altitude grazing conditions (Leiber, Kreuzer, Nigg, Leuenberger, & Wettstein, 2006). Proportions of unsaturated fatty acids and carotenoids in milk fat from cows grazing highland pastures strongly affect the cheese texture and colour (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005; Nozière et al., 2006). However, variations in diet quality when changing pasture concern the entire alpine grazing season and are mostly associated with positive cheese quality (more yellowish, for example). Extensive studies on alpine systems

* Corresponding author. Tel.: +41 52 354 9201.

E-mail address: joel-berard@ethz.ch (J. Berard).

with transhumance showed that the milk yield, milk protein content and rennet coagulation properties are impaired on highland pastures (Leiber, Kreuzer, Leuenberger, & Wettstein, 2006) especially at very high altitude (Koczura et al., 2019; Leiber et al., 2006; Zendri, Ramanzin, Cipolat-Gotet, & Sturaro, 2016). In addition, Zendri et al. (2016) demonstrated that highland pastures decreased cheese making efficiency with a reduction of cheese yield. There is also a higher percentage of milk samples that do not coagulate in the highlands (Niero et al., 2018). These differences are partly due to the higher somatic cell count (SCC) in milk (Lamarche, Martin, Hauwuy, Coulon, & Poutrel, 2000; Niero et al., 2018). Milk with high SCC is also considered to be associated with defects in cheeses, including texture (decrease in firmness and elasticity, increase in stickiness) and taste (rancid and bitter taste due to pronounced lipolysis and proteolysis, respectively) (Coulon, Delacroix-Buchet, Martin, & Pirisi, 2004). These studies typically gave cows a sufficiently long period of adaptation to the pastures before investigating milk properties.

To the knowledge of the authors, direct effects of the transhumance on milk and cheese quality have not yet been investigated. The difficulties in cheese-making encountered immediately after transhumance may rather result from changes in milk composition caused by moving the animals and their arrival in a new environment. Indeed, it has previously been demonstrated that milk of walking cows had a higher fat content and SCC (D'Hour, Hauwuy, Coulon, & Garel, 1994; Coulon, Pradel, Cochard, & Poutrel, 1998). However, even transport to high alpine pastures by truck represents a major stress exposure to dairy cows (Kreuzer et al., 1998). These observations were, however, obtained under experimental conditions and without measuring coagulation properties of milk, cheese manufacturing and analyses.

The aim of the present study was to investigate the short-term changes in milk quality directly after transhumance and identify consequences for rennet coagulation properties and cheese quality. The following hypotheses were tested: (i) an increase in milk fat content and SCC occurs immediately after the transhumance, (ii) the change in milk composition impairs milk coagulation properties and leads to higher fat cheeses with taste and texture defects and (iii) these changes are no longer observed after an adaptation time of 5 days.

2. Materials and methods

2.1. Experimental design

The experiment took place in summer 2016 in Aosta Valley, Italy. All animal-related procedures were in compliance with EU Directive 2010/63/E. The first part of the study involved 16 autochthonous Valdostana Red Pied cows (172 ± 30 days in milk, 8.9 ± 2.3 kg milk per milking at the beginning of the experiment) that were randomly selected from one herd (Institut Agricole Régional d'Aoste (IAR), Aosta, Italy) such that half of the cows were primiparous (150 ± 8 days in milk) and the other half multiparous (195 ± 26 days in milk). Cows were kept outside 24 h a day and managed under the strip-grazing technique. They were milked with a mobile milking parlour (Eliar 4, Eli IAR, Aosta, Italy) at 5.00 a.m. (morning) and 4.00 p.m. (evening), where they received 2 kg day⁻¹ of dairy concentrate (Mangime Settebello Ma. Co. Pa., Mareine & Cie, Bosconero, Italy). During three walked transhumances (duration of 1.5 h, 300 m of difference in height), milk yield and flow were recorded as well as individual milk samples taken at: (i) morning and evening milking of 5 days before transhumance (Day -5); (ii) on the evening and the following morning after transhumance (Day 1); (iii) evening and morning milking of the second day after transhumance (Day 2);

and (iv) morning and evening milking on the fifth day after transhumance (Day 5).

In the second part of the study, bulk milk samples from the IAR herd and two additional herds were collected across five walked transhumances according to the description of the events reported in Table 1. Bulk milk was collected at 7.00 a.m. (morning) and 6.00 p.m. (evening), applying the same four-day sampling scheme as in the first part of the study. In the IAR farm, cows were managed exclusively outside using a mobile milking parlour, whereas in the other farms, they were milked and housed in tied-stall barns overnight, grazing outside during the day and receiving 2 kg d⁻¹ of the concentrate. The management system (outside or barn) did not change within transhumance. Transhumances lasted for between 1 and 1.5 h and were always performed after morning milking. Bulk milk samples from the respective entire herds were collected in amounts allowing milk quality analyses and the manufacturing of Fontina PDO cheese.

2.2. Milk analysis

The milk samples, preserved at 4 °C with the antimicrobial preservative Bronopol® (2-bromo-2-nitro-1,3-propanediol; D&F Inc., Dublin, CA, USA), were analysed for content of fat, protein, lactose, casein, β -hydroxybutyrate (BHB) and urea by NIRS (MilkoScan FT6000) and SCC by a fluorimetric method (Fossomatic®) (both devices from Foss Electric A/S, Hillerød, Denmark). The latter was transformed with a decimal logarithmic function to reach normal distribution. Rennet coagulation time (RCT), curd firmness 30 min after rennet addition (a_{30}) and curd-firming time (k_{20} ; time when the device reports 20 mm of extension) were measured using a Formagraph (Foss Electric A/S) according to the methods of Penasa, De Marchi, Ton, Ancilotto, and Cassandro (2015). Samples that did not coagulate within 30 min were considered as 'non-coagulating' (NC). The proportion of NC samples was calculated and in statistical analysis they were considered as missing values.

2.3. Cheese-making

The raw whole bulk milk obtained from the herds of the second study part was used to produce Fontina PDO cheese at each milking according to the official European specifications (Disciplinare di Produzione della DOP "Fontina", 1995) by the farmhouse cheesemakers. The dairies where the cheeses were manufactured changed along within the transitions. Except for one farm (transition event 2), autochthonous starter cultures were added to the milk before cheese manufacture (16F248 Bioagro SARL). Before rennet addition, milk was heated to 36 °C in copper vats. After around 45 min of coagulation time at 36 °C, the curd was cut to produce maize grain-sized chunks. The curdled mass was then mixed and slowly heated to 48 °C. The curd was subsequently mixed again without additional heating to drain the chunks, and a 10–15 min resting phase was applied. Then, the drained curd was shaped and pressed for

Table 1
Description of the transhumance events.^a

Transhumance event	Date	Altitude range	Milking system	Number of cows per herd	Duration of transhumance (h)
1	10/06/2016	1500–1800	Mobile	60	1.5
2	14/06/2016	1400–1600	Barn	38	1.5
3	05/07/2016	1800–2100	Mobile	55	1
4	23/07/2016	1700–2100	Barn	90	1.5
5	06/09/2016	1800–2100	Mobile	50	1

^a Altitude range is given as metres above sea level.

approximately 24 h, using a wooden mould with a diameter varying between 35 and 45 cm and a height of 7–10 cm. Cheese forms stayed in brine for about 12 h before being moved to ripening cellars. In the latter, they were ripened for 115 ± 5 days at a humidity of 90% and a temperature between 8 and 10 °C. All cheeses within one transhumance were ripened under the same conditions.

2.4. Cheese analysis

Morning and evening of each day of the transhumance event, a cheese was manufactured and sampled ($n = 2$ times of manufacturing \times 4 days \times 5 transhumance events). The pH of the fresh cheeses was measured using a pH-meter 24 h after manufacturing (pH 24 h). The ripened cheeses were cut in half and a rectangular sample of 100 g was drawn from the core and immediately analysed for content of dry matter (DM), fat, protein, ash, calcium and NaCl using NIRSystems 5000 (Foss Electric A/S) according to infrared technologies (De Marchi, Penasa, Zidi, & Manuelian, 2018). Two further rectangular replicates of 50 g from the core were stored under vacuum at -20 °C for later analyses of variables describing proteolysis, colour and rheological properties. After thawing, contents of total nitrogen (N), soluble nitrogen (SN), and phosphotungstic acid-soluble nitrogen (PTSN) were measured using the methods described by Ardö (1999). The following ratios were then calculated: WSN-to-N, PTSN-to-N and PTSN-to-WSN. Colour measurements were performed on five random points on each cheese sample using a CM-2600d spectrophotometer (Konica Minolta, Ramsey, NJ, USA). Results were expressed using the $L^* a^* b^*$ system, where L^* reflects brightness, a^* redness (on a green-red scale) and b^* yellowness (on a blue-yellow scale).

In addition, three circular pieces with a diameter of 10 mm were cut out from the core of the 50 g samples. The rheological method used consisted of an uniaxial compression at a constant displacement rate (Instron 5543 compressor coupled with Instron BlueHill software, ITW Inc., Glenview, IL, USA). Force at failure, Young's module, resistance to compression by 20, 40 and 60% of the height of the cheese core piece were recorded.

Sensory analysis was performed by a trained panel of six assessors who were used to perform routine sensory analysis of Fontina cheeses. They had to rate three texture attributes and four specific tastes (sweetness, saltiness, sour taste and bitterness) applying an intensity score from 1 to 7, with 1 being the lowest level and 7 the highest. Two visual aspects (appearance of the eyes and colour of the core) were also rated from 1 to 7, 1 being least typical for Fontina and 7 being most typical. Usually, eyes of Fontina should be small, regular and round-shaped, few in number. The colour of the core should be straw-yellow. Assessors were asked to spontaneously identify abnormal flavours in the cheeses and describe them using pungent, rancid, propionic, butyric, stable, animal, and putrid, etc., as attributes. The number of such abnormal tastes observed per cheese sample was recorded and the proportion of judges finding at least one abnormal taste was calculated. During one session, the panellists were individually assessing all cheeses from one transition event where cheeses were coded with random numbers in a varying order. Water and dry bread sticks ('Grissini') were provided to each panellist if requested in between the samples.

2.5. Statistical analyses

Data were analysed using SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA). Normality of the residuals was checked using the Shapiro–Wilk test. For SCC, statistics were performed on the logarithmically transformed data, but arithmetic means of SCC are

presented in the tables. For all models, the level of significance was set to $P < 0.05$, and $0.05 < P < 0.10$ was considered as a trend.

A first repeated mixed model was applied when analysing the milk data from the 16 individual cows, with the fixed effects of the day of transhumance (−5, 1, 2, or 5), time of milking (evening or morning), parity (primiparous or multiparous), and interactions between day of transhumance \times time of milking and day of transhumance \times parity. All other interactions were excluded from the model as they turned out to never be significant. Day in relation to transhumance was considered as the repeated factor, with the cow nested as subject, and cow was considered as a random factor. Multiple comparisons of means were performed using Tukey's correction.

The NC samples were gathered in a separate dataset. The effects of the day in relation to transhumance, time of milking and parity on the proportion of NC samples were assessed using a Chi-square test through the FREQ procedure of SAS. Then, a two-sided Student t-test (TTEST procedure of SAS) was performed to compare the gross composition of NC samples with that of the coagulating (C) samples.

For bulk milk and cheeses there were five replicates (transition events; Table 1) per each of the milking of the four sampling days. Multiple data obtained on colorimetric and rheological variables were averaged per cheese. In this second mixed model, effects of day of transhumance, time of milking and, with that, time of cheese manufacturing and their interaction were integrated as fixed factors. Day was used as repeated factor and transition event as subject and random factor. Homogeneity of the sensory panel was assessed for each sensory attribute by calculating Pearson correlation coefficients (CORR procedure of SAS) between the notes given by the individual panellist and the average note given by the entire panel. In a third mixed model, the fixed effects of day in relation to transhumance, time of cheese manufacturing and their interaction were tested, using the day in relation to transhumance as repeated factor and the individual panellist (1–6) nested in the transition event as subject. Individual panellists were considered as random effects. Multiple comparisons among means with the bulk milk and cheese data were made with Dunnett's correction. In the tables, the significance of the difference to the mean value of Day −5 is indicated.

3. Results

3.1. Individual milk properties

All variables determined were affected by the day of transhumance (Table 2). Individual milk yield declined by 44% in the evening and 36% in the morning on Day 1 (Fig. 1). In the morning of Day 2, it still had not increased again, while this was the case in the evening of Day 2. In the 5 days investigated, milk yield did not return to the level measured before transhumance. Milk flow followed the same pattern as milk yield.

Milk fat content initially increased after transhumance, significantly so in the morning of Day 2 ($+9.2$ g kg^{-1} , Fig. 1). Milk protein content increased by 1.2 g kg^{-1} on Day 1. However, the increase in milk fat was more pronounced, leading to an increase in fat-to-protein ratio on Days 1 and 2. Casein followed principally the same pattern as protein, but still casein-to-protein ratio slightly decreased. Lactose content dropped on the evening of Day 1 and was lower than on the evening of Day 5 (data on milking events not shown). The SCC increased by 71×10^3 cells mL^{-1} on Day 1 and by 45×10^3 cells mL^{-1} on Day 2, but had normalised on Day 5. Transhumance event increased β -hydroxybutyrate (BHB) concentration in milk, an indicator of hyperketonaemia in dairy cows directly related to ketosis (Benedet, Manuelian, Zidi, Penasa, & De

Table 2
Effect of the day of transhumance, time of milking, parity of the cow and their interactions on milk yield and flow as well as composition and coagulation properties of the milk of the individual cows.^a

Parameter	Day in relation to transhumance				Time of milking		Parity		SEM	P-values				
	-5	1	2	5	Evening	Morning	Primiparous	Multiparous		Day	Time	Parity	Day × Time	Day × Parity
Milk yield (kg milking ⁻¹)	6.92	5.18***	5.88***	5.56***	5.67	6.10	6.23	5.54	0.350	<0.001	0.005	0.171	0.007	0.142
Milk flow (kg min ⁻¹)	0.72	0.54***	0.57***	0.54***	0.56	0.62	0.62	0.57	0.041	<0.001	0.014	0.400	0.350	0.602
Composition														
Fat (g kg ⁻¹)	37.4	41.5**	42.5***	39.8	41.2	39.4	39.7	40.9	1.43	<0.001	0.034	0.587	<0.001	0.480
Protein (g kg ⁻¹)	34.5	35.7***	34.8	35.0*	35.1	34.9	35.2	34.9	0.71	<0.001	0.418	0.765	0.433	0.333
Casein (g kg ⁻¹)	27.1	27.9***	27.5	27.6*	27.5	27.6	27.4	27.7	0.63	<0.001	0.415	0.766	0.203	0.581
Lactose (g kg ⁻¹)	47.0	46.7	47.2	47.3	46.9	47.2	45.7	48.4	0.53	0.001	0.002	0.003	0.026	0.005
Fat-to-protein ratio	1.09	1.17 ^(*)	1.22***	1.14	1.18	1.13	1.18	1.13	0.032	0.002	0.039	0.297	<0.001	0.679
Casein-to-protein ratio	0.787	0.783**	0.788	0.790*	0.784	0.789	0.794	0.779	0.002	<0.001	<0.001	0.005	<0.001	0.104
SCC (× 10 ³ cells mL ⁻¹)	136	207***	181***	159	172	170	105	250	68.5	<0.001	0.448	0.004	0.759	0.351
BHB (mmol L ⁻¹)	0.085	0.118***	0.099*	0.095 ^(*)	0.098	0.101	0.092	0.107	0.016	<0.001	0.138	0.133	0.049	0.200
Urea (mg kg ⁻¹)	219	259***	239 ^(*)	185***	236	216	219	232	12.2	<0.001	0.001	0.456	0.108	0.573
pH	6.64	6.62*	6.60***	6.64	6.62	6.62	6.62	6.63	0.014	<0.001	0.587	0.492	0.118	0.143
Coagulation properties														
RCT (min)	19.4	18.7	18.7	21.1**	19.4	19.6	21.1	17.9	1.39	<0.001	0.637	0.131	0.333	0.529
a ₃₀ (mm)	25.4	28.6*	29.5**	23.9	26.7	27.0	23.0	30.7	4.12	<0.001	0.653	0.205	0.222	0.149
k ₂₀ (min)	5.4	4.7***	4.7***	5.3	5.1	4.9	5.4	4.6	0.38	<0.001	0.068	0.166	0.462	0.949

^a Abbreviations are: SCC, somatic cell count; BHB, β-hydroxybutyrate; RCT, rennet coagulation time; a₃₀, curd firmness 30 min after rennet addition; k₂₀, curd-firming time until 20 mm firmness is reached. Asterisks indicate that values differ from those of Day -5 at ****P* < 0.001, ***P* < 0.01, **P* < 0.05 and ^(*)*P* < 0.10 (*n* = 16 per day and per milking).

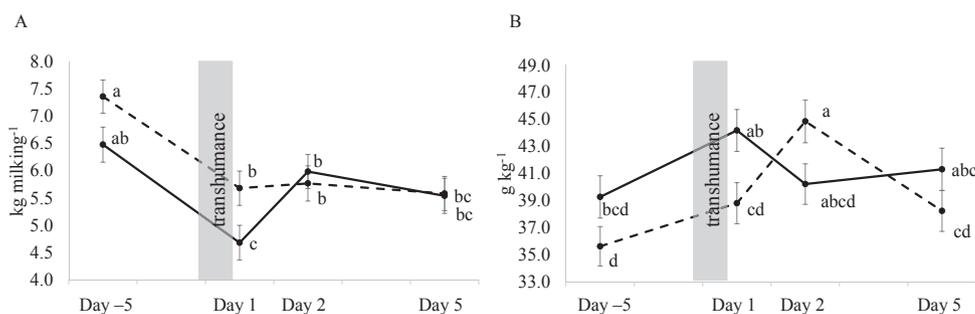


Fig. 1. Effect of the day of transhumance on evening (—) and morning (---) individual milk yield (A) and fat content (B). Means not marked with the same letter are different at *P* < 0.05 (day × time).

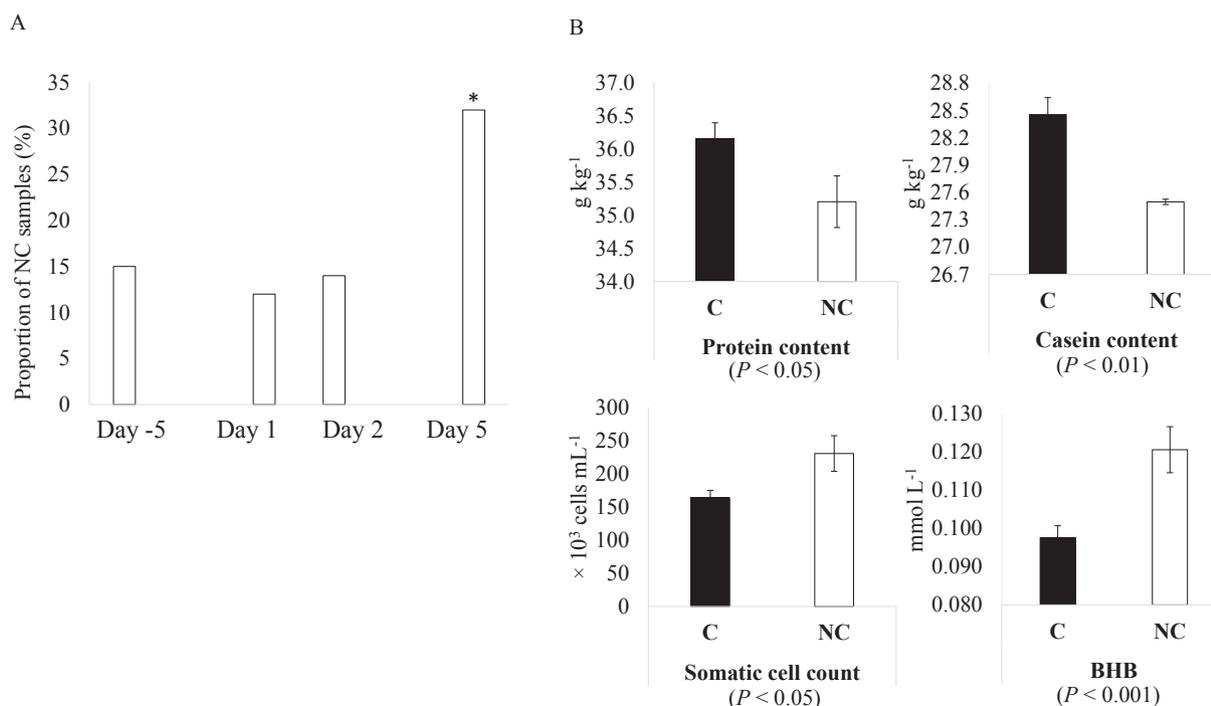


Fig. 2. Proportion of non-coagulated milk samples (A) and differences of non-coagulated and coagulated (NC and C, respectively) milk samples in composition (B) (BHB, β-hydroxybutyrate).

Table 3
Effect of the day of transhumance, time of milking and their interaction on composition and coagulation properties of the bulk milk.^a

Parameter	Day in relation to transhumance				Time of milking		SEM	P-values		
	-5	1	2	5	Evening	Morning		Day	Time	Day × Time
Composition										
Fat (g kg ⁻¹)	38.4	42.6*	42.1(*)	40.0	42.4	39.1	1.36	0.046	0.005	0.587
Protein (g kg ⁻¹)	34.2	35.1*	34.4	34.4	34.6	34.5	0.33	0.026	0.672	0.599
Casein (g kg ⁻¹)	26.8	27.5*	27.0	27.1	27.1	27.1	0.25	0.080	0.737	0.707
Lactose (g kg ⁻¹)	46.9	46.9	47.2	47.0	46.8	47.2	0.64	0.550	0.033	0.451
Fat-to-protein ratio	1.12	1.21	1.22(*)	1.16	1.22	1.13	0.033	0.109	0.005	0.717
Casein-to-protein ratio	0.784	0.784	0.785	0.787	0.783	0.787	0.003	0.515	0.022	0.659
SCC (× 10 ³ cells mL ⁻¹)	192	353**	230	259	255	258	42.1	0.013	0.591	0.311
BHB (mmol L ⁻¹)	0.090	0.102	0.089	0.089	0.088	0.096	0.007	0.526	0.251	0.071
Urea (mg kg ⁻¹)	240	232	224	202	232	217	25.4	0.292	0.350	0.563
pH	6.62	6.62	6.63	6.65	6.63	6.63	0.015	0.164	0.466	0.729
Coagulation properties										
RCT (min)	18.8	18.2	19.1	20.6	19.3	19.1	0.72	0.067	0.759	0.894
a ₃₀ (mm)	25.4	28.9	25.6	22.5	25.5	25.7	1.31	0.015	0.903	0.226
k ₂₀ (min)	6.2	5.9	6.1	6.5	6.2	6.1	0.26	0.430	0.775	0.378

^a Abbreviations are: SCC, somatic cell count; BHB, β-hydroxybutyrate; RCT, rennet coagulation time; a₃₀, curd firmness 30 min after rennet addition; k₂₀, curd-firming time until 20 mm firmness is reached. Asterisks indicate that values differ from those of Day -5 at **P < 0.01, *P < 0.05 and (*)P < 0.10 (n = 5 per day and per milking).

Marchi, 2019), by 0.039 mmol L⁻¹ in the evening and 0.027 mmol L⁻¹ in the morning of Day 1. It gradually decreased thereafter but not fully to pre-transhumance levels. Compared with Day -5, milk urea content was higher by 40 mg kg⁻¹ on Day 1 and by 20 mg kg⁻¹ on Day 2 but lower by 34 mg kg⁻¹ on Day 5. Milk pH was lower by 0.02 and 0.04 on Days 1 and 2 compared with Day -5, respectively. Compared with Day -5, RCT increased by 1.7 min on Day 5 whereas it did not change on Days 1 and 2. The a₃₀ increased by 3.2 and 4.1 mm on Days 1 and 2, respectively, while k₂₀ decreased by 0.7 min on both days.

Across all days in relation to transhumance, milk yield, flow, lactose content and casein-to-protein ratio were lower in the evening than in the morning milking, while milk fat, fat-to-protein ratio and urea content were greater in the evening (Table 2). Lactose content of milk of multiparous cows was lower than that of primiparous cows and decreased on Day 1 compared

with Day -5, whereas this did not happen in primiparous cows (data not shown). The casein-to-protein ratio was lower in multiparous compared with primiparous cows, whereas their SCC was 2.4 times higher.

The proportion of NC milk samples doubled on Day 5 compared with Day -5 but did not change on Days 1 and 2 (Fig. 2A). No significant differences were observed between morning and evening milk (15.1 and 21.9% of NC samples, respectively) and between milk from multiparous and primiparous cows (22.7 and 15.3%, respectively). Overall, 18.6% of the milk samples did not coagulate. The NC samples had lower protein and casein contents and higher SCC and BHB concentration than C samples (Fig. 2B). The lactose content was lower by 1.3 g kg⁻¹ and the pH higher by 0.02 in NC samples compared with C samples (data not shown). No other variable of milk composition differed between NC and C samples.

Table 4
Effect of the day of transhumance, time of manufacturing and their interaction on composition, colour, proteolytic and rheological characteristics of the Fontina cheese.^a

Parameter	Day in relation to transhumance				Time of manufacturing		SEM	P-values		
	-5	1	2	5	Evening	Morning		Day	Time	Day × Time
pH 24 h after manufacturing	6.41	6.55(*)	6.38	6.46	6.46	6.45	0.172	0.058	0.843	0.286
Chemical composition										
Dry matter (DM; g kg ⁻¹)	597	599	603	601	602	598	9.7	0.675	0.387	0.846
Fat (g kg ⁻¹)	306	313	311	302	316	301	34.0	0.431	0.038	0.228
Protein (g kg ⁻¹)	247	243	246	252	243	251	4.7	0.370	0.030	0.767
Ash (g kg ⁻¹)	36.8	35.7	35.8	34.7	35.5	36.0	1.16	0.307	0.569	0.097
NaCl (g kg ⁻¹)	18.7	18.7	17.5	16.2	18.4	17.1	1.25	0.147	0.143	0.850
Ca (g kg ⁻¹)	8.28	7.99	8.21	8.47	8.05	8.42	0.24	0.364	0.056	0.715
Fat (g kg ⁻¹ of DM)	512	526	519	506	526	505	9.5	0.248	0.007	0.124
NaCl (g kg ⁻¹ of DM)	31.3	31.5	29.5	27.2	30.8	29.0	2.34	0.171	0.222	0.799
Colour of cheese core										
Brightness (L*)	75.5	77.1	74.5	73.7	76.4	74.1	1.23	0.097	0.017	0.380
Red index (a*)	1.91	1.65	2.13	1.94	1.97	1.85	0.486	0.681	0.637	0.723
Yellow index (b*)	18.3	19.8	19.0	18.5	19.0	18.8	1.16	0.423	0.730	0.602
Proteolysis (ratios in %)^a										
WSN-to-N ratio	18.6	16.6	15.8	16.4	16.8	16.9	1.38	0.107	0.893	0.013
PTSN-to-N ratio	3.88	3.03	2.86	3.65	3.42	3.29	0.383	0.116	0.708	0.568
PTSN-to-WSN ratio	21.2	18.3	19.3	24.0	21.4	20.0	3.6	0.329	0.581	0.052
Rheological variables										
Force at failure (N)	265	211	239	250	213	270	38.2	0.473	0.023	0.673
Young module (MPa)	0.298	0.291	0.276	0.308	0.251	0.335	0.041	0.939	0.043	0.945
Stress 20% (N cm ⁻²)	2.07	1.60	2.12	1.93	1.63	2.22	0.411	0.540	0.030	0.188
Stress 40% (N cm ⁻²)	4.56	3.83	4.64	4.23	3.61	5.02	0.819	0.779	0.024	0.344
Stress 60% (N cm ⁻²)	8.80	8.11	8.71	8.44	7.30	9.73	1.283	0.972	0.033	0.821

^a Abbreviations are: WSN, water soluble nitrogen; N, total nitrogen; PTSN, phosphotungstic acid-soluble nitrogen. An asterisk indicates that values differ from those of Day -5 at (*)P < 0.10 (n = 5 per day and per milking).

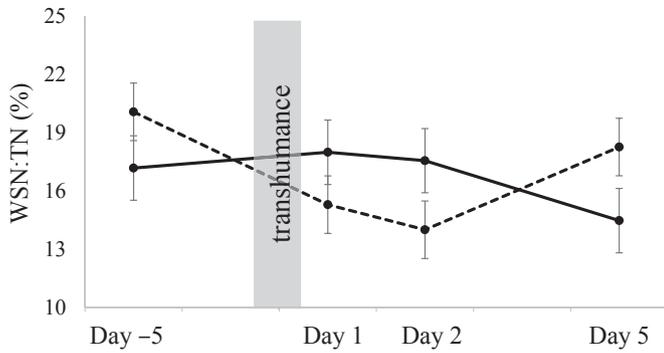


Fig. 3. Effect of day of transhumance on proteolysis (ratio of water-soluble nitrogen, WSN, to total nitrogen, TN) in cheeses produced from evening (—) and morning (---) milk. Means not marked with the same letter are different at $P < 0.05$ (day \times time).

3.2. Bulk milk properties

Contents of fat, protein and lactose in the bulk milk increased by 4.2, 0.9 and 0.7 g kg⁻¹, respectively, on the day after transhumance (Table 3). While protein and casein contents returned to pre-transhumance levels on Day 2, fat content tended to stay higher by 3.7 g kg⁻¹ and the fat-to-protein ratio was increased in the same way. The casein-to-protein ratio was not affected by transhumance. On Day 1, the SCC increased by 161 $\times 10^3$ cells mL⁻¹ compared with Day -5. All samples did coagulate. Curd firmness (a_{30}) was greater by 6.4 mm on Day 1 compared with Day 5, but did not differ from the value found on Day -5. All milk composition traits that were affected by the transhumance returned to their previous level within 5 days. The fat content of the bulk milk was higher by 3.3 g kg⁻¹ in the evening milk than in the morning milk across all days, and the same was true for fat-to-protein ratio. Lactose content was higher by 0.4 g kg⁻¹ in morning

Table 5

Effect of the day in relation to transhumance, time of milking and their interaction on sensorial properties and abnormalities found in the Fontina cheese.^a

Parameter	Day in relation to transhumance				Time of manufacturing		SEM	P-values			
	-5	1	2	5	Evening	Morning		Day	Time	Day \times Time	
Texture											
Elasticity	4.80	4.49	4.83	4.82	4.75	4.73	0.281	0.115	0.857	0.652	
Hardness	2.66	2.74	2.89	2.56	2.39	3.04	0.228	0.286	<0.001	0.001	
Melting	4.54	4.41	4.25	4.53	4.69	4.17	0.224	0.440	<0.001	0.026	
Taste											
Sweetness	2.61	2.68	2.42	2.46	2.43	2.66	0.302	0.527	0.090	0.918	
Saltiness	2.55	2.38	2.67	2.37	2.45	2.53	0.269	0.314	0.554	0.999	
Sour	2.00	2.12	1.73	1.77	1.97	1.84	0.345	0.047	0.233	0.848	
Bitterness	1.58	1.53	1.22	1.47	1.39	1.51	0.179	0.149	0.332	0.090	
Visual aspect											
Appearance of the eyes	3.23	3.20	3.89*	3.56	3.35	3.59	0.263	0.012	0.162	0.011	
Perceived colour of the core	4.32	4.25	4.88**	4.65	4.53	4.52	0.305	0.004	0.953	0.024	
Abnormalities found											
Proportion of judges (%)	60.0	54.2	40.3	45.0	51.5	48.3	1.39	0.356	0.718	0.616	
Number of abnormal tastes	1.70	1.31	1.37	2.20	1.84	1.45	0.686	0.469	0.383	0.952	

^a Grades from 1 to 7, 1 = low and 7 = high. Asterisks indicate that values differ from those of Day -5 at ** $P < 0.01$ and * $P < 0.05$ (n = 5 per day and per milking).

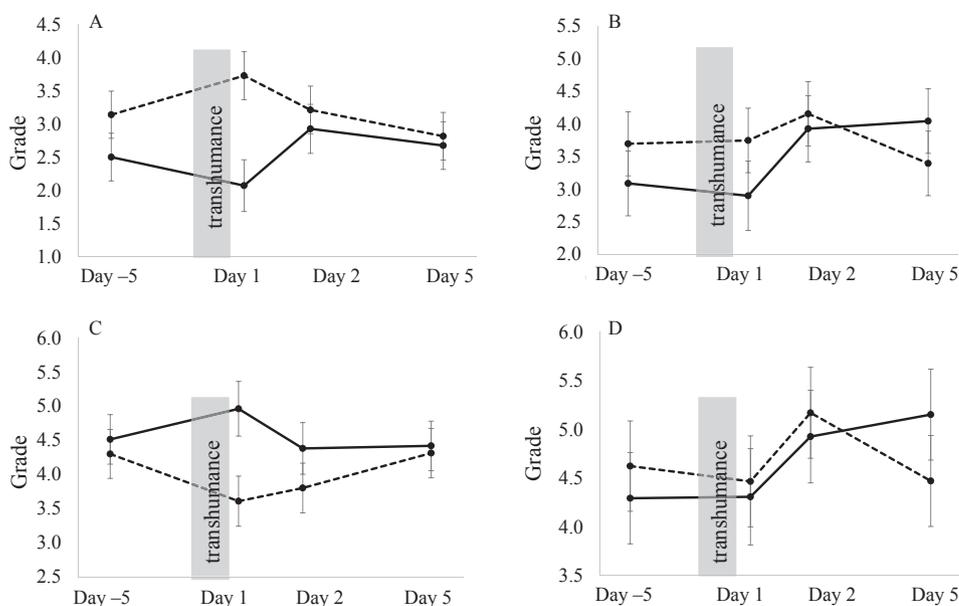


Fig. 4. Effect of the day of transhumance on texture (A, hardness; C, melting texture) and visual aspects (B, appearance of the eyes; D, colour of the core) of cheeses produced from evening (—) and morning (---) milk (grades from 1 to 7, where 1 = low and 7 = high) Means not marked with the same letter are different at $P < 0.05$ (day \times time).

than in the evening milk and the casein-to-protein ratio was slightly lower in the morning.

3.3. Cheese quality

The day of transhumance did not affect composition, colour or rheological properties of the cheeses (Table 4). The cheese manufacturing time, however, affected several variables across all days. The fat content was higher in the evening than in the morning by 21 g kg⁻¹ DM and the protein content was lower by 8 g kg⁻¹ DM in the evening. Cheeses from the evening were brighter than those from the morning (+2.3 in L*). The WSN-to-N ratio was higher in the cheeses manufactured from the morning milk compared with those prepared from the evening milk on Days -5 and 5, whereas the contrary was found on Days 1 and 2 (Fig. 3). Force at failure and Young's module were lower in the evening compared with the morning by 21 and 26%, respectively, and the resistance to compression at 20, 40 and 60% of the cheese's height was lower by 26, 28 and 25%, respectively (Table 4).

The sensory panel was not able to detect differences between days of transhumance in most sensory variables (Table 5). However, cheeses from Day 1 had a more sour taste than those from Day 2, but this attribute was not perceived differently from cheeses from Day -5. The visual appearance of the eyes of the cheeses globally improved on Day 2 after transhumance, and the same change was observed for the perceived colour of the core. In both traits there was an interaction between Day and manufacturing time (Fig. 4). The morning cheeses were harder and less melting in the mouth on average, and on Day 1 the gap in cheese texture between evening and morning manufacturing increased. The panel did not report differences in abnormal tastes or defects between the days in relation to transhumance.

4. Discussion

4.1. Effects of transhumance and their persistence

4.1.1. Milk composition

The increase in milk fat content and SCC right after transhumance found in both individual and bulk milk confirmed the first part of our hypothesis, the changes are most probably linked to the walking effort of the cows, as previously shown by D'Hour et al. (1994) and Coulon and Pradel (1997). Movement stress causes a mobilisation of body fat stores (Coulon & Pradel, 1997) which increases free fatty acids in blood and ketone bodies in blood (Kreuzer et al., 1998) and in milk (BHB in the present study) indicating energy deficiency (Ježek et al., 2017). Part of the effect on milk fat can be explained by the decline in milk yield and the consequently lower dilution of the fat. In these low energy supply conditions, milk protein content was expected to decrease, as previously observed (Coulon & Rémond, 1991). In the present study, however, the great decline in milk yield seemed to have counteracted a reduction in milk protein. The increase in milk urea content points towards a lower protein use efficiency as well (Coulon et al., 1998). The decreasing casein-to-protein ratio in the day immediately after transhumance could mean that milk had a higher proportion of soluble proteins, which is usually linked to a higher SCC (Coulon et al., 2002). The increase in SCC was lower than previously observed during long walks of cows (Coulon & Pradel, 1997), probably because cows in the present experiment had shorter walks and were already experienced in walking as they were reared in alpine transhumant systems. Also, lower increases in milk SCC were found in cows that were not primarily infected by minor or major pathogens (Coulon et al., 1998; Lamarche et al.,

2000), which might have been the case in the present study for the primiparous cows. Indeed, the increase in SCC on Day 1 was numerically lower for the primiparous compared with the multiparous cows. The mammary gland of primiparous cows has yet experienced less movement and milking events, and therefore probably presents fewer injuries and opportunities for primary infections (Martin, Stocker, Sauerwein, Buscher, & Muller, 2018). The lower SCC found in individual milk compared with bulk milk can also be related to the primiparous cows as in the individual part of the study half of the cows were primiparous, whereas in typical herds this proportion usually do not exceed 30% of the animals. The present study showed that there is really an immediate but transient impairment of milk quality associated with transhumance and that this lasts for less than 5 days on the new alpine pasture for most variables.

4.1.2. Milk coagulation properties

Alpine grazing is typically associated with impaired milk coagulation properties (Leiber et al., 2006; Niero et al., 2018) and our results were consistent with the latter studies. The enhancement of RCT and a₃₀ during day 1 and 2 was probably related to lower pH values compared with Day -5 and 5. Indeed, this relationship is well known, as well as the relationship between milk coagulation traits and casein content (Tiezzi, Pretto, De Marchi, Penasa, & Cassandro, 2013), even though the latter was not so clear in our study. As already observed in the same breed, parity did not affect milk coagulation properties (Niero et al., 2018). The proportion of individual milk samples that did not coagulate unexpectedly only increased with a delay (Day 5). In general, the proportion is consistent with previous studies on other breeds. Tyrisevä, Vahlsten, Ruottinen, and Ojala (2004) described that 30% of Finnish Ayrshire cows produced NC samples at least once in the lactation, 12.9% of Holstein cows in an intensive system produced NC milk (Toffanin, De Marchi, Lopez-Villalobos, & Cassandro, 2015) and 13.2% of Valdostana cows managed in extensive conditions had NC milk (Niero et al., 2018). Individual milk does not always coagulate because of different characteristics like casein genetic polymorphism (Coulon et al., 2004), but this was not investigated here. Moreover, soluble proteins and enzymes found in milk include plasmin, which is increased at high altitude and with sward maturity (Leiber et al., 2006). Plasmin may hydrolyse casein and impair coagulation through hydrolysis of the micelles (Bhatt et al., 2017). Differences in milk gross composition between NC and C samples in the present study confirmed that high SCC and slow coagulation are associated with each other (Coulon et al., 2004; Niero et al., 2018). Only the link between coagulation ability and BHB concentration is unclear and may be indirect as Sundekilde, Poulsen, Larsen, and Bertram (2013) showed that BHB concentration is usually increased in milk with high SCC. The pH was slightly higher in NC compared with C samples. This may have been sufficient to slow down coagulation as pH is inversely related to RCT (Shalabi & Fox, 1982). In general, the comparison of the results obtained with milk from individual cows and from bulk milk show that much of the individual variation is counterbalanced.

4.1.3. Cheese

The gross composition and minerals of Fontina cheese reported in the present study were consistent with the data reported by Manuelian, Currò, Penasa, Cassandro, and De Marchi. (2017) in a study aimed to characterise chemical composition, mineral, fatty acid and cholesterol content of more than 100 commercial cheese samples. Unlike our hypothesis, day of transhumance hardly affected compositional and sensory properties of the cheeses. First, unlike milk fat, fat content in the cheese dry matter did not significantly increase the day right after transhumance, but actually

followed the evolution of the fat-to-protein ratio, which was consistent with previous observations (Coppa et al., 2011; Walstra, Wouters, & Geurts, 2006). Unexpectedly, the high SCC in the milk of Day 1 was not associated with a higher level of proteolysis in the cheeses. A high SCC is known to induce an increase in soluble proteins and enzymes in milk, due to a mammary gland dysfunction that is responsible for the transfer of compounds from the blood (Coulon et al., 2004). In this context, the presence of plasmin and the higher moisture content of the cheeses manufactured from high SCC milk is usually associated with an increase in proteolysis (Coulon et al., 2004). Nevertheless, the reduced primary proteolysis (WSN-to-N ratio) in morning cheeses from Days 1 and 2 was in line with the concomitantly decreased melting texture and increased hardness highlighted by the sensory panel.

In the evening cheeses, changes opposite to that were found in these variables. A more intensive proteolysis, which degrades the protein matrix, results in a weaker cheese structure (Bugaud, Buchin, Noel, Tessier, & Pochet, 2001). In regards cheese taste, direct effects of days in relation to transhumance were observed by the sensory panel (and not through analytical methods). Sensory characteristics are human responses to perception of stimuli that are experienced with the cheeses (Delahunty & Drake, 2004). The interaction between tongue receptors, flavour perception and actual cheese composition is a complex phenomenon. In the present study, panellists found cheeses from Day 1 to be more sour, which could be partly explained by the numerically lower proteolysis at that time, but might also have resulted from the acidification process or other multifactorial effects. The slightly higher pH in the 24 h old cheeses found on Day 1 suggests a slower acidification in the moulds, which could result from poorly drained cheeses. The arrival at a new manufacturing room with a low temperature in the first evening affects the drainage; the lactose content of such fresh cheeses is then higher and induces post acidification, and therefore more acidic ripened cheeses. When moving to the high altitude pastures, changes in the microbial environment may also play a role. Some other defects in taste were observed by the sensory panel across all cheeses and could not be linked to the day of transhumance. Therefore, they were more probably related to the different farms' typical environments, as previously underlined by Giannino, Marzotto, Dellaglio, and Feligini (2009) for production of Fontina cheese. Eventually, the general improvement in visual appearance of the cheeses 2 and 5 days after transhumance associated with yellower paste in the cheese's core may be due to the younger phaeological stage of the fresh grass offered after transhumance, leading to a higher carotene incorporation into milk and cheese (Nozière et al., 2006).

4.2. Differences between morning and evening milk and cheese manufactured therefrom

In the present study, milk fat content and SCC decreased when the preceding milking interval increased, as previously shown by Rémond, Pomiès, Julien, and Guinard-Flament (2009). The similarity in coagulation properties of morning and evening milk confirms earlier findings (Niero et al., 2018). The guidelines of Fontina cheese production specifically demand that this cheese has to be prepared from each milking and not after collecting milk of one or more days. Although the differences in composition and properties of morning and evening milk are well-documented, the consequences on the resulting cheeses have received little attention so far. Martin, Pomiès, Pradel, Verdier-Metz, and Rémond (2009) found only very small effects of milking frequency (once or twice daily) on cheeses properties, but this did not include a comparison of cheeses from morning and evening cheeses. In the present study, it seems that the higher fat content and fat-to-protein ratio of the

evening milk led to cheeses with higher fat content. Accordingly, evening cheeses were softer and melt more in the mouth, which is concomitant with a lower mechanical resistance to fracture stress (Bugaud et al., 2001). However, this phenomenon was not related to the other important factors influencing texture, namely dry matter content and primary proteolysis. Therefore, it could also be partly due to milk fat composition. Indeed, milk from the evening has a lower content of mostly saturated de novo synthesised fatty acids (e.g., C16:0) and is richer in C18:1 *cis* 9 than that from the morning, which increases fat fluidity due to their respectively low and high melting points (Ferlay et al., 2010; Lerch et al., 2015). This leads to softer cheeses (Hurtaud et al., 2001). Fatter cheeses with a softer fat content may have a more pronounced oiling-off during processing and thus reflect light (Lerch et al., 2015). This might explain the brighter appearance of the evening cheeses compared with the morning cheeses in the present study. Eventually, it also seems that transhumance enhanced the general morning-evening difference in cheese texture.

5. Conclusions

The present study confirmed that alpine transhumance influences milk quality on the day after walking to the new alpine pasture, and our results provide first indications that allow quantification the short-term changes in milk during this transition period. Almost all changes disappeared after 5 days. Results on bulk milk and cheese properties were not as distinct as those found in milk of individual cows. It seems that the time of milking and manufacturing of the cheeses (morning or evening) influences cheese properties more than the actual day of transhumance. Nevertheless, walking transhumance may indirectly increase the difference between morning and evening cheese texture in the days after the transition. Being aware of these differences in milk quality, farmers could adapt their transformation process during the days following transhumance to optimise production. Further studies should investigate the role of the severity of the transhumance event for the cows, such as duration, steepness and climate during the walk, as well as production environment and possible microbial problems causing faulty cheese qualities, especially taste, during transhumance.

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