INDIGENOUS COW BREEDS IN TRANSHUMANT MOUNTAIN GRAZING SYSTEMS

Madeline Koczura

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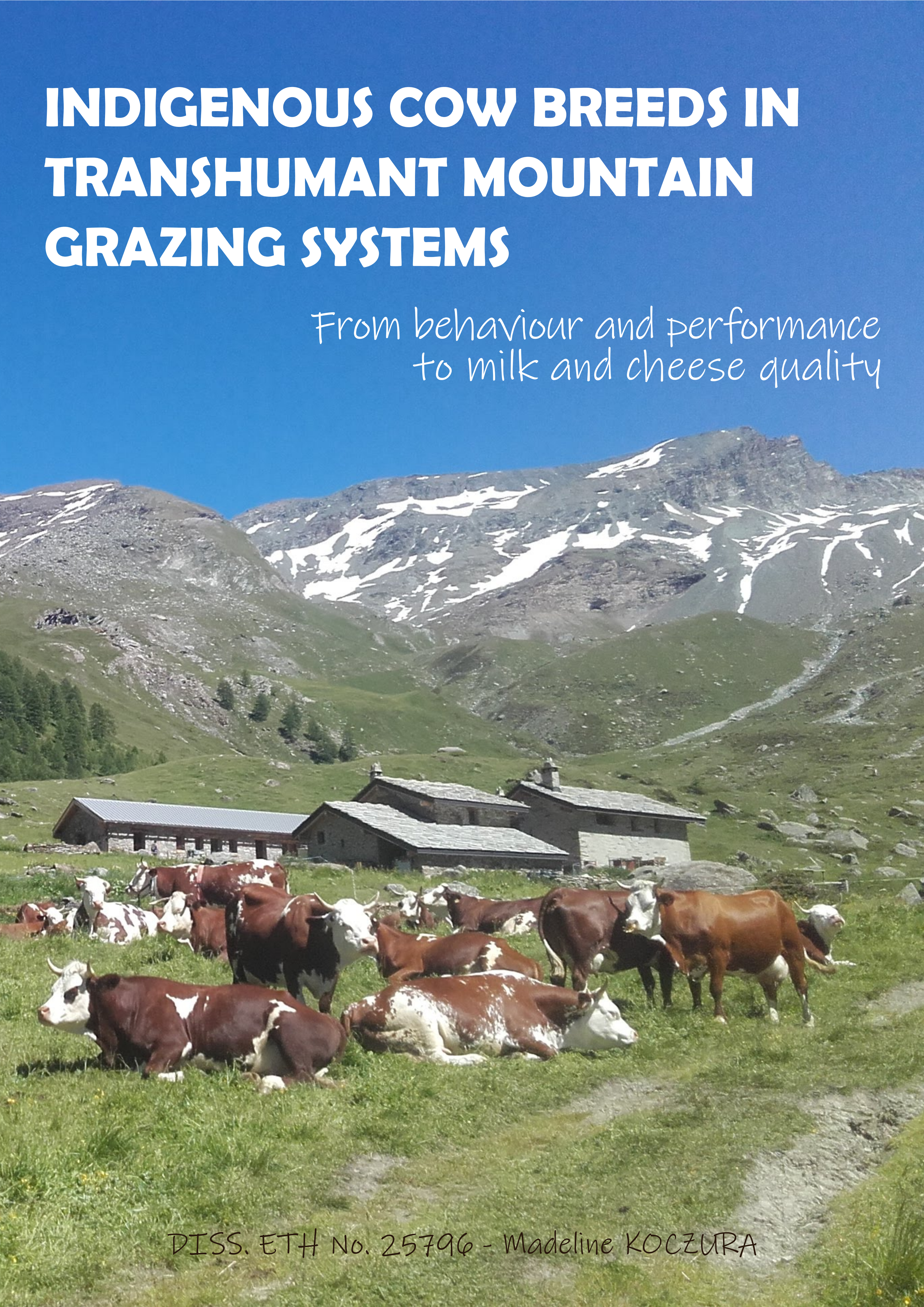
From behaviour and performance to milk and cheese quality



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INDIGENOUS COW BREEDS IN TRANSHUMANT MOUNTAIN GRAZING SYSTEMS: FROM BEHAVIOUR AND PERFORMANCE TO MILK AND CHEESE QUALITY.

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Presented by

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List of abbreviations

a_{30}	Curd firmness 30 min after rennet addition
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
Alp	Mountain transhumant dairy farm
ANA.Bo.Ra.Va.	National Association of the Breeders of Valdostana Red Pied breed
Asp	Asparagine
BHB	β-hydroxybutyrate
BW	Body Weight
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1 *cis* 9	Oleic acid
C18:1 *trans* 11	Vaccenic acid
C18:2 n-6	Linoleic acid
C18:3 n-3	Alpha-linolenic acid
Ca	Calcium
CAP	Common Agricultural Policy
CLA	Conjugated Linoleic Acid
CMP	Caseinomacropeptid
CN	Casein Number
CP	Crude Protein
DIM	Days In Milk
DM	Dry Matter
EU	European Union
FA	Fatty acid(s)
FAO	Food and Agriculture Organisation
Glu	Glutamate
H_{22}	Pasture with High botanical diversity and high slope (22°)
H_7	Pasture with High botanical diversity and low slope (7°)
HI	Highland
Ho	Holstein
IAR	Institut Agricole Régional
IS	Jacob's Indice of Selectivity
k_{20}	Curd-firming time (until 20 mm)
L	Control pasture with Low botanical diversity
LFA	Less Favoured Areas
LO	Lowland
m a.s.l.	Meters above sea level
MCP	Milk Coagulation Properties
Met	Methionine
Mo	Montbéliarde
MUFA	Mono-Unsaturated Fatty Acids
MUN	Milk Urea Nitrogen
MY	Milk Yield
MY_{pot}	Potential Milk Yield
ΔMY	Difference between MY and MY_{pot}

N	Nitrogen
n-3	Omega 3
n-6	Omega 6
NaCl	Sodium Chloride
NC	Non-coagulating
NDF	Neutral Detergent Fibre
NEFA	Non Esterified Fatty Acids
NE_L	Net Energy for Lactation
OMD	Organic Matter Digestibility
P	Phosphorus
PDIE	Absorbable protein at the duodenum according to supply with fermentable energy and rumen undegradable protein
PDIN	Absorbable protein at the duodenum according to supply with rumen degradable protein and rumen undegradable protein
PDO	Protected Designation of Origin
Phe	Phenylalanine
PSC	Plant Secondary Compounds
PTSN	Phosphotungstic acid-soluble nitrogen
PUFA	Poly-unsaturated Fatty Acids
RCT	Rennet Coagulation Time
SCC	Somatic Cell Count
SCS	Somatic Cell Score = $3 + \log_2(SCC/100\,000)$
SD	Standard Deviation
SN	Soluble Nitrogen
UV	Ultra Violet
Va	Valdostana Red Pied
Z1	Lower zone of the mountain pastures, zone 1
Z2	Middle zone of the mountain pastures, zone 2
Z3	Upper zone of the mountain pastures, zone 3

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Summary

Because of the lack of arable lands and the compromised use of mechanical engines, dairy production is predominant in the European mountains. Local actors made the choice to develop tradition and local knowledge, focusing on the *terroir* concept. Transhumance from the lowlands to the highland pastures is a tradition, and a key element allowing valorisation of the local forage resources through highland grazing. In such systems, mostly autochthonous cow breeds are used, because of their presumed robustness and adaptation to the system, and are often prescribed in Protected Designation of Origin (PDO) product specifications. In the frame of a need for improved sustainability of agricultural systems, the importance of these indigenous breeds in the valorisation of highland pastures for production of high quality dairy food still needs to be better understood. The investigation of their level of adaptation in terms of behaviour, performance, milk quality etc. compared to specialised dairy breeds is necessary.

Therefore, the present doctoral thesis aimed at experimentally investigating these aspects on the short (around transhumance events) and medium term (season), by researching the autochthonous Valdostana Red Pied (Va) breed. First, the qualification of the adaptation of Va to their mountainous environment through behaviour, performance and milk quality was performed, in order to compare them with more specialised dairy breeds (namely, Montbéliardes (Mo) and Holstein (Ho)) on mountain pastures. Then, an on farm study initiated by local farmer's empiric observations and the literature aimed at identifying the direct effects of walked transhumance on milk composition and locally produced cheese (Fontina) quality. Afterwards, a comparison with truck transportation and more specialised dairy breeds was performed.

Our experiments with Va in their native environment demonstrated that, even though they are indigenous cows, they are also adversely affected to some extent by transhumance and highland grazing. Milk yield (MY) of Va decreased while grazing in the highlands more than expected from progressing lactation alone. The rennet coagulation time (RCT) of their milk was higher in the highlands, leading to an increased number of non-coagulating samples. This poorer ability to coagulate in the highlands was linked to an increased somatic cell count (SCC). Age or previous site-specific experience of Va cows did not cause a better performance in their local mountain environment, but seemed to decrease their susceptibility to develop clinical mastitis. When moved to a new biodiverse mountain pasture and compared with more specialised dairy breeds, Va decreased their MY by the same proportion than Ho and Mo. Only few differences in diet selection were observed, with Ho showing the highest preference for grasses and Va being indifferent to forbs. However, it remained unclear if this was linked to the genotype, the previous experience or the interaction between both. Regardless of breed, on a steep slope, cows stayed lower and selected forbs, whereas on a low slope, they went up and selected grasses. These results question the choice for trade-off made by cows according to their breed, and highlight the high importance of understanding the interaction between genotype, experience and environment in cow's behaviour and performance.

In the days around walked transhumance, the milk of Va cows was impaired in the same way in their local environment as in experimental conditions on a new mountain pasture. Milk had a higher fat content and SCC. These changes in composition did not affect the milk coagulation properties and barely had effects on the quality of Fontina cheese, which suggest that the difficulties encountered around transhumance in cheese manufacturing are not due to the cows or the quality of their milk, but rather to the changing environment, and could be counteracted by an adaptation of the process. Besides, transportation by truck had the same consequences on milk quality than walk, regardless of breed, even though it did not lead to a MY decrease the evening after in late-lactating cows. In general, Va and Mo showed better milk coagulation properties than Ho.

In conclusion, the direct link between indigenous cow breeds and a better valorisation of mountain pastures is more complex and difficult than expected. The local adaptation of the cows might be of higher importance than its breed. Further experiments must investigate broader criteria (such as reproduction performance) and long term aspects, taking into account the coevolution of the breeds with their native environment. Research must focus on determining the adequacy of the animal for its native environment to support future choices for genetic selection, sustainable dairy systems or even PDO policies. This would lead to the optimisation of local forage resources in high quality dairy products, a higher autonomy and therefore better social, economic and environmental sustainability of mountainous dairy production systems.

Résumé

En raison du manque de terres arables et des difficultés de mécanisation, la production laitière est prédominante dans les montagnes européennes. Dans ces zones, les acteurs locaux ont souvent fait le choix de développer les produits de *terroir* qui valorisent la tradition et les savoir-faire locaux. La transhumance des vallées vers les alpages est une pratique ancestrale et permet de valoriser les ressources fourragères d'altitude. Les vaches utilisées dans ces systèmes sont souvent des races autochtones réputées robustes et bien adaptées aux systèmes locaux. Elles sont souvent privilégiées dans les cahiers des charges des produits bénéficiant d'une Appellation d'Origine Protégée (AOP). Dans un contexte où l'amélioration de la durabilité de la production est devenu un enjeu majeur, la place des races locales pour la valorisation des alpages et pour la production de produits laitiers de qualité doit être mieux comprise. Cela nécessite la mise en place de travaux de recherche concernant leur adaptation au contexte local, sur les plans de leur comportement, de leurs performances, de la qualité de leur lait, etc. comparativement à des races laitières spécialisées.

La présente thèse de doctorat visait donc à explorer expérimentalement ces aspects à court (autour de la transhumance) et à moyen terme (à l'échelle de la saison) en utilisant la race autochtone Valdôtaine Pie Rouge (Va) comme modèle. Les premiers travaux ont permis de caractériser le comportement, les performances et la qualité du lait de la race Va lorsqu'elle est conduite sur des pâturages de montagne, comparativement à des races laitières plus spécialisées (Montbéliardes (Mo) et Holstein (Ho)). Ensuite, une étude dans des fermes locales a été mise en place pour identifier les effets de la marche lors de la transhumance sur la composition du lait et la qualité du fromage local (Fontina). Enfin, une comparaison des effets de la marche avec le transport en camion a été réalisée avec des vaches de race Va et des races laitières plus spécialisées.

Nos travaux menés sur les vaches Va conduites dans leur environnement natif ont bien démontré que bien qu'elles soient indigènes, ces vaches sont affectées à la fois par la transhumance et par l'alpage. Leur production laitière (PL) a diminué au cours de la saison d'alpage de façon plus accentuée qu'attendu, compte tenu de l'avancement de leur stade de lactation. La coagulation enzymatique de leur lait a été rallongée en alpage où le nombre de laits qui ne coagulaient pas était plus élevé. Cette moindre aptitude à la coagulation en alpage était liée principalement à l'augmentation des comptages en cellules somatiques (CCS) observée. La parité ou l'expérience antérieure de l'alpage n'ont pas modifié les performances des vaches Va conduites dans environnement classique mais ont eu tendance à réduire leur susceptibilité au développement de mammites cliniques. Lorsqu'elles étaient déplacées sur des nouveaux pâturages de montagne où elles étaient comparées à des vaches plus spécialisées, la baisse de PL observée au cours de la saison de pâturage pour les vaches Va a été similaire en proportion à celle des Ho et Mo. Les différences raciales de choix alimentaire au pâturage ont été faibles ; les Ho ont cependant montré une préférence accrue pour les graminées alors que les Va ont consommé indifféremment les plantes diverses. Cependant, il était difficile de savoir si ces choix différents sont liés au génotype, à l'expérience antérieure ou à l'interaction entre les deux. Indépendamment de la race, sur une parcelle avec une forte

pente, les vaches sont restées sur les parties les plus basses et ont consommé des plantes diverses alors que lorsque la pente était plus faible, les vaches ont préféré monter sur les parties hautes pour sélectionner des graminées. Ces résultats montrent que les compromis réalisés par les animaux semblent peu dépendre de la race et ils soulignent l'importance de comprendre l'interaction entre le génotype, l'expérience antérieure et l'environnement pour expliquer le comportement et les performances des vaches.

Dans les jours suivant la transhumance, le lait des vaches Va a été modifié de la même manière lorsque les vaches marchaient pour aller sur un nouvel alpage ou lorsque la marche était reproduite en conditions expérimentales. Le lait avait alors un taux de matières grasses et des CCS plus élevés. Ces modifications de composition n'ont pas altéré la coagulation du lait et ont eu des conséquences faibles sur la qualité de la Fontine. Ce résultat suggère que les défauts de qualité classiquement rencontrés par les fromagers lors de la transhumance ne sont pas liés aux vaches ou à la qualité de leur lait mais plutôt au changement de locaux. Ils pourraient être évités grâce à une adaptation des procédés de transformation. En outre, le transport en camion a eu des effets similaires à ceux de la marche, quelle que soit la race. Il n'a cependant pas entraîné de diminution de la PL, contrairement à la marche. De façon générale, le lait des vaches Va et Mo avait une meilleure aptitude à la coagulation que celui des vaches Ho.

En conclusion, l'intérêt supposé des vaches de race locale pour la valorisation des alpages n'est pas direct ; il est plus complexe à mettre en évidence qu'attendu. A l'échelle de la saison, l'adaptation des vaches aux conditions locales semble plus importante que la race en tant que telle. Des travaux conduits à plus long terme devront prendre en compte des critères plus larges (incluant la santé ou les performances reproductives) et s'intéresser à la coévolution des races avec leur environnement natif. Définir l'adéquation des animaux avec leur environnement semble prioritaire pour choisir les futures orientations en matière de sélection génétique, de développement de systèmes laitiers durables ou de cahiers des charges des AOP. Ces orientations permettront d'optimiser la transformation des ressources fourragères locales en produits laitiers de qualité, d'améliorer l'autonomie et la durabilité sociale, économique et environnementale des systèmes laitiers de montagne.

Riassunto

Le zone montane europee sono caratterizzate da un'orografia tale da rendere scarsamente praticabile la meccanizzabilità di larga parte delle superfici agricole. Conseguentemente, i pascoli coprono la quasi totalità delle aree agricole e risultano utilizzati da ruminanti. La migliore valorizzazione di tali aree agricole è legata alla produzione lattiero-casearia. Le filiere lattiero casearie di montagna hanno scelto di sviluppare la tradizione e le conoscenze locali come elemento caratterizzante il valore aggiunto dei loro prodotti attraverso la tutela del loro consolidato legame con il *terroir*. La transumanza dalla pianura ai pascoli d'alpeggio è parte integrante di tale tradizione ed un elemento chiave che permette di valorizzare le risorse foraggere locali attraverso il pascolo di alta montagna. In sistemi transumanti di montagna si allevano prevalentemente razze bovine autoctone per la loro presunta robustezza e il loro adattamento al sistema. Inoltre il loro utilizzo è spesso prescritto dai disciplinari di produzione dei formaggi a Denominazione di Origine Protetta (DOP). Il ruolo delle razze autoctone nella valorizzazione dei pascoli d'alpeggio per la produzione di prodotti caseari di alta qualità richiede ulteriori approfondimenti. È quindi necessario studiare il loro livello di adattamento rispetto alle razze cosmopolite specializzate da latte.

Nello specifico la presente tesi di dottorato ha indagato sperimentalmente il comportamento, le performance produttive e la qualità del latte della razza autoctona Valdostana Pezzata Rossa (Va) nel breve (durante gli eventi di transumanza) e medio termine (durante la stagione di monticazione). In primo luogo, è stata eseguita la qualificazione dell'adattamento della Va all'alpeggio attraverso lo studio del comportamento, il monitoraggio della produzione e della qualità del latte, in confronto con le razze da latte più specializzate (Montbéliardes (Mo) e Holstein (Ho)). Successivamente, è stato eseguito uno studio volto ad identificare gli effetti diretti della transumanza tradizionale (a piedi) sulla composizione del latte e sulla qualità del formaggio prodotto localmente (Fontina DOP). Infine a seguito della recente crescita dell'uso di mezzi motorizzati di trasporto per la transumanza, è stato effettuato un confronto sugli effetti della transumanza a piedi rispetto al trasporto su camion, sempre comparando la Va con razze specializzate da latte.

Gli esperimenti condotti con vacche della razza Va nel loro ambiente d'origine hanno dimostrato che, pur essendo animali rustici ed abituati a importanti spostamenti a piedi per la monticazione in alpe, le loro performances sono comunque influenzate dalla transumanza e dal pascolo d'alpeggio. La produzione di latte (MY) delle Va è diminuita successivamente alla monticazione in alpe, ben più di quanto ci si potesse aspettare da una normale progressione della lattazione. Anche le caratteristiche qualitative del latte sono state influenzate dalla transumanza. In particolare, il tempo di coagulazione (RCT) del latte è aumentato in alpeggio, con un numero più elevato di campioni non coagulanti entro il tempo standard di analisi. La minor attitudine casearia in alpeggio è stata associata ad un parallelo aumento del numero di cellule somatiche (SCC). L'età o la precedente esperienza in alpeggio delle Va non ha migliorato le prestazioni in alpeggio, ma sembra diminuire la loro suscettibilità a sviluppare una mastite clinica. Quando sono state trasferite in un pascolo con un'alta biodiversità e confrontate con razze da latte più specializzate, le Va hanno subito un calo percentuale della

loro produzione simile alle Ho e Mo. La transumanza in camion ha avuto le stesse conseguenze sulla qualità del latte che la transumanza a piedi, indipendentemente dalla razza, con l'unica differenza di una perdita di produzione trascurabile la sera successiva al trasporto nelle vacche in tarda lattazione, rispetto alla transumanza a piedi. Le Va e le Mo hanno mostrato delle proprietà di coagulazione del latte migliori rispetto alle Ho. Sono state osservate anche poche differenze nella selezione al pascolo: la composizione dei patches delle Ho ha registrato una preferenza maggiore per le Gramineae, mentre le Va hanno espresso un minor rifiuto per le dicotiledoni non leguminose. Tali differenze nel comportamento alimentare possono in parte essere legate al genotipo, in parte a esperienze pregresse legate a divergenti abitudini a tecniche di pascolamento che consentono l'espressione più o meno marcata della selezione. Indipendentemente dalla razza, su un pendio ripido, le vacche sono rimaste più tempo nelle parti pianeggianti alla base del pendio selezionando le div, mentre su un pendio con pendenza minore, hanno preferito esplorare le parti pendenti del pascolo alla ricerca di graminacee. Questi risultati evidenziano l'importanza della comprensione dell'interazione tra genotipo, esperienza e ambiente nel comportamento e nelle prestazioni delle vacche da latte.

La tipologia di pascolo o di ambiente montano durante la transumanza non hanno avuto effetti differenti sulle performance o sulle caratteristiche del latte. A seguito della transumanza a piedi la qualità del latte delle vacche Va è peggiorata nello stesso modo sia in condizioni aziendali in pascoli conosciuti dagli animali, sia sperimentali in un nuovo pascolo. Il latte in giorno seguente la transumanza ha registrato un contenuto di grasso e di cellule somatiche più elevato. Questi cambiamenti di composizione hanno influito in maniera trascurabile sulle proprietà di coagulazione del latte e non sono stati direttamente associabili ad alterazioni del profilo sensoriale del formaggio Fontina DOP. Rallentamenti nella coagulazione e difetti sensoriali sono comunque stati registrati a seguito delle monticazioni in condizioni aziendali. I risultati dei presenti esperimenti suggeriscono che l'origine di tali problematiche sia da ricercare maggiormente in un cambio dell'ambiente di trasformazione del latte con la salita in alpe, piuttosto che a una differente composizione del latte o un'alterazione dello status degli animali.

In conclusione, il legame diretto tra le razze bovine autoctone e una migliore valorizzazione degli alpeggi è legato a dinamiche complesse e parzialmente ancora non note. L'adattamento alle condizioni locali da parte delle vacche potrebbe avere una maggiore importanza rispetto alla loro razza. Ulteriori studi saranno necessari per indagare i sistemi di alpeggio per valutare gli effetti più a lungo termine (come ad esempio le prestazioni riproduttive) e tenendo conto della coevoluzione delle razze con il loro ambiente di origine. In futuro la ricerca potrà indirizzarsi sulla misurazione del livello di adeguatezza dell'animale per il suo ambiente d'origine per orientare le scelte in materia di selezione genetica, considerando anche la sostenibilità dei sistemi lattiero-caseari, specialmente se in filiere DOP. La selezione verso animali più adatti ai territori montani porterebbe all'ottimizzazione dell'utilizzo delle risorse foraggiere locali e della loro trasformazione in prodotti lattiero-caseari di alta qualità, garantendo una maggiore autonomia e quindi uno sviluppo sostenibile in termini sociali, economici e ambientali delle zone rurali e dei sistemi di produzione di montagna che le caratterizzano e rafforzerebbe il legame con il *terroir* d'origine.

CHAPTER I
Mountains and agriculture

"Working with researchers, we learned that tradition and modernity are not contradictory and should be intertwined. What matters is respecting life, respecting consumers, respecting the purpose of all things."

(Maxime Viallet)

A) Mountain agriculture: specificity, context and challenges

1. Definition of a mountainous area

1.1. Geographical definition

Geographers have always been referring to "mountains" in several different ways (Bras et al. 1984). From a strictly structural point of view, this terminology obviously refers to an elevated territory circled by peripheral valleys, or protruding reliefs. However, "mountain" also defines a global environment: the latter morphological characteristics, but also specific landscapes and people living there, their activities, lifestyle and difficulties. It appears that the definition of a mountainous area is quite unclear and subjective, and varies between regions, countries or even culture and history. The peculiarities of such an environment had to be officially defined, for further recognition in agriculture.

1.2. Administrative and political definition

Since 1985, mountainous areas are administratively defined by altitude and slope thresholds in the "Loi Montagne" in France (Lascoumes, 1995), but also by liabilities linked to a low potential for land use and increased production costs. Indeed, mountainous areas combine high altitude, harsh climatic conditions, protruding reliefs and decreased mechanisation possibilities (Martin et al. 2014). Through this definition, the disadvantageous conditions for the development of agriculture were explicitly recognised (Table I.1.).

Table I.1. Strengths, weaknesses, opportunities and threats of mountain for the development of agriculture (based on Santini et al. (2013), Martin et al. (2014), Lauber et al. (2014)).

Strengths	**Weaknesses**
Water resource (for cattle, food processing)	High altitude (500 to 1000 m a.s.l. minimum)
Autochthonous diversity of cattle breeds	Climate (increased number of days of frost)
Development of dairy industries	Decreased vegetation period
Biodiversity of mountain pastures	Need of important forage stock
Environmental externalities	Topography (slope 15–25%)
Low level of inputs	Compromised use of mechanical engines
Traditional know-how	Increased collection and transport costs
Winter and summer tourism	Low labour productivity
Opportunities	**Threats**
Increasing interest of consumers for *terroir*	Isolation → migration to urban areas
Development of tourism	Global warming inhibiting winter tourism
Development of niches markets	Growing interest of consumers for veganism
Resilience of pastures to climate change	Attractiveness → excessive urbanisation

In Europe, 14 states out of 27 members have mountainous areas. They represent 40% of the territory and 18.5 % of the total European Union (EU) area (Santini et al. 2013). That is why the EU Common Agricultural Policy (CAP) also recognised the specificities of those territories (article 18.1 of EC N° 1257/1999). In 1951, the Swiss law on agriculture stated that it was mandatory to take into account the difficulty of mountain conditions (Lauber et al. 2014). In the EU, a compensation for natural handicaps (Less Favoured Areas, LFA) was implemented (Santini et al. 2013). In France, farms in the mountains received 43 % more financial support than farms in the lowlands in 2011 (Martin et al. 2014). From then on, national and global measures were developed in order to help mountain territories and agriculture, with the EU playing a huge role in them. The combination of strengths and weaknesses of mountain territories does not only lead to limitations, but also to several opportunities for the development of such regions (Table I.1.). For instance, altitude and climate allowed the development of summer and winter tourism, the second main activity of mountain regions, which can work sometimes in synergy, sometimes in an antagonistic way with agriculture (Santini et al. 2013).

2. Mountain agriculture

2.1. Importance of livestock systems

Due to the previously described weaknesses and threats, farmers in the mountains had fewer production options than in the lowland areas. In the 1950's, agriculture in the lowlands faced a fast and global modernisation. Productivity increased, and the gap between the lowlands and the highlands widened, due to the lack of competitiveness of mountainous systems. Indeed, the land was parcelled up, and soils are heterogeneous and mostly not arable (Martin et al. 2014). This situation led to high production costs, more work and less income. Indeed, even though production costs per livestock unit are similar in the mountains and in the lowlands, lower yields in the mountains result in higher production costs per unit of product (Santini et al. 2013). In addition, there are higher transport and collection costs, as well as higher processing costs (due to small structures). In reaction to these conditions and to counteract the migration of people to more urbanised areas, mountain regions promoted the use of specific resources of their territories, privileging quality over productivity and emerging the *terroir* concept (Martin et al. 2014). The latter combines notions of local habits, tradition and typical characteristics of regional products. In the *Beaufortain* massif for instance, farmers, researchers and technicians worked together in order to adapt to the market and maintain food production in the mountains. They aimed at optimising their systems without losing sight of tradition and local knowledge (Exhibition "Milking machine on alpine pastures: 30 years of technology, reflexion and cooperation", Beaufort, France, 2004).

Because of the lack of arable lands and the evolution of mountain agriculture, animal products are thus predominant in the European mountains nowadays (54 % of the total turnover are coming from livestock activities). Among these, the dairy and grazing livestock meat sectors represent 28 % and 16 % of the total income of European mountains, respectively (Santini et al, 2013). All these production systems are related to ruminants, as monogastric animals are less present in the mountains than in the lowlands. Indeed, a significant proportion of sheep and goat products (34 % of milk and 25 % of meat) is produced in mountainous regions, while bovine milk and meat products represent 9.5 % and 12 % of the global EU production, respectively (Santini et al. 2013). Ruminant's feed is largely obtained from local pastures and forages (hay and grass silage). In the Alps and central Europe, it has been estimated that 70 % of the feed of dairy cows comes from mountain areas (Santini et al. 2013). Mountain pastures are the main forage resource of mountain livestock systems, and a key element to them.

2.2. A focus on mountain pastures

The omnipresence of pastures and their higher botanical diversity, compared to the lowlands, can actually be considered one of the biggest strength of the mountain agricultural system. Besides being the main local forage resource, permanent grasslands also are reservoirs of biodiversity and produce fundamental positive externalities. Each grassland is unique with up to 80 different plant species. This diversity is linked to a decrease in proportion of grass species coupled with an increase in dicotyledonous species such as those from the *Asteraceae, Rosaceae* or *Plantaginacae* families (Jeangros et al. 1999). Not only the botanical diversity is enhanced and preserved, but also that of pollinating insects, which are attracted by plant secondary metabolites (Farruggia et al. 2008). The list of ecosystem services provided by permanent grassland is even larger: conservation of genetic resources, water flow regulation and landscape maintenance (Battaglini et al. 2014) but also prevention of soil erosion (Zhu et al. 2015). Grasslands also play a role in carbon sequestration, but the latter fluctuates depending on soil depth, altitude, temperature and precipitation (Garcia-Pausas et al. 2007). Although, when modelling a 100 % forage self-sufficiency, it has been demonstrated that small-scale dairy farms reduced their greenhouse gas emissions by 28 % in average, thanks to carbon sequestration (Salvador et al. 2017). Eventually, secondary compounds contained in biodiverse pastures (tannins) have been proved to mitigate methane emissions when included in the diet of ruminants (Jayanegara et al. 2012).

2.3. Diversity of mountain dairy farms

Dairy systems can differ considerably from one mountain to another. The case of Switzerland will be exemplified here. There, 465 500 ha (1/3 of the usable agricultural area) are located in the mountains and in 2012, 100 869 dairy cows grazed on mountain pastures. Lauber et al. (2014) were able to identify four different ways to manage a not permanently operated mountain dairy farm, denominated as an *alp* (Table I.2.).

Table I.2. Proportion of the possible combinations of ownership and management of an alp: the case of Switzerland (adapted from Lauber et al. 2014).

	Private alp	Collective or public alp
Individual management	**38.0 %** Mountain pastures and building owned by a farmer using or leasing them to one farm only (Berner Oberland, Luzern, Appenzell, Suisse Romande).	**40.0 %** Mountain pastures owned collectively, building most often owned by a farmer managing its own herd (frequently observed in central and eastern Switzerland, and Valais).
Collective management	**0.5%** Mountain pastures and buildings owned by a farmer, occupied by animals from several farms as a consortium.	**21.5 %** Mountain pastures owned collectively, managed by several farms in consortium, with recruited staff (frequently observed in Grisons).

In the management system, also the milking system can differ. Cows can either be milked in a stable (where they are sometimes also sheltered for the night), or directly on the pasture (where they are kept 24 h a day). The development of mobile milking in the *Beaufort* cheese sector is a good example of the contemporary evolution of mountain dairy systems. In the 1950's, cows were milked by hand on the pasture, and milk pots were carried back to the farm where the cheese was produced. Then, donkeys carried cheeses down to the valley. In the 1970's, mechanical mobile milking was developed in order to decrease the arduousness of this work and make the young generation of farmers want to stay. The aim was to follow the herd by making a motorised milking equipment move, instead of the cows. Then, in the 1990's, technicians and researchers improved the comfort of mobile milking parlours and included the distribution of concentrate. At the beginning of the 2000's, they were able to decrease workload and improve milking and washing conditions, whilst decreasing the time needed to install or move the parlour. Eventually, they managed to mechanise milking, adapt it to their specific mountain pastures and keep their product's quality, in order to stay competitive even with the mountain's limits and constraints (resumed from the exhibition "Milking machine on alpine pastures: 30 years of technology, reflexion and cooperation", Beaufort, France, 2004). Mobile milking is nowadays common in several mountainous regions in France and Switzerland, and in some cases in Italy.

3. The use of local mountain forage resources through transhumance

3.1. Development of transhumant systems

In the mountains, the practice of moving people and animals from the lowlands in winter to the uplands in summer is commonly known as "transhumance". It has always relied on an extensive management of animals with outdoor grazing and the valorisation of natural grassland resources. The anthropologist and historian Anatoly Mikhailovich Khazanov (1994) categorises "seasonal transhumance" as one of the five nomadic forms. The aim of this practice is to follow the herbage growth seasonally, by vertically or horizontally moving the herds accordingly. On one hand, vertical transhumance refers to the successive movements of herds from the valley at the bottom of the mountain to the upland pastures (typical from the Alps). On the other hand, horizontal transhumance refers to the very long journeys of herds from the coasts and lowlands to the mountains. Actually, the seasonal and altitudinal relocation of the herds between two or more established permanent homes differentiate transhumance from nomadism, where people follow an irregular pattern (Evans 1940).

Vertical and horizontal transhumance are animal husbandry systems that have always been widespread in Europe and the rest of the world (Ntassiou and Doukas 2018). Isotope studies of bones of livestock found in the mountains showed early proofs of animals seasonally moved (Costello and Svensson 2018). In the Swiss Alps, several hundred objects from the Neolithic period, the Bronze and Iron Ages and early medieval times were discovered on the Schnidejoch Pass (2756 m a.s.l.). Radiocarbon dating confirmed the early use of this mountain pass, and the combined archaeological and palaeoecological data allowed providing evidence of early mountain pastoralism and vertical transhumance (Hafner and Schwörer 2018). Several other European countries were already concerned by transhumant farming systems in the Neolithic and Middle Age. Such systems strongly depend on the culture and ethnicities and are usually described and considered case by case. In Scandinavian countries, reindeer and cattle breeders moved to the *seter* (common mountain pasture used in summer), which was first mentioned in medieval poems (Sturluson, around 1230). Transhumance was also common in Great Britain: it was mentioned in ancient writings in Wales, southern England, Scotland and Ireland, cited as *booleying* in the Brehon laws, around 1155 (Ginnell 2010). In Switzerland, the *Acta Murensia*, oldest law ruling the collective management of the mountain area dates back to 1160 approximately (Hitz 2009). The entire Mediterranean area also has a strong transhumance history. In Greece, old traditional routes for horizontal transhumance were recorded and mapped (Ntassiou and Doukas, 2018). In southern and central Italy, these transhumant roads were called *tratturi*, and became exclusive for shepherds and regulated under the reign of King Alfonso V of Aragon in 1442 (Wright 2018). They are now being integrated as touristic resource and restored (Meini et al. 2014), and requested to be recognised as Unesco human heritage. Such organised transhumant routes existed with different names according to the region: *canadas* in Castile, *camis ramaders* in eastern Pyrenees, *trazzere* in Sicily etc (Wright, 2018). In Southern France, the ancient network of droving roads (*drailles*) from the coast to the mountains dates back to antiquity (Cleary 1988). With the development of railroads in the 1950's, the transition from winter to summer pastures also evolved. People started to use vehicles and wagons to move animals and this progressively replaced the long walks. Traditional routes are no longer used and animals are more often moved by truck or trains on long distances.

If the history and culture of horizontal transhumance is already well known, the specific ancient history of vertical transhumance is still not so well documented. The early development of the vertical transhumant pastoral system that has shaped the iconic mountain landscapes and the kind of animals used at that time still have to be investigated. Projects on region-wide study of transhumance in the Western Alps are still currently being developed (University of York 2018). Through a multi-disciplinary approach (integrating archaeological, faunal and paleo-environmental archives, including ancient DNA), they might clarify some aspects of the origins of transhumance in the Alps and address the lack of information concerning dairy cattle. Archaeologists aim at examining animal bones from lowland and high altitude sites from French, Italian and Swiss valleys in order to characterise the composition of flocks and herds.

3.2. Shaping current pastures and landscapes by vertical transhumance

Cattle is usually moved in late spring (May) from the lowland pastures to mountain sites in altitude, where the herbage growth is delayed. Animals progressively graze at several different elevations, and then the herd returns to lowland in fall (October). This practice has shaped the mountain landscapes: without animal grazing, upland pastures below 2000 m a.s.l. would actually be just forests. As mentioned earlier, the transhumant systems have to be taken case by case. The development of mountain pastures through transhumance will be exemplified here using the case of the *Beaufortain*, in France. In this massif, grasslands were naturally found at high altitudes (> 2000 m a.s.l., rarely lower except for some special cases such as windy ridge, holes in the forest due to avalanches, or soils that prevent trees from growing). Farmers created “artificial pastures” by deforestation in order to let the herds graze or to produce hay. The majority of deforestation occurred in the 12th and 13th centuries. Creation of upland pastures led to different forage potentials, due to the diversity of soils, slopes, rocks etc. Even at the parcel scale, botanical diversity and forage potential is highly variable. These originally “artificial” pastures were mostly grazed, which led to their conservation. The use of grassland by animals induced a selection of adapted and competitive plants (Farruggia et al. 2008). Shorter stems, buds near the ground, rosettes leaves are strategies adopted by the vegetation in order to optimise nutrients’ uptake, growth rate and organ renewal. Altitude enhanced this selection, because of the decreased oxygen availability and higher UV radiation that also affect vegetation (Kohler et al. 2014). Moreover, trampling, repeated cuts (through grazing) and organic matter input through dejections caused the elimination (or at least limitation) of ligneous, and the diversification of herbaceous species. Lowland pastures, on the contrary, were cultivated. The most productive ones were selected and a lower diversity resulted (resumed from Dorioz 1998).

4. Current challenges for mountain dairy systems

Even though the dynamics of mountain areas is improving since the last decades, they experience different rates of development according to the region (Gløersen et al. 2016). Different projects were implemented at a national or European level in order to clarify the current challenges of mountain agriculture, identify the political actions needed, analyse the profitability, understand the consumers’ point of view and estimate the needs and role of landscapes in mountain grazing systems (AlpFUTUR in Switzerland (Lauber et al. 2014); Euromontana with Euromarc project (www.euromontana.org); Eurobarometer study (ec.europa.eu)). Moreover, several reports produced by the EU analysed the case of mountain areas in order to better define mountain conditions and resulting products. As a first step, according to the Regulation (EU) N°1151/2012, the “mountain product” terminology can now only be used when feed and raw materials come from mountain areas, and if processing is also performed in mountain areas (Santini et al. 2013). However, several other parameters still have to be investigated, taken into account and improved.

4.1. Workload in mountain systems

Despite the latest improvements, the biggest issue in the mountains currently is the workload. Lauber et al. (2014) estimated that the average workload per week in the alp of Switzerland is 86 hours. In an increasing number of mountains, shepherds are no longer families moving from the plain to the upland pastures, but also agricultural workers hired seasonally to group and look after the cattle (Table I.2.). During the summer season, this can lead to social conflicts between owners and staff: actually, 45% of the mountain workers are ready to stop their work in the middle of the summer, most of them because of social conflicts, followed by the immense workload (Figure I.1.). The younger generation is forsaking the previously family-managed highlands, which was highlighted in 2007 by the Swiss national research program on landscapes and habitat in the alpine arc (PNR48). It indeed concluded that many mountain pastures are abandoned and invaded by bushes (Lauber et al. 2014). In other grazing systems where mobile milking was not developed or even not possible, the work and costs to maintain and renovate old farming building are high.

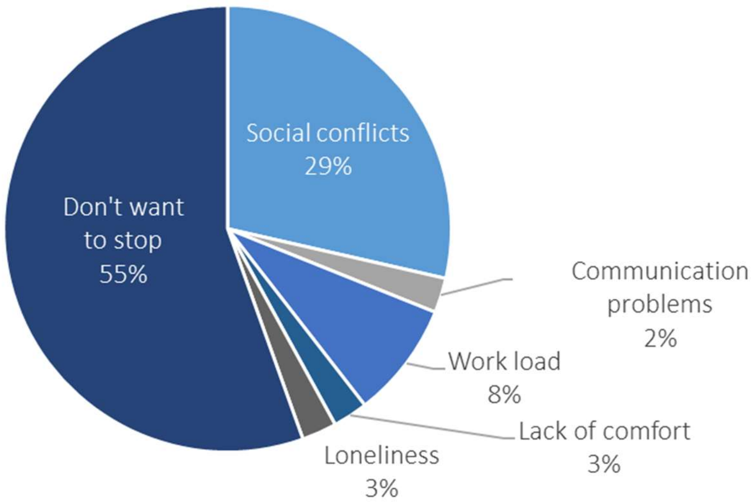

Figure I.1. Proportions of the answers of Swiss alp caretakers on their motives to interrupt their stay during summer (n=119) (adapted from Lauber et al. 2014).

4.2. Biodiversity and environmental sustainability

As underlined in I.2.2., in addition to the fact that mountain grasslands are a local resource for quality forages, they can provide a large range of ecosystem services. Their carbon sequestration capacity, botanical diversity, diversity of flower colours and capacity to host pollinating insects are several examples of different services delivered by such grasslands (Balay et al. 2015). When comparing a high diversity mountain pasture to a more productive and less biodiverse one, Farruggia et al. (2014) indeed highlighted a positive effect of the first one on the abundance of *Lepidoptera, Orthoptera, Heteroptera, Arachnida* and *Coleoptera*. In the mountains, the landscape mosaic between pastures, bushes and low trees is actually very important to enhance this diversity. Indeed, some species of insects or plants do not live in either one or the other, but needs the combination of both habitats to exist (Lauber et al. 2014).

The environmental role of transhumance and mountain pastures were quantified by the Mediterranean Consortium for Nature and Culture. Transhumance facilitates the adaptation to climate change, the creation of carbon sinks in the ground (up to 100 tons of CO_2 per ha), contributes to forest management, fertilises the soil (3 tons of manure daily per 100 cows) and spread seeds over long distances (5 million seeds spread within a radius of 20 km every day per herd). Nowadays, the preservation of this biodiversity and ecosystem services through transhumance and mountain agriculture is thus a huge challenge, in the context of global warming and agricultural intensification.

4.3. Image of mountain products and transhumance

Resulting from the previously described ecosystem services and preserved landscapes, the image of mountain products is an important issue for mountain agriculture. Several studies showed the evolution of the perception of the products by consumers. In 2011, the Eurobarometer study showed that 65 % of the asked panel members had a positive image of mountain products. In France, this proportion is even beyond 80 %. European consumers are nowadays growing interest in local and organic products, low-input systems but are also increasingly questioning animal welfare. Indeed, the demand for sustainable or organic food products is constantly growing, which increases the market potential of high-priced mountain products (Santini et al. 2013). Consumers perceive mountain products as pure, authentic and important for the rural development. Mountain food products are associated with sustainable agriculture (Lauber et al, 2014) and therefore consumers are willing to pay a higher prize for them (McMorran et al. 2015). The Eurobarometer study also highlighted that consumers judge sensorial and nutritional quality of mountain products higher, but their availability insufficient. Thus, from an economic point of view, it is important to understand and preserve the special quality of mountain food products (Martin et al. 2005; Leiber et al. 2005; Farruggia et al. 2014).

Besides, in the Alps, the complete agriculture and tradition is rhythmed by the transhumance. Since the 12^{th} and 13^{th} centuries, a very rich culture was developed around the transhumance practice: songs, books, drawings, engravings, cowbells, decoration etc. Nowadays, the image and culture of transhumance is of very high importance for agriculture and tourism (Magrin et al. 2015). In several mountain areas, the day of transhumance is often associated to a celebration, during which farmers are parading with their decorated cows down from the alps (Aosta Valley, Beaufortain, Valais, Grisons, Bavaria etc.). Moreover, in some regions, transhumance is also strongly associated with other cultural practices such as *Combats de Reines* in the first three previously mentioned valleys. Cows of autochthonous breeds from these mountain regions are by nature warring animals, therefore farmers developed and organised competitions between them. The cultural background behind transhumant systems and mountain products, together with the image of cows grazing in idyllic landscapes, make mountain agriculture a key-element for the touristic sector (Ochsenbein 2017). In the mountains, it is necessary for agriculture and tourism to work in synergy.

4.4. Implications for the political and research context

Policy and researchers investigated the specificity of mountain agriculture and the options to sustain it. Switzerland for example financially supports farming methods that have an ecological or cultural value with direct payments (Bötsch 2004). However, this financial help from agricultural policies might still not be enough in our current context. Indeed, the gap between lowlands and highlands still increases. More specifically, the European Parliament reported in 2016 the contrasts happening already between urban and rural areas in the mountains territories themselves. Actually, the image of mountains as attractive living environments and the growing interest for mountain products led to an increased migration to urban areas at the bottom of mountains, leading to competition between agricultural and residential lands. Agricultural policy must now consider actions to promote integrated value-chains, encourage entrepreneurship, innovation, targeted education and training programs in the mountains (Gløersen et al. 2016). Besides, research must focus on the importance of low-input systems such as mountainous areas for the future. It is important to collect more data and develop new methods that help increase the autonomy of systems, through a better use and valorisation of forage resources. The importance of the local mountain breeds in the latter valorisation and the production of high quality products still needs to be better understood and highlighted. Finally, to develop mountain territories and support future policy decisions, it is important to get a deeper understanding of the role of transhumant farming systems and their economical, ecological and cultural impact.

B) Quality of dairy products from the mountains

1. Prevalence of cheese manufacturing

1.1. Importance of cheese in human nutrition history

Cheese is one of the most ancient manufactured foods on earth. It is difficult to date back with accuracy the first cheeses. They most probably appeared already in the Neolithic era in Europe, central Asia and Middle East (Gerbault et al. 2011). Old drainers in clay from around 5000 years ago were recently discovered in Poland along the Vistula River, and were associated with cheese production thanks to the identification of fatty acids characteristic for ruminant’s milk (Salque et al. 2012). These discoveries highlight the ancient importance of cheese in human nutrition. If babies are able to digest milk thanks to a specific enzyme, the lactase, the capacity to synthesise it disappears when they become adults. However, it was demonstrated that milk consumption led to a genetic mutation inducing persistence of the lactase even in adults (Gerbault et al. 2011). Nevertheless, this mutation needed thousand years to settle in populations, and still not all humans are able to digest lactose (Figure I.2.). In order to valorise milk obtained from ruminants, it was essential in the past and is still currently necessary to transform it through fermentation or coagulation. The first cheese may actually have been discovered randomly while using animals' rumen to transport milk. It had the advantage of being an important source of fat and proteins that could easily be transported.

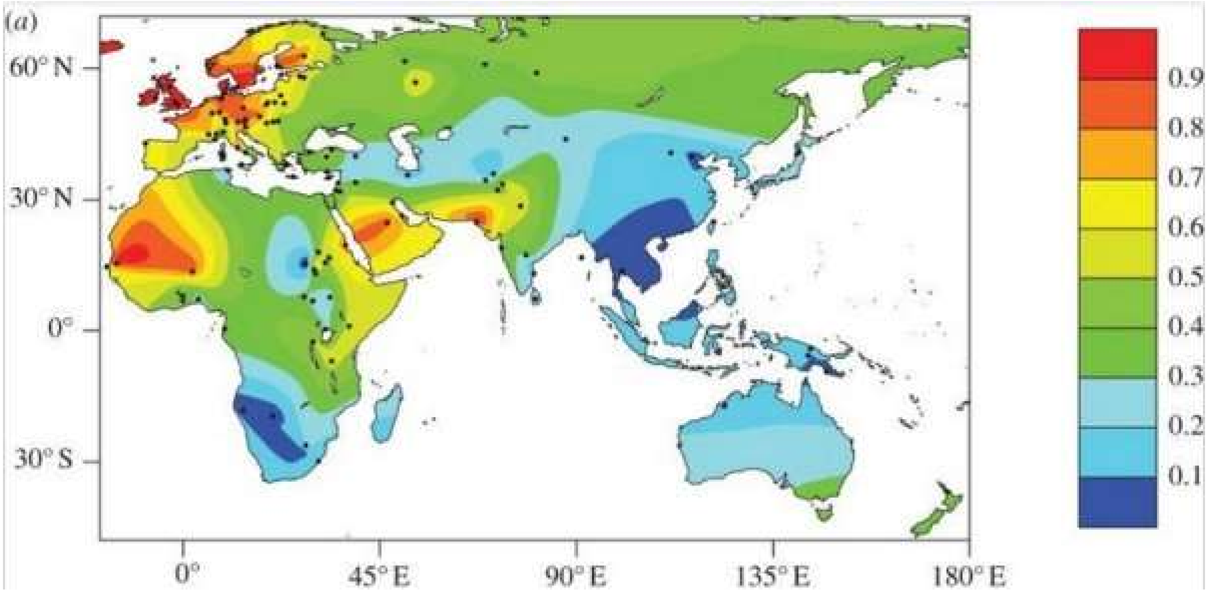

Figure I.2. Frequency of the phenotype “persistant lactase” in Europe, Africa and Asia *(source: Gerbault et al. 2011).*

Cheeses are actually very diverse and, unlike many other foods, biologically and biochemically dynamic (McSweeney 2007). Cheese production consists in two distinct phases: manufacture and ripening. During manufacture, the aim is to dehydrate the matter and concentrate the fat and casein found in milk. The lactose is fermented into lactic acid through bacteria, and often “starter” cultures are added to the milk to enhance this process. After milk has coagulated (thanks to enzymes or acidification), the gel is cut and the pieces express liquid, known as whey. After cooking, the curd is usually separated from the whey and transferred to moulds of different shapes and sizes according to the cheese variety. It is then pressed in order to extract as much whey as possible. Salt is added during the manufacture as a preservative and flavouring agent (most of the time by immersing the cheese in brine after moulding). Subsequently, the cheese is ripened, and during this period the specific flavour and texture are developing. However, the aroma of the finished product is largely pre-determined by the manufacturing process.

1.2. Cheese manufacturing in the mountains: a tradition arising from a need

Because of its convenience for storage and transport, cheese has traditionally always been produced in mountainous regions. In the Swiss Alps, an analysis of chemical residue on pottery shards in several sites across the mountains suggested that people from the Iron Age were already producing and eating cheese there (Carrer et al. 2016). According to Paul Kindstedt from the University of Vermont (Thompson 2007), all cheese types arose from the unique constraints forced by geography and the human efforts to preserve milk. This combination led to traditional cheeses, which are all different according to the region. Hence, in the mountains, farmers started to make cheese for the universal reason of storing and transporting milk. But in transhumant systems, cheese had to be kept up in the mountains until the end of summer. Therefore, it had to be large and durable, to avoid chipping and crackling. This was enabled through a low moisture content. As salt was not found easily in the mountains, the manufacture had to be adapted. Consequently, cheesemakers started to adapt the cutting of the curd, producing very small-sized particles (often called pea-sized or maize-sized grains). They also cooked and stirred the cheese at high temperatures and for long periods, in order to further reduce water content.

The production of cooked and hard cheeses is currently widely developed in the mountains. For instance, in Switzerland, 5200 tons of mountain cheese are yearly produced (Lauber et al. 2014). Globally, mountain areas are characterised by more on-farm processing than lowland ones. Structures are smaller and less modern. Actually, only 65 to 70% of mountain milk production is processed in the mountains in France and Austria, the rest of the milk has to be processed in the lowlands (Santini et al. 2013).

1.3. Milk coagulation as a key step of cheese making

One of the key steps between milk and cheese is milk coagulation, which can be influenced by several parameters. In order to better understand the influence of the various factors, we need to get back to the basics and keep in mind that there are different ways to coagulate milk. Actually, there are three types of coagulation: acidic, enzymatic and mixed.

The aim of the acidic coagulation is to precipitate the κ-casein at its iso-electric point (pHi = 4.6) thanks to *Lactobacilli*. The production of lactic acid by the microorganisms from lactose decreases the negative charges of the micelles. Consequently, the hydration level also decreases, which creates electrostatic repulsion with solubilised Ca and P. The micelles are then unstructured. The curd obtained is not very elastic; the hydrophobic bonds do not have a high mechanical resistance. Moreover, this reaction takes time: between 3 and 24 hours. This type of coagulation is exclusively used for yoghurt production.

The second way to obtain curd for cheese production is enzymatic coagulation, which is much faster than the acidic one. Three kinds of curdling agents can be used: from animal origin (rennet), plant-derived (ficin (fig tree) or bromelain (pineapple) for instance), or enzymes (pepsin or chymosin) produced thanks to genetically modified microorganisms (*E. coli, Bacillus subtilis* or *Aspergillus sp.*). The curdling agents used in PDO transformation are traditionally animal-derived ones, like rennet. The latter is a solution made out of the calves' stomachs, containing a high amount of chymosin (around 520 milligrams of active chymosin per litre) and 10% NaCl. The enzymatic environment is rich in amino acids and some other enzymes such as pepsin (the chymosin/pepsin ratio is usually supposed to be higher than 1.38). There are numerous kinds of solutions characterised by their *force* (the number of litres of milk that curdle with 1 litre of rennet, at 35°C). The enzymatic coagulation can be divided into three parts (Mahaut 2000):

- *Primary phase*: the κ-casein is hydrolysed by chymosin (bond Phe-Met), forming para-κ-casein on one hand and a caseinomacropeptid (CMP) on the other hand.
- *Secondary phase*: this phase starts when 80% of the κ-casein has been hydrolysed (*i.e.* pH ≈ 6.6). Para-κ-caseins form micelles and associate with Ca^{2+} cations, forming electrostatic bounds and Ca^{2+} bridges between Asp and Glu amino acids.
- *Tertiary phase (syneresis)*: the curd firmness is achieved as a result of the casein micelle skeleton contraction, spontaneously delivering the whey. Phosphocalcic bonds are created between the casein molecules. The enzymatic activity will continue during ripening.

The combination of rennet with the natural milk acidification is also possible (the so-called *mixed* coagulation). The large number of potential combinations explains the diversity of cheeses (soft cheese and uncooked pressed cheese more specifically). Indeed, at the end of the coagulation, the chemical and physical properties of the curd induce variations in drainability and final characteristics of the ripened cheese.

Titratable acidity and pH are obviously the first factors influencing coagulation (De Marchi et al. 2009). Milk coagulates faster with a low pH (Shalabi & Fox 1982) and high acidity, which is related to the aggregation rate of para-casein micelles and the reactivity of rennet (De Marchi et al. 2009). Then, caseins and the size of their micelles are of high importance: the aggregation speed increases with casein content, leading to a firmer curd. Furthermore, the genetic variants of caseins play a role on the coagulation properties of milk (Delacroix-Buchet et al. 1993) and will be developed later. The protein fraction of the milk also contains soluble proteins and enzymes originating from blood. Among them, plasmin can impair coagulation by denaturing the micelles (Bhatt et al. 2017). A high somatic cell count of the milk is usually related to a slow coagulation and poor drip-dry (Coulon et al. 2004). Then, a high fat content leads to higher water retention of the gel, with further consequences on dripping. Eventually, the temperature during coagulation also affects the final product by influencing the enzymatic activity. Besides, if milk is kept more than 48 hours in a cold environment, then the saline balance and the structure of micelles change, inducing a higher coagulation time and a softer gel. Actually, the majority of the variation factors of milk coagulation are linked to the animal, its physiology, nutrition, management and breed. The interaction between the animal and its environment must then play a huge role in the quality and specificity of dairy products, especially in the case of mountain systems.

1.4. Ripening of the cheeses

During the ripening, a number of microbiological and biochemical changes occur, which are caused by enzymes from the rennet, the milk and the microorganisms. It involves three main metabolic pathways: glycolysis of lactose, lipolysis and oxidation of the fat and proteolysis of the caseins. Glycolysis in not the most important metabolic pathway for the sensory properties of the final product. During this process, lactose is converted into lactic acid by lactic ferments, which increase the speed of coagulation of the caseins and the eviction of lactoserum (Fox and Wallace 1997). This production of lactic acid allows the colonisation of the surface of the cheese by yeast and fungi, establishing the cheese rind. Short-chain fatty acids (FA) are liberated through lipolysis. The latter can lead to volatile compounds such as methyl ketones, esters, or lactones (Collins et al. 2003). Besides, a lipase lipoprotein can be found in milk, because of a transfer from blood through the mammary gland (Collins et al. 2003). This enzyme plays a role in the production of short-chain FA. The latter are highly flavoured, and a high level can cause rancidity. Eventually, proteolysis is the most complex and important metabolic pathway during ripening. Indeed, caseins are broken down by chymosin and plasmin to large and intermediate-sized peptides (primary proteolysis), which are degraded further by the enzymes of microorganisms (secondary proteolysis). It ultimately leads to free amino acids, which can be responsible for various tastes, such as bitterness (Fox and Wallace 1997, McSweeney 2007). The hydrolysis of caseins also gives the cheese its texture.

1.5. The case of Fontina PDO

In the present doctoral thesis, the cheese type investigated was the PDO Fontina Valle d'Aosta (Fontina). This semi-hard cylindrical cheese weighing 8 to 12 kg is the main product in the Aosta Valley (North-Western Italy). Fontina is produced from raw milk exclusively, and the native-bred cows (Valdostana Red Pied, Valdostana Black Pied and Valdostana Chestnut) that produce the milk needed for Fontina must be fed with forages coming exclusively from the valley (Disciplinare di Produzione della Fontina Valle d'Aosta, 1996). Other feeds are only allowed in defined quantities. Four different starter cultures can be added to the milk, but are not mandatory:

- FT-1 D IAR: *Streptococcus thermophilus* (M17PTZA4'96, MTH17CL3'96, M17BA7'96) *Lactococcus lactis* (M17LEF24' 04 1), *Lactobacillus delbrueckii lactis* (MRSBAF24' 04 3).
- FT-N D RAVA: *Streptococcus thermophilus* (121UC, 122UC, 124UC), *Lactobacillus rhamnosus* (317 UC), *Lactococcus lactis* (211 UC).
- FT-AE D RAVA: *Streptococcus thermophilus* (C242, C531), *Lactococcus lactis* (C278), *Lactobacillus paracasei* (B262).
- FT-AI D RAVA: *Streptococcus thermophilus* (C212), *Streptococcus macedonicus* (C276), *Lactococcus lactis* (C218), *Lactobacillus paracasei* (B220).

One of the peculiarities of Fontina is that the milk has to be processed within 4 h after the milking, which leads to both morning and evening cheeses. In 1957, the Cooperative of milk and Fontina producers has been created, aiming at collecting, ageing and marketing the Fontina. At the beginning, there were 46 founding members. Nowadays, the Cooperative counts up to 200 members, including private companies, cheese manufactures, alps… They deliver around 300 000 Fontina cheeses per year.

During the incubation time of the cultures, milk temperature must not be higher than 36°C (Figure I.3.). After rennet addition, coagulation is most of the time carried out in copper vats. The curd is then cut into pieces of the size of maize grains and is slowly heated up to 48°C. Finally, the curd is wrapped in line cloth and placed in round, concave moulds, which are pressed afterwards. Cheese wheels are put into brine and stored afterwards in a traditional cave (directly on the alp or in the Cooperative caves), at 90 % humidity and at a temperature varying between 5 and 12°C. Fontina is then ripened between 3 and 6 months until it has a compact, brown colour, an elastic and soft consistence, a fat content of at least 45 % and a high abundance of living *Lactobacilli* (Consorzio Produttori Fontina 2008).

The first iconographic evidence of this type of cheese in Aosta Valley dates back to 1270, in a mural from the Issogne Castle (www.fontina-valledaosta.it). The term "Fontina" appeared for the first time only years later in the *Summa Lacticinorum* (Pantaleone di Confienza 1447). The first classification dates back to 1887, by the Experimental Station of Lodi's dairy factory. Then, in 1955, Fontina earned its Control Designation of Origin status thanks to a presidential decree. Finally, EU awarded in 1992 its PDO status (EC No 2081/92). Since the 1960's and the revolution of agriculture, Fontina was part of a continuous search for quality and authenticity, even though not intensively studied. The cheese was more precisely characterised and described by Pillonel et al. (2004) and Bérard et al. (2007). A total of 74 volatile compounds were identified, including branched chain aldehydes, which can be considered key flavour compounds in some cheese varieties (Bérard et al. 2007).

However, in the last decade, an increase in the number of Fontina cheeses with abnormalities such as colour and opening defects has been reported by the farmers. Due to huge variations between cheese manufacturing locations and cheese producers (around 200 different producers on mountain pastures), it is difficult to keep a constant and homogenous quality in Fontina cheeses. More than 300 000 cheeses are yearly delivered to the Cooperative, representing a turnover of 20 millions euros. Fontina is mostly consumed in Northern Italy (80% of the income). Foreign markets stand for 10 % of the sales volume, and include USA, Germany, Switzerland, France, Belgium and Great Britain (www.fontina-valledaosta.it). In order to be able to satisfy the consumers and maintain the good image of mountain products, further investigation is needed to improve and stabilise Fontina quality.

a) Rennet addition, T=36°C

b) After 45 min, the curd is sliced, T=36°C

c) Sliced curd looking like maize grains, T=36°C

d) Mixing and heating the curd up to T=48°C

e) Curd wrapped in line cloth and put in mould

f) The cheese is pressed for 24h

Figure I.3. Different steps of Fontina production (*Photos N. Hermann 2016*).

2. Interaction between animal and environment as influencing product specificity

2.1. Dairy cows' digestion and the effects of feed on the quality of dairy products

2.1.1. Ruminants' diet and digestion

After being chewed by the dairy cow a first time, plants are chemically broken down by microorganisms in the rumen (bacteria, protozoa and fungi). Rumen fluid contains up to 10^{11} bacteria/mL, 10^{6} protozoa/mL and 10^{3} fungi/mL (Denman et al. 2007). These microorganisms digest carbohydrates, lipids and proteins and produce short-chain FA. The latter, that are volatile, are adsorbed through the rumen wall and used as main energy source by the cow. Preformed long-chain FA are obtained from plant lipids (galactolipids, phospholipids, triacylglycerols etc) in the rumen (Figure I.4.), where they can later be hydrogenated by the microorganisms (described later in 2.1.2.).

Dairy cows regularly regurgitate the rumen's content to ruminate it, *i.e.* chewing it again in order to break the fibre and large particles remaining after the first microbial lysis. Rumination is possible thanks to the contraction of the reticulum and the opening of the cardia (the sphincter at the entrance of the oesophagus). In addition to this, the digesta is carried by expiration and gas eructation. After being degraded to fine enough particles, the digesta is then transported to the omasum, where it is physically digested thanks to a highly keratinised mucosa. Afterwards, the remaining digesta goes through the abomasum, duodenum, jejunum and ileum, to end in the large intestine, as for single-stomached animals. The main peculiarity of dairy cow's digestion is thus the rumination process and the fermentation of their feed in their rumen. The complex cata- and anabolism of the molecules (especially FA) from the digestive tract to the milk, illustrated in Figure I.4., explains why it is not possible to directy link the composition of the forage to milk chemical composition and quality.

Amongst all molecules, cows also ingest plant secondary compounds (PSC), especially when grazing on biodiverse highland pastures. Dicotyledons are richer in PSC than grasses: all these compounds represent 1.5 to 2.5 % of the grasses' dry matter in temperate regions, whereas in *Polygonum bistorta* for instance (a plant commonly found on mountain pastures), this content can go up to 6 % of the dry matter (Farruggia et al. 2008). Thanks to this botanical diversity, upland extensive pastures have higher contents of soluble phenolic compounds and terpenes (Cornu et al. 2001; Falchero et al. 2008, 2009a and 2009b; Reynaud et al. 2010). It has been confirmed that upland biodiverse pastures also have higher contents of several minerals such as zinc or manganese (Coulon et al. 2000). The latter differences in chemical composition with altitude can be partly explained by differences in abundance between the main botanical families, but also by the vegetation stage. Indeed, frost episodes and snow challenge the growth of mountain plant species, which leads them to develop specific adaptation strategies. Among them, the synthesis of terpenoids is a response to oxidative and abiotic stressors such as cold, high UV-radiation or water deficiency (Sing and Sharma 2015). Polyphenol synthesis is also sensitive to variations in herbage phenological stage: on the same pasture but at different vegetation growth stages, Fraisse et al. (2007) identified 170 different compounds, of which only 30 were common to all vegetation species and growth stages. These PSC are of high interest for animal's health. Carotenoids, polyphenols and tocopherols have an antioxidant capacity (Aurousseau et al. 2002) and tannins were found to have antihelminthic properties (Hoste et al. 2006). Moreover, carotenoids are precursors to tocopherols, fat-soluble molecules involved in several biological functions such as embryo development, growth or gene regulation (Farruggia et al. 2008).

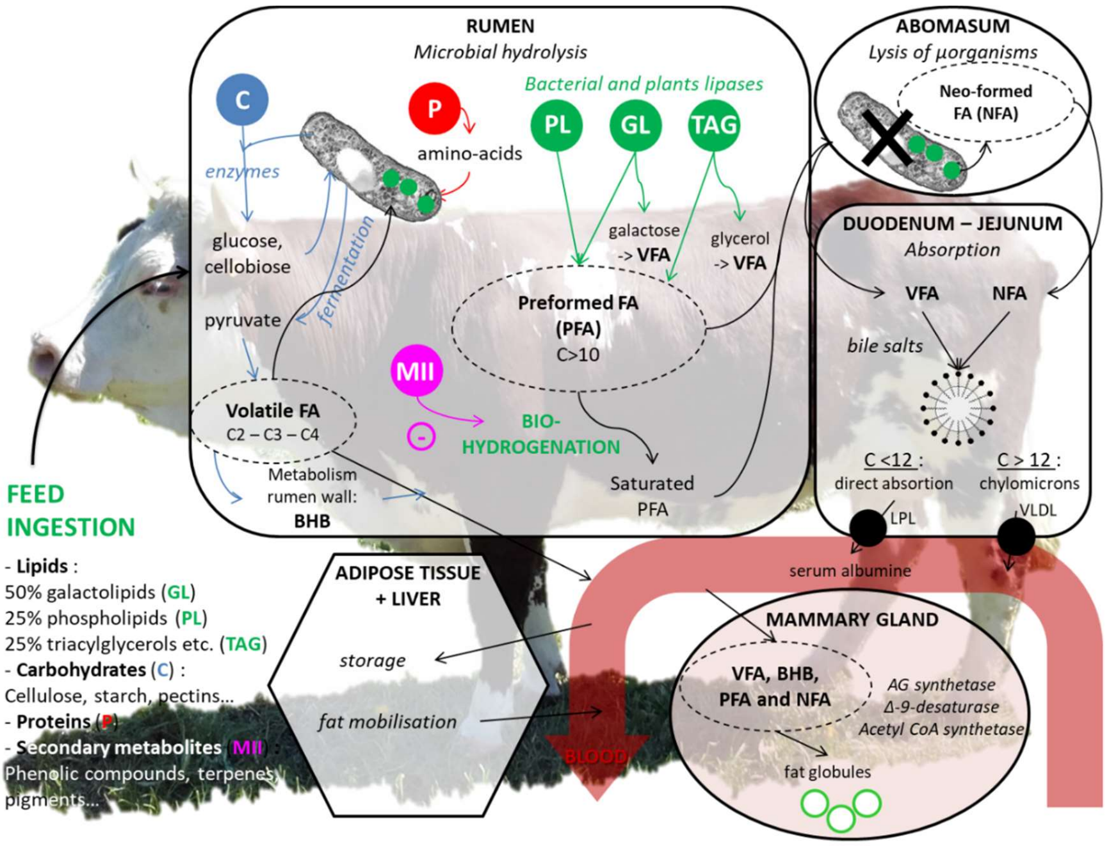

Figure I.4. Overview of the digestion and metabolism of the fatty acids in a dairy cow (M. Koczura).

2.1.2. *Ruminal bio-hydrogenation and its effects on the fatty acid profile of mountain food*

Fatty acids found in milk and cheese can have different origins. On one hand, FA with short or medium aliphatic chains (C4 to C14 and around 50% of C16:0) are synthesised *de novo* in the mammary gland from volatiles FA, which were produced by ruminal fermentation. These FA represent 40% of the total milk fat. On the other hand, FA with long chains (some of C16:0 and chains with more than 18 carbons) originate from the mobilisation of adipose tissue or from the diet.

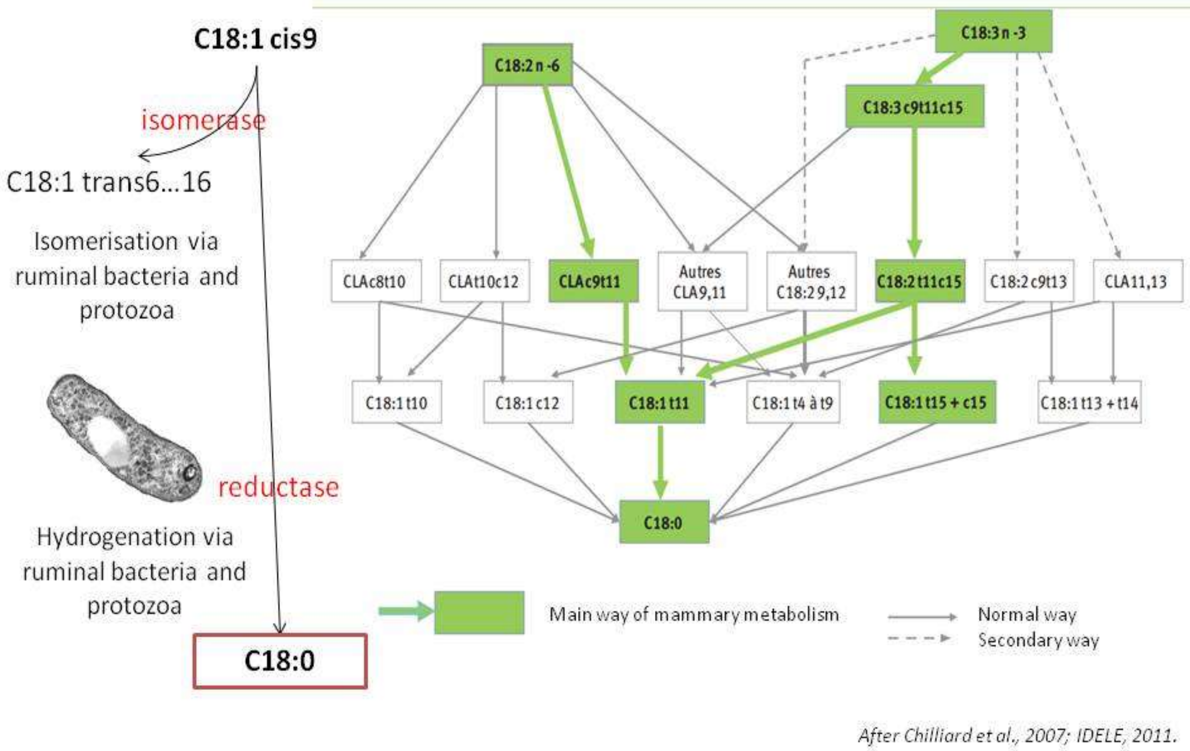

Figure I.5. Main ways of ruminal bio-hydrogenation for C18:1 *cis* 9, C18:2 n-6 and C18:3 n-3.

Long-chain unsaturated FA may undergo a bio-hydrogenation in the rumen: an isomerisation and reduction of the ingested unsaturated FA by the rumen bacteria (Khiaosa-ard et al. 2011). Most of the poly-unsaturated FA (PUFA) have a *cis* configuration. However, in ruminants, *trans* FA are produced through the first phase of bio-hydrogenation and transferred to adipose tissue and milk fats. In Figure I.5., the bio-hydrogenation of the three most abundant unsaturated FA (C18:1 *cis* 9, C18:2 n-6 and C18:3 n-3) is illustrated. This chain of reactions produces *trans* FA and conjugated linoleic acids (CLA). The PSC (such as tannins, terpenes, polyphenols etc), which are more prevalent in mountain than lowland pastures, may be involved in the inhibition of the final reduction step of the bio-hydrogenation, leading to the reduction of C18:1 *trans* 11 to stearic acid C18:0 (Khiaosa-ard et al. 2011). Changes in the ruminal metabolism of FA involving PSC have been demonstrated in vitro (Cabiddu et al. 2010; Khiaosa-ard et al. 2009; Vasta et al. 2009) and also in vivo (Cabiddu et al. 2009 and 2010), concerning the possible inhibition of the first step of bio-hydrogenation. The exact PSC in mountain pastures responsible for this inhibition have not been identified yet (Leiber et al. 2005; Chilliard et al. 2007). Mountain pasture milk is then richer in MUFA and PUFA, CLA and n-3 FA such as ALA (Eyer et al. 2002; Leiber et al. 2005; Wehrmueller et al. 2008). Actually, Lucas et al. (2006) could even establish a gradient of richness in CLA in the milk depending on the nature and the characteristics of the main forage fed to cow: mountain pasture > permanent grassland (first cut) > permanent grassland (second cut) > temporary grassland > grass silage > hay > maize silage.

The effect of the grass-based diet on FA can also be observed in processed milk such as cheese. Indeed, cheeses produced out of milk from grazing cows are in general richer in unsaturated FA than cheeses produced by cows fed with hay (Martin et al. 2002, Leiber at al., 2005). In the case of mountain pastures, milk fat has a high level of C18:3 n-3, C18:1 *trans* 11 (Khiaosa-ard et al. 2011), *cis* 9, *trans* 11 CLA, and a low proportion of medium-chain saturated FA (*i.e.* 10 to 16 carbons) (Collomb et al. 2002; Povolo et al. 2013; Contarini et al. 2014), which is recovered in cheese. In the case of transhumance, the proportion of C16:0 is lower for highland pastures (Gorlier et al. 2012). Besides, Hauswirth et al. (2004) showed that the proportion of C18:3 n-3 in mountain cheese was four times higher than that of cheddar. The higher C18:1 *cis* 9 to C16:0 ratio (spreadability index) that results from the inhibition of ruminal bio-hydrogenation is related to a low fat melting point, *i.e.* less firm texture for cheese (Bugaud et al. 2001).

The n-6 and n-3 FA (C18:2 n-6 and C18:3 n-3 are the most abundant in milk) are essential because the mammalian body cannot synthesise them by itself, and yet they are necessary for growth and physiology. The inhibition of the ruminal bio-hydrogenation is thus a factor enhancing the transfer of unsaturated FA to dairy products; and mountain cheese and milk may be relevant sources of C18:3 n-3 and other probable cardio-protective, anti-carcerigen and healthier FA for humans (ANSES, 2011).

2.1.3. *Additional consequences of grass-based diets for milk and cheese quality*

Cheesemakers were the first to frequently observe differences in sensorial qualities of their cheeses according to the forages the cows were fed. Martin et al. (1995) found significant differences in terms of colour or sensorial characteristics in *Reblochon fermier* produced from milk of cows fed with hay or on pasture. Monnet and Gaiffe (1998) even associated a floristic typology to the sensorial properties of *Comté*. In the case of *Abondance* cheese, a difference between the cheese produced from the milk of cows grazing on the northern slope of the mountain or the southern one was found (Buchin et al. 1999): the cheeses from the northern slope were less firm, creamier and stickier. In addition, the flavour was more salty and bitter, unlike the cheeses from the southern slope which were sweeter. Eventually, *Beaufort* cheeses produced with the milk of cows grazing on highland pastures (altitude of 2200 m a.s.l., 20 different botanical species) were more salty, more “spicy” and more sour than the ones from middle pastures (lowlands, 42 botanical species including 12 *Poaceae*) (Martin et al. 2005).

The previously described effects are mostly due to PSC found in the diet that have been transferred to the milk, or to the action of these molecules on the ruminal microorganisms (see 2.1.2.). These compounds do not affect only the FA profile: terpene composition of the pasture was linked to that of the milk. Indeed, milk from pastures rich in dicotyledons, (particularly *Apiaceae, Asteraceae* and *Lamiaceae;* Cornu et al. 2001) contains a greater quantity and a wider variety of terpenes than milk from pastures rich in *Gramineae* (Bugaud et al. 2001). It was also demonstrated that cheeses from mountain pastures are richer in terpenes than cheeses from the lowlands (Martin et al. 2002), because cows are grazing in the highlands, whereas they are fed on hay in the lowlands. Milk from pasture-fed cows contained 6 to 23 times more terpenoids than that obtained from cows fed with hay from the same grass (Martin et al. 2005). Terpenes are absorbed directly from the diet (Serrano et al. 2007), but can also be hydrogenated and isomerised by the rumen microflora, yielding additional terpenes to the milk (Schichterle-Cerny et al. 2007). These molecules have been recognised since Ancient times for their antiseptic properties, and are the main bioactive compounds of essential oils (Calsamiglia et al. 2007). However, their effect on sensory properties is marginal (Tornambé et al. 2008; Martin et al. 2002), and the effect of terpenoid-rich pasture on cheese flavour must still be investigated as it may also involve effects on the cheese microbiota (Buchin et al. 2006).

Phenolic compounds (such as flavonoids or tannins), carotenoids and vitamins contribute to the nutritional value of the milk. They could be of high interest for human health, as they may be involved in the prevention of cancer and cardiovascular diseases (Carocho et al. 2013). An inverse evolution exists between the stage of development of forage grass species and carotenoid levels, and a grassland with a lower botanical diversity used in a more intensive way has higher carotenoid levels but lower polyphenol levels than a high diversity one (Graulet et al. 2012). Besides, β-carotene, xanthophylls, retinol and α-tocopherol concentrations were higher in the cheese fat of pasture-based diets, compared to preserved forage-based diets, regardless of cow breed (Nozière et al. 2006). Transfer of polyphenols from feed to milk must also be investigated more in details, as well as the degradation of these compounds along storage.

2.2. Variations in grazing behaviour and consequences for milk and cheese quality

2.2.1. *Monitoring the individual grazing behaviour*

Different methods have been implemented in order to investigate the cow's individual feeding behaviour. Animals need to get adapted to the equipment and sampling methods before. First, visual observations are providing the highest amount of information. Usually, scan sampling every 5 min is the most used method (Dumont et al. 2007a; Coppa et al. 2011a). However, this method is difficult to implement because of the huge amount of work and people involved. In order to simplify the measurements, experimental sensors were developed. The three main methods consist in acoustic signals (Clapham et al. 2011; Milone et al. 2009), measurements of the electrical resistance or pressure differences in a noseband sensor (Rutter et al. 1997), or by movement sensors in cow's ear (Bikker et al. 2014).

In the present doctoral thesis, the MSR chewing sensors (MSR Electronics, Seuzach, CH) were chosen to investigate the individual grazing behaviour of cow's on mountain pastures. They record at a 10 Hz-frequency changes in pressure due to movements of the cow's jaw. An oil-filled tube is included in the noseband of a cow halter (Figure I.6.), and gives an electric feedback to a pressure data logger (MSR 145) attached to it. This method was developed and patented by Agroscope and MSR Electronics (Patent CH 700 494 B1).

Chewing movements during rumination are regular and generate uniform amplitudes (Figure I.7., in red). On the contrary, ingestion generates irregular waveforms with varying amplitude (Figure I.7., in green) (Braun et al. 2013). Together with the corresponding software, ingestion and rumination time, bites during ingestion and during rumination as well as the number of rumination boli can be quantified (Nydegger et al. 2011).

Figure I.6. Head collars containing MSR pressure sensors: a flexible tube containing oil (1) is linked to a MSR 145 electronic sensor (2) and inserted in the noseband (3a = oil tube, 3b = sensor) of a regular head collar (3). (*Photos S. Zurmühle 2017 and Braun et al. 2013*).

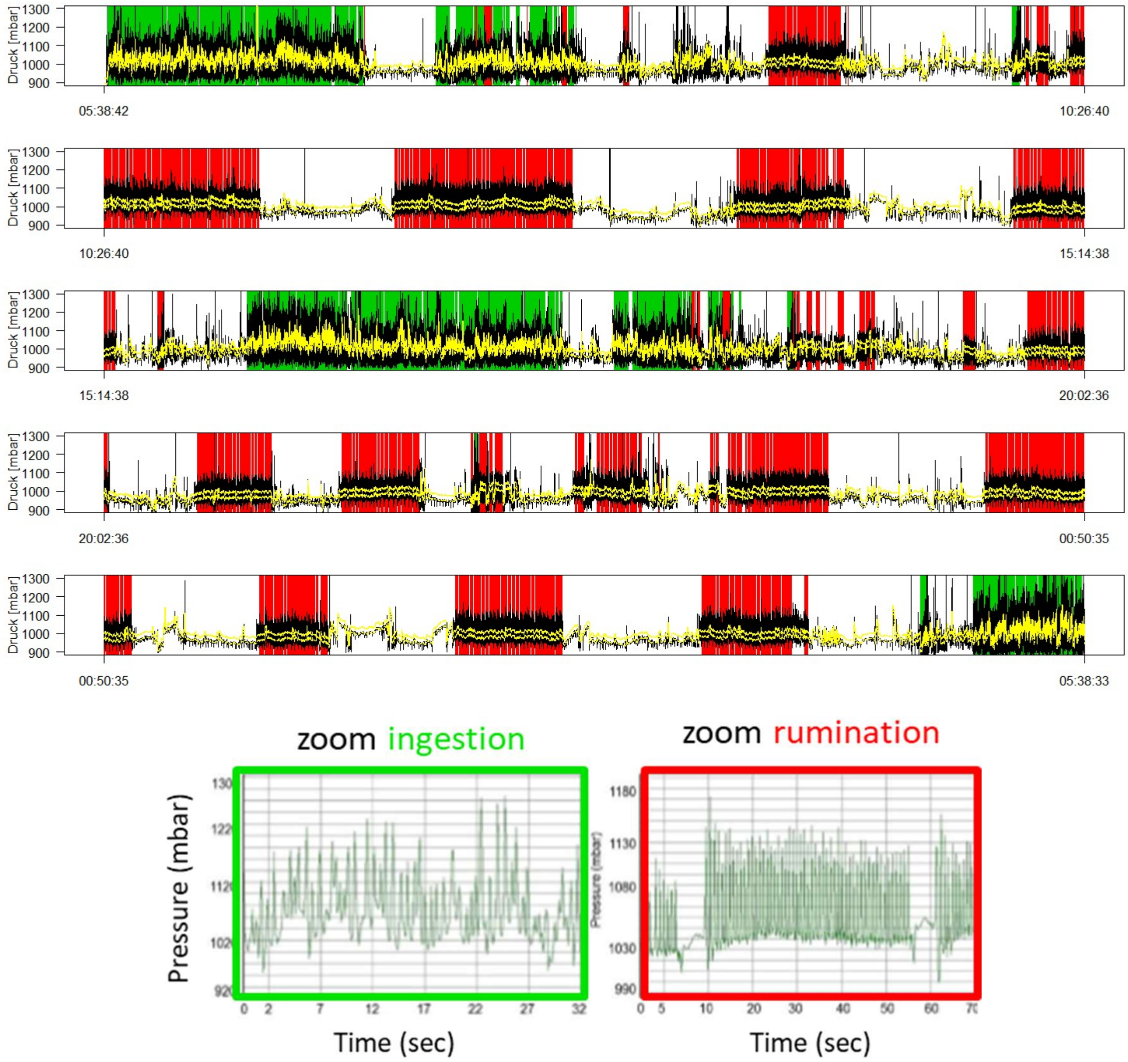

Figure I.7. Pressure variations recorded by a MSR sensor on an individual cow during 24 h with a magnification on the ingestion and rumination patterns. Ingestion phases are highlighted in green, while rumination phases are red (Koczura 2015).

2.2.2. *Factors influencing diet selection on the pasture*

Dairy cattle usually spend a large proportion of time grazing: between 6 and 8 hours a day (Delagarde et al. 2001). When ingesting, cows chose bites of grass that they swallow without much chewing. They will ruminate it later while resting. Grazing behaviour is affected by several factors, among which environmental and management conditions, plant species, and the animal's needs and experience. It has been demonstrated that when the access time on pasture is limited, cows would adapt their amount of bites per min and bite size (Kennedy et al. 2009). Under barn-feeding conditions, Cortes et al. (2006) found that the diversity of individual plants and feeds increases the animals' motivation to eat and then their level of feed intake.

When available, dairy cattle actually prefer open areas with botanically rich vegetation, dominated by grass species (Meisser et al. 2014; Sickel et al. 2014). It is however not possible to distinguish if this effect is rather linked to the large number of species, the presence of particular species or the phenological stage of the plants (Farruggia et al. 2008). Dorioz (1998) observed a specific diet selection on mountain pastures. Indeed, cows express a global aversion for some plant species because of their toxicity, taste, thorns or smell (*Veratrum album*, *Cirsium spinosissimum* or *Aconitum sp.*, for example). A temporary aversion is also observed for some plants, regardless of their palatability, but because they were soiled by dejections (cows will avoid for 2 to 4 weeks spots where urine can be found). Eventually, the phenology of plants also influences diet selection. For instance, *Phleum alpinum* or *Dactylis glomerata*, which are basically of high nutritive value, could be left uneaten when they are past the flowering stage (Dorioz 1998). Farmers report that cows would go for *Geranium sylvaticum* if already used to it (in the *Beaufortain* massif), and observe behavioural differences between cows experienced with the specific conditions of an alp or others that arrive here for the first time (Silbernagel 2002). Lopes et al. (2013) confirmed in experimental conditions that grazing experience acquired at a juvenile age affects diet selection years later in cows.

Actually, seasonal evolution or morphology of the pasture also affect diet selection of cows. Large seasonal variations in grazing behaviour were observed along with herbage evolution and availability (Farruggia et al. 2014), and animals take larger bite masses as the vegetation size and structure decline (Agreil et al. 2005). Besides, Dorioz (1998) reported that the same plant species are less consumed when growing in clumps than when dispersed among grasses, and that some species impair consumption of the neighbouring plants. Plant morphology plays a global role, linked to all these previous preferences: cows would rather go for leaves than stems, young and green parts rather than old and lignified parts (Dorioz 1998).

In conclusion, herbivores exploit the heterogeneity of the resources provided by selective grazing, choosing a diet of better quality than what the average vegetation offers (Prache et al. 1998). The decisions are made within the morpho-physiological, digestive and behavioural constraints that the animals face on the pasture (Prache et al. 1998), and also the interaction between the taste and smell of plants and their digestive consequences (Ginane et al. 2005). However, even though it is possible to estimate the species selection and dry matter intake of animals on the pasture (Coppa et al. 2011a), it remains a rather simple approach at the moment compared to the complexity of the behaviour on large spatio-temporal scales. Cows consume first the preferred patches, but because of the combination of all parameters described, it is very difficult to draw universal rules to exactly define these patches.

2.2.3. *Impact of diet selection on milk and cheese quality*

The cows' grazing activities and their choices influence their own physiology and the flora on the pastures, but also the final dairy product (Bugaud et al. 2001; Farruggia et al. 2014). Because herbivores can eat a diet of better quality than the average vegetation in the pasture through selective grazing (II.2.2.2.), and because biodiverse pastures strongly affect milk and cheese quality through several different molecules (II.2.1.3.), it can be assumed that the variations in behaviour would impact milk and cheese quality. Coppa et al. (2015) demonstrated that significant day-to-day variations in the milk FA profile can be observed in relation to the grazing behaviour of cows. Indeed, the bites of cows grazing the new layer of vegetation on a new pasture were richer in crude protein and poorer in fibre, which was related to an increased proportion of C18:0, C18:1 *cis* 9, C18:2 n-6 and *de novo* synthesised FA. Therefore, the evolution of herbage phenology during the grazing season combined with the botanical composition of the pasture and the animal behaviour is a key element to understand the variability of milk and cheese quality on mountain pastures (Farruggia et al. 2014), and must be further investigated.

2.3. Effects of transhumance on ruminants metabolism and dairy products

In the mountains, not only the pasture's biodiversity and morphology can affect the animal. The physical effort required to move to mountain pastures by walking, the stress of truck transportation and eventually the specific conditions of altitude and mountain systems strongly affect the animals, with consequences for the quality of dairy products.

2.3.1. *Effects of transportation and walk on cows and their milk*

During transhumance, animals traditionally move from the lowlands to the mountain pastures, by either walk or truck. Some authors demonstrated under experimental conditions that milk quality is impaired by walking in dairy cows, resulting in a higher fat content and somatic cell count (SCC) (D'Hour et al. 1994; Coulon et al. 1998). Besides, a single walking of 12.8 km distance decreased the cows' feed intake and milk yield (MY), while provoking a rise in body temperature and in concentrations of non-esterified fatty acids (NEFA) and glucose in plasma (Coulon and Pradel, 1997). Magrin et al. (2015) confirmed this effect on blood metabolites during a 40 km long transhumance. Kreuzer et al. (1998) found signs of immediate stress response during truck transportation. Plasma and saliva cortisol concentration increased, and so did lactate and NEFA concentration in plasma. Moreover, the animals still had higher thyroid hormone levels after transportation, an indicator of the animals' metabolic rate, probably due to stress. The β-hydroxybutyrate (BHB) levels were also still elevated, which could indicate an energy shortage. Reduced plasma insulin and glucose levels in blood samples of the transported cows, also observed by Leiber et al. (2004) and Zemp et al. (1989b), could be due to a limited energy intake. Physical exercise or limited energy intake lead to body fat mobilisation. It results in an increase in C18:1 *cis* 9 and decrease in *de novo* synthesised FA in milk (Pires et al. 2015).

All these metabolic and milk composition changes observed shortly after transportation and physical exercise of the cows might lead to several variations in the cheeses. Indeed, farmers in Aosta Valley reported facing difficulties in cheese manufacturing right after transhumance, specifically after the animals moved. The specific effect of walk and truck transportation on milk and cheese was poorly studied in the last decade and needs to be further investigated, in order to better understand and qualify the variations of mountain dairy products in transhumant dairy systems.

2.3.2. Links between mountain conditions, management system and dairy products

Right after moving to mountain pastures, cows need to cope with the hypoxic environment at high altitude. Higher levels of erythrocytes, leucocytes, hemoglobin, and pH in the blood were observed as well as increased respiration and heart rates (Zemp et al. 1989b). Leiber et al. (2004) suggested that these changes in metabolism, which are occurring from 2000 m a.s.l., explain the decreased feed intake at the start of the mountain grazing season. Additionally, cows need to cope with general changes in environment such as unknown housing, different and possibly harsher climatic conditions, increased solar radiation, and a more difficult, steeper topography. Zendri et al. (2016a) observed increased movement in herds on mountain pastures, associated with grazing and interactions with unknown individuals in the case of mixed herds. The metabolic adaptation to the immediate energy deficit on high altitude pasture seems to require at least three weeks (Kreuzer et al. 1998). Directly linked to this acclimation, a decline in MY (and usually the incapability of the animals to recover their previous milk productivity) can be observed over the duration of the summer grazing season (Zemp et al. 1989a; Leiber et al. 2004). Larger studies involving the medium-term effect of transhumance on milk and cheese quality showed that MY and milk quality decrease in highland pastures (Bergamaschi et al. 2016; Zendri et al. 2016a). Mountain conditions other than feed composition are thus strongly related to changes in milk and might be related to changes in cheeses too.

Even though a high diversity of mountain dairy farm management measures exists, their relation with animals and dairy products has been poorly studied yet. Costa et al. (1990) compared two different mountain grazing systems in Piedmont (North-West of Italy) with Piemontese cows (usually beef cattle): the traditional one with the cows sheltered overnight in the barn, and the full open-air system. Milk production of the group in the barn was higher than that of the group in open air, also with a higher daily variability (the variability could be related to thunderstorms *inter alia*). They also showed that there was a significant difference in terms of animal dejections on the plots: 2.3 kg DM per livestock unit and per day for the group sheltered in barn vs. 4.8 kg DM per livestock unit and per day for the group in open-air system. However, they did not find any difference in terms of grass ingestion between the two systems. According to the diversity of mountain dairy farms, it would be interesting to further investigate the differences in milk composition that may occur between different types of management.

2.3.3. Impact of the physiological stage and sanitary state of cows on dairy products

Summer grazing in the mountains is usually carried out at medium to late lactation stage because of seasonal calving. Besides, it is associated with increased movement of the mammary gland because of the need to walk, on pastures and during transhumance. These parameters induce a higher milk SCC. Indeed, it was repeatedly demonstrated that milk of cows grazing on mountain pastures during the summer season was richer in somatic cells (Lamarche et al. 2000; Leiber et al. 2006; Bergamaschi et al. 2016). High SCC are regularly synonyms of mastitis, which affects the microbiological quality of milk through the transfer of infectious germs (Coulon et al. 2004). Besides, mastitis and high SCC also result in a decreased lactose content, alterations of the fat globules (promoting lipolysis), decreased casein content and increased soluble proteins and enzymes concentrations (Munro et al. 1984; Coulon et al. 2002). Soluble compounds are transferred from blood to milk because of a dysfunction in the mammary gland. Among them, plasmin can be found, as well as white blood cells, which are responsible for the increase in SCC in milk (Coulon et al. 2004). Somatic cells also possess an enzymatic complex involving plasmin.

Plasmin is a protease synthesised from the plasminogen, a plasmin precursor, and plasmin activity depends on the activation of this plasminogen (Figure I.8.). The first role of plasmin is to break blood clots by cleaving the fibrin. In milk, the role of plasmin is similar, the difference being its contribution to the hydrolysis of casein instead of fibrin. Milk with high SCC and therefore high plasmin activity is usually associated with slow coagulation and poor drip-dry (Coulon et al. 2004). The consequence is low cheese yield, and higher moisture content in the final product. These changes are also associated with an increased proteolysis rate and modification of the proteolysis pattern (Cooney et al. 2000; O'Farrell et al. 2002).

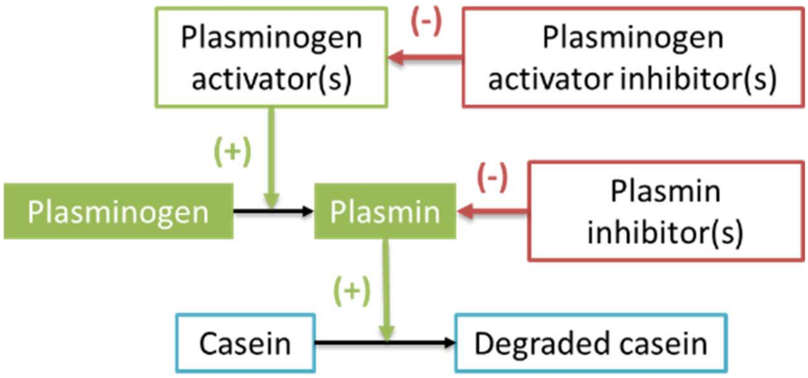

Figure I.8. Schematic representation of the plasmin enzyme system in milk (adapted from Bastian and Brown 1996).

An increased SCC (and therefore increased moisture content, plasmin activity and proteolysis rate) in milk was associated with cheeses being less firm and elastic, more sticky (Grandison and Ford 1986; Auldist et al. 1996). Bugaud et al. (2001) observed a higher plasmin activity in milk produced in mountain pastures (1 500 and 1 850 m a.s.l.) than in milk from valley pastures (850 and 1 100 m a.s.l.). In fact, differences found between *Abondance* cheeses from northern and southern slopes may be related to this plasmin activity: there was twice more plasmin in cheeses from the northern slope, which means that the proteolysis would be faster, which can explain the higher sticky and creamy characteristics (Buchin et al. 1999). A high SCC in milk does not only impair cheese texture, but also the general flavour (Coulon et al. 2004). Indeed, high SCC was associated with a “rancid” and “oxidised” taste, which can be linked to lipolysis (Auldist et al. 1996), or “bitter” taste, linked to proteolysis (Cooney et al. 2000). Besides SCC and mountain grazing, parity or breed of the cow can also be factors influencing plasmin activity (Bastian and Brown 1996). Actually, several previously described parameters such as metabolism, behaviour or milk quality might also be affected by the cow breed.

3. Autochthonous cow breeds used in the mountain areas

3.1. Characteristics of autochthonous cow breeds

There is no unambiguous definition of autochthonous breeds. The FAO defines it as an “indigenous” or “native” species, which means that it originates from, is adapted to and is used in a particular geographical region. It is noteworthy that autochthonous breeds are considered as a sub-set from the “locally adapted breeds”. The latter are defined as breeds “which have been in the country for sufficient time to be genetically adapted to one or more of the traditional systems or environments in the country”. Most of the time, autochthonous breeds are described as to be robust. However, robustness is also a controversial and extensive concept, which involves several different parameters. Star et al. (2008) consider that robust animals are able to adapt to and produce in a wide variety of environmental conditions. Optimally, they have a high production potential and show resilience (*i.e.* maintain homeostasis and only take short periods to recover from adverse effects). More recently, Friggens et al. (2017) summarised robustness as an ability including numerous traits, which allow carrying on casual activities in the face of environmental constraints. The key concept behind all these denominations and definitions actually is adaptation to the local territory.

The subject of autochthonous and locally adapted breeds is currently being intensively investigated in the frame of future sustainable dairy systems. In the mountains, the ability of such breeds to traditionally develop a natural resilience to their local environment over time seems to be a key element. For instance, the Brown Swiss cattle got improved for over 1000 years without active breeding efforts (Spengler Neff et al. 2007). Swedish mountain cows spent less time in grass dominated pastures than Holstein cattle and travelled a longer distance (Hessle et al. 2014), suggesting that using traditional breeds can result in a better management of diverse vegetation types. In transhumant systems, Zendri et al. (2016a) concluded that local and dual-purpose breeds might adapt better than specialised breeds to the mountain sojourn, with lower variations in milk quality and better maintenance of body fat reserves.

3.2. Breed effects on milk and cheese

It is difficult to directly link differences in milk and cheese quality of different cows to their breed, because effects are most of the time intertwined between the environment and the behaviour of the animal. The genetic variants of caseins are actually one of the best known direct genetics effect for milk and cheese manufacturing. Indeed, variant B of κ and β-caseins reduces clotting and curd-firming times, and leads to firmer rennet curd than variant A (Coulon et al. 2004). More specifically, the BB homozygote genotype for κ-casein leads to faster gelation and higher curd firmness than AA, AC, BC and AB genotypes (Delacroix-Buchet et al.

1993). The cheese yield of milk from cows with variant B is higher, also because of a better retention of fat matter in the casein network. The genetic variants of milk proteins strongly influence the milk coagulation properties (MCP), and the heritability of MCP is higher than that of MY (Bittante et al. 2012). The *Tarentaise* breed, which originates from the *Beaufortain* massif and is included in the PDO *Beaufort* specifications, shows a higher frequency of rare variants of α_{s2}, β and κ-caseins (Grosclaude 1988). Even though not clearly described and understood yet, other genetic effects and differences between breeds have been observed. Indeed, in a comparison between *Abondance* and *Montbéliarde* herds, the carotenoid content of cheese was higher when using milk from *Montbéliarde* than from *Abondance* (Lucas et al. 2006). The same authors found that *Abondance* and *Montbéliarde* breeds were associated with a higher CLA percentage in the cheese than *Holstein*. Moreover, differences in FA profile were found between *Valdostana Red Pied* and *Valdostana Chestnut* cows managed in the same conditions (Renna et al. 2010). These results suggest that milk and cheese obtained from autochthonous mountain breeds may have a certain specificity.

3.3. The Valdostana Red Pied cow

In the Aosta Valley, there are three local dual-purpose cow breeds: *Valdostana Chestnut*, *Valdostana Black Pied* and *Valdostana Red Pied* (Va) (ANA.Bo.Ra.Va., 2010). The latter breed is investigated in the present doctoral thesis. The Va cows have a higher MY (Renna et al. 2010) and lower muscular mass (ANA.Bo.Ra.Va., 2010) than *Chestnut* cows. The cows from the *Valdostana Black Pied* and *Chestnut* breeds actually belong to the same Genealogic Book, their main difference being their colour. They are the original cattle from Aosta Valley (ANA.Bo.Ra.Va., 2010). Historically, the first aim of Va was to produce both milk and meat. However, milk products prevailed in altitude, which led to an increase in the MY potential of the Va. In the 1800's, even though it was controversial in the beginning, *Simmental* bulls from Switzerland were used to improve Va's MY potential. The dynamism and the robustness of the breed remained the most important though. Nowadays, Va is considered as a dual-purpose animal, agile and versatile, supposed to give adequate results in terms of both milk and meat production performance. Main characteristics taken into account in the selection of the breed were also its high fertility, relative resistance to mastitis and diseases in general. The typical cow has a robust condition, hard hooves, medium size and weight with beefy extremities. Usually, its coat is red and white, with white underside and head. The chest is low and broad, and the ears are red while the horns are yellowish. The animals from this breed are supposed to show resilience and valorise roughage efficiently (resumed from the ANA.Bo.Ra.Va., 2010).

In 2014, Va was represented by 12868 animals, whereas the *Valdostana Black Pied* and *Chestnut* counted 5594 registered animals. This breed is included within the Domestic Animal Diversity Information System (DAD-IS) of FAO. Currently, the Va's average MY is about 4000 kg/year (ANA.Bo.Ra.Va., 2010). Under barn-feeding conditions, Renna et al. (2014) showed that their feed intake, performance and milk FA profile is not influenced by offering a total mixed ration or separate ingredients. As this breed was selected aiming at both milk and beef performance, which are antagonistic targets, its yield is quantitatively lower compared to respectively specialised breeds (Mazza et al. 2016). A Piano di Sviluppo Rurale was implemented, helping at conserving the genetic resources of this breed. Traditionally, Va cows are kept in tied-stall barns during winter where they are fed hay and concentrate. In the spring, they start grazing lowland pastures near their barn during the day, whereas they are still fed on hay and sheltered in the barn during night. In some farms, the fresh grass is cut and directly given to the animals in the tied-stall barn. Then, in the end of spring, cows experience a first transhumance to high mountain pastures. There, animals are still sheltered in barns during the night and the warmest hours of the day, and for milkings. After successive transhumances to high altitude, cows finally come back to the valley in late summer/early autumn.

C) Objectives of the present doctoral thesis

As demonstrated, mountain dairy systems need to be investigated in more detail in the current economic, social, political and research context. In such systems, tradition and modernity are deeply intertwined. On one hand, a deeper understanding of transhumant dairy systems in their diversity is needed. On the other hand, a better comprehension of the importance of autochthonous breeds and at which level (behaviour, performance, milk quality) they might be better adapted to their local territory is necessary. Therefore, the present doctoral thesis aimed at experimentally investigating these aspects on the short and medium term, through the case of the autochthonous Va breed. The following issues were addressed:

- Qualification and quantification of the adaptation of Va cows to their mountainous environment through behaviour, performance and milk quality, and comparison with more specialised dairy breeds on mountain pastures.

- Based on the literature, local farmers' empiric observations and an on farm study, identification of the short-term effects of walked transhumance on milk composition and Fontina quality, and comparison with truck transport and more specialised dairy breeds.

On one hand (chapters II to IV), the effects of the highland sojourn on MY, milk quality and coagulation properties of primiparous and multiparous Va cows are firstly described. Then, the effects of previous mountain grazing experience of primiparous and multiparous Va cows on their behaviour, MY and milk quality are presented. Finally, an in depth and comparative study of the behaviour and performance of multiparous Va cows on biodiverse mountain pastures to Montbéliarde (Mo) and Hosltein (Ho) cows is described. On the other hand (chapters V and VI), the short-term impact of transhumance on milk composition and Fontina cheese investigated in three transhumant dairy farms in Aosta Valley is presented. The effects of transport during transhumance on the blood metabolites and milk quality of the three dairy breeds are clarified. Ultimately, the objectives, results of and perspectives from the research carried out in present doctoral thesis are discussed and illustrated in the seventh and eighth chapters.

CHAPTER II

Are cheese making properties of dual-purpose cattle impaired by highland grazing?

A case study using Valdostana Red Pied cows

Based on Niero G, Koczura M, De Marchi M, Currò S, Kreuzer M, Turille G, Berard J..
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ABSTRACT

Summer transhumance is often practiced in mountainous farming systems. It includes moving dairy cows from lowland (LO) to highland (HI) pastures during summer. It is known that high genetic merit cows are susceptible to the HI conditions, but it is unclear if this also applies to more adapted, regional cow types. The present study aimed to investigate the effect of HI sojourn on cheese-making properties of Valdostana Red Pied cows, a dual-purpose cattle type. Milk coagulation properties were measured in the milk of 47 cows before and after transhumance. Sources of variation were investigated using linear mixed models, including parity, site, milking time, the interaction parity × site, milking time × site and milking time × parity. Cow was nested within site, and used as subject for repetition, and sampling date was included as repeated factor. Curd-firming time and curd firmness did not vary between LO and HI, whereas rennet coagulation time was prolonged in HI compared to LO in both primiparous (16.4 vs. 18.5min) and multiparous cows (17.5 vs. 21.1min, respectively). The percentage of non-coagulating samples was greater in HI (15.0%) compared to LO (8.5%). The lower milk reactivity to rennet addition in HI seems to be mostly related to the simultaneously increasing somatic cell score. Morning and evening milk were similar in coagulation properties. In conclusion, even indigenous dual-purpose cows were affected by HI conditions and the experience the multiparous cows had with the transhumance was not helpful either.

HIGHLIGHTS

- The rennet coagulation time of Valdostana Red Pied cows was higher in highland.
- There was a higher number of non-coagulating samples in highland.
- The poorer ability to coagulate in highland was mostly due to a higher somatic cell score.
- Neither parity nor time of milking affected the milk coagulation properties.

1. Introduction

In European mountainous regions, dairy cows are often moved from lowland (LO) to highland (HI) pastures in late spring or early summer in transhumance systems established since centuries (Zendri et al. 2016a). The maintenance of this activity is threatened by high labor load and low income. However, since recently, producing milk and cheeses on HI pastures is gaining attention again for various reasons. Firstly, for consumers these foods are attractive due to the presumed higher health value. This perception is based on their high proportions of n-3 fatty acids in the food lipids and on their great percentage of conjugated linoleic acids in total fat, compared to products from LO (Leiber et al. 2005a). Secondly, HI pastures products are characterized by specific sensory properties (Buchin et al. 1999) and are well suited to market by defining restricted geographical areas and typical manufacturing as Protected Designation of Origin (PDO) (Buchin et al. 1999). Thirdly, public authorities and organizations are promoting agricultural use of HI pastures through several conservation actions in order to preserve marginal landscapes and biodiversity (Maretto & Cassandro, 2014; Niero et al. 2016b).

However, in order to be able to profit from the added value of the dairy foods and thus help maintaining the management of these areas, premium prices have to be realized (Pretto et al. 2009; Zendri et al. 2016b). One of the drawbacks of the HI dairy systems is the impairment of milk coagulation properties (MCP) reported from studies with specialized dairy cattle breeds, such as Holstein Friesian, Brown Swiss and Brown Italian (Leiber et al. 2005b, 2006; Bovolenta et al. 2008). The main reason explaining the longer rennet coagulation time (RCT) and the less favourable curd properties, measured as curd firming time (k_{20}) and curd firmness (a_{30}), is the energy deficit these cows are experiencing at high altitude, harsh climatic conditions and steep slopes (Christen et al. 1996; Kreuzer et al. 1998). This results in a lower milk protein content (Leiber et al. 2005b) which reduces both cheese yield and milk coagulation performance, with the latter due to the lower density of the casein (CN) molecules in milk. It is yet unclear if the use of autochthonous or local dual purpose cattle breeds, which would fit better to extensive grazing systems, help to preserve biodiversity and have a lower impact on the environment (Visentin et al. 2015b), may better tolerate the harsh conditions due to their comparably lower milk yield and, possibly, due to an inherited adaptation.

This research question was investigated through the example of summer grazing systems using Valdostana Red Pied (Va) cows, an autochthonous dual purpose cattle breed, reared in north-western Italy. Fontina cheese, a PDO product, is manufactured from full-fat and unpasteurized Va milk, from LO or HI pastures, within 2 h after every single morning and evening milking (Mazza et al. 2016). For this purpose, milk from 47 Va cows was repeatedly sampled from May to July, including both LO to HI pastures.

2. Materials and methods

2.1. Experimental design and milk sample collection

All procedures involving animals, including individual milk sampling, were approved by the Italian veterinarian authorities. The herd of 47 Va cows belonged to the Institut Agricole Régional (Aosta, Italy) and was subjected to the official milk recording system.

In May 2016, milk sampling started in the LO barn (Montfleury, Aosta, Italy, 580 m a.s.l.). Milking took place in the morning from 5.00 a.m. to 7.30 a.m. and in the evening from 4.00 p.m. to 6.30 p.m. All cows were sampled during two morning milkings. In addition, 14 cows out of this group, half of them primiparous and half of them multiparous, were sampled also at two evening milkings. The total amount of milk samples collected in LO was 122. In the barn animals were tethered during the night and during milking, while during the day they were grazing LO pastures. In the barn, animals were additionally fed with local hay offered at an amount of 10 to 12 kg per cow per day, and with 3 kg of concentrate per cow and per day during milking.

Milk from the same Va cows was sampled in the highlands (Val di Rhêmes, Rhêmes Notre-Dame, Italy, from 1800 to 2100 m a.s.l.) between June and July 2016. At that site, milking was performed from 4.30 a.m. to 7.00 a.m. and from 3.30 p.m. to 6.00 p.m. At HI, cows had permanent and free access to pasture and water, using the strip grazing technique to provide them daily fresh grass. Milking was accomplished directly on the pasture with a mobile milking parlour (Eliar 4, Eli IAR, Institut Agricole Régional, 4 milking places). During milking, 2 kg of concentrate per cow per day was provided. The same 47 cows as in LO were sampled two times during the morning milkings and the same 14 cows were sampled additionally during one more morning milking and three evening milkings. The total amount of milk samples collected in HI was 150.

The LO barn and the HI mobile milking parlour were equipped with the same milking device (Afimilk Ltd, Kibbutz Afikim, Israel). The device recorded milk yield (MY) for each cow at each milking and sampled 50 mL of milk. Immediately afterwards 18 mg preservative tablet containing 8 mg Bronopol (2–bromo-2–nitropropan-1,3–diol) and 0.30 mg Natamycin were added to inhibit bacteria, yeast and mould growth. Samples were straightaway stored at 4° C and successively transferred by refrigerated shipping, to the laboratory of the Breeders Association of Veneto Region (ARAV, Padova, Italy) for analysis of milk composition and MCP.

2.2. Analysis of milk composition and milk coagulation properties

Analysis of contents of fat, protein, CN, lactose and milk urea nitrogen (MUN) were carried out using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). Determination of somatic cell count (SCC) was accomplished through a Fossomatic (Foss Electric). Values of SCC were transformed to somatic cell score (SCS) as $3 + \log_2(SCC/100{,}000)$ to achieve normality and homogeneity of variances. The MCP were determined using the Formagraph (Foss Electric A/S Hillerød, Denmark) as lactodynamographic tool, following the method proposed by McMahon & Brown (1982). The coagulation properties measured were RCT (min) as the time from rennet addition to the beginning of coagulation, k_{20} (min) as the time from the gel development to a width of 20 mm of the bell shaped graph created by the Formagraph and a_{30} (mm) as the graph width at 30 min after rennet addition. Samples that did not coagulate within 30 min were considered as non-coagulating.

2.3. Grass sampling and analysis

Two replicates of 10 cm × 10 m strips were randomly chosen on 2 different pastures in LO and HI each resulting in 4 samples of fresh grass from both site. Samples were weighed, then dried for 120 h at 60 °C and milled through a 5 mm screen. These pre-dried samples were weighed and analysed for contents of dry matter (DM), crude protein (CP), ether extract, total ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin, Ca and P by NIRSystems 5000 (Foss Electric A/S, Hillerød, Denmark). From weights of fresh and pre-dried samples as well as DM content, the biomass per m^2 on the pastures was calculated. Contents of net energy for lactation (NE_L), PDIE and PDIN (for definitions see footnotes of Table II.1) of the samples were estimated using the regressions underlying the calculation module of the official Swiss feeding recommendations for ruminants (Agroscope, 2017).

2.4. Statistical analysis

By using the Shapiro-Wilk's test, it was first confirmed that all variables were normally distributed. Pearson correlations between traits were calculated by the CORR procedure of SAS (SAS Institute Inc., Cary, NC). Data from the 47 and 14 cows, respectively, were analysed by different models for analysis of variance using the MIXED procedure of SAS. In the first model, 188 samples from 47 cows and morning milkings only were included. Parity (primiparous and multiparous), site (LO and HI) and the interaction between parity and site were used as fixed effects. In the second model, 140 samples from the 14 cows with data from morning and evening milkings were used to assess milking time effect. Parity (primiparous and multiparous), site (LO and HI), milking time (morning and evening), and the interactions between milking time with site and parity were considered as fixed effects. The interaction between site and parity and the three-way interactions were excluded after determining that they were not significant. From the results obtained from Model 2 only means of milking time and site were presented as parity effects were presented with the larger dataset in Model 1. In both models, cow was nested within site, and used as subject for repetition, whereas date of sampling was included as repeated factor. Multiple comparisons among least square means were tested using Bonferroni's correction, and effects were considered significant at $p < 0.05$. Non coagulating samples were considered in the statistical evaluation of milk yield and milk composition, whereas they were treated as missing values in case of RCT, k_{20} and a_{30}.

3. Results and discussion

3.1. Grass quality on the lowland and highland pastures

Table II.1. shows grass composition from LO and HI pastures. The mean biomass of grass from the LO and HI was not different. This is due to the great variability (SD) of the available biomass of the grasslands from the HI. Indeed, the 4 LO pastures were more homogenous than the 4 HI pastures, which represent the range over the whole summer grazing season. Contents of CP, Ca and P were higher by 36, 51 and 65 % in the LO compared to the HI grass. The HI grass was more fibrous than the LO grass (24, 32 and 58 % higher on average for contents of NDF, ADF and acid detergent lignin, respectively). The average NE_L content was quite similar for LO and HI grass, although it varied more in the HI grass. The PDIN content was higher by 41 % for LO compared to HI grass.

Table II.1. Composition of the grass from the lowland (n = 4) and the highland (n = 4) pastures.

Site	Lowland		Highland	
	Mean	SD	Mean	SD
Biomass, g dry matter/m^2	139	26	253	106
Chemical composition, g/kg dry matter				
Crude protein	202	12	149	20
Ether extract	31.9	2.0	30.4	2.9
Total ash	123	7	74	5
Neutral detergent fiber	354	29	439	39
Acid detergent fiber	237	28	312	11
Acid detergent lignin	41.1	10.9	65.0	4.8
Ca	12.7	1.0	8.4	1.6
P	4.12	0.27	2.50	0.43
Energy and protein per kg dry matter				
NE_L, MJ	6.32	0.10	6.05	0.59
PDIE, g	109	2	100	12
PDIN, g	135	8	96	17

NE_L, net energy for lactation; PDIE, absorbable protein at the duodenum according to supply with fermentable energy and rumen undegradable protein; PDIN, absorbable protein at the duodenum according to supply with rumen degradable protein and rumen undegradable protein; SD, standard deviation.

3.2. Variation and correlations of milk quality and technological traits in Valdostana Red Pied cows

Table II.2. Descriptive statistics of production and milk-related traits.

Item	No of samples[a]	Mean	SD	Minimum	Maximum
Production-related traits					
Days in milk, d	264	176	48	42	269
Parity, n	271	3.29	2.33	1.00	10.0
Milk yield, kg/d	197	15.5	4.6	5.6	27.8
Milk chemical composition					
Fat, %	258	3.80	0.81	1.84	8.09
Protein, %	258	3.34	0.25	2.79	4.00
Casein, %	258	2.62	0.21	2.12	3.15
Lactose, %	258	4.74	0.20	4.06	5.12
MUN, mg/dL	257	19.2	5.4	4.0	35.3
SCS	258	2.73	1.70	-1.64	7.79
Milk acidity (pH)	258	6.62	0.06	6.44	6.83
Milk coagulation traits					
RCT, min	236	18.8	4.9	4.5	29.0
k_{20}, min	164	5.16	1.44	2.45	9.15
a_{30}, mm	237	27.4	12.9	2.4	58.4

MUN, milk urea nitrogen; SCS, somatic cell score; RCT, rennet coagulation time; k_{20}, curd firming time; a_{30}, curd firmness 30 min after rennet addition; SD, standard deviation.
[a]Number of cows: 47.

In terms of milk yield and milk gross composition, results of the present study are comparable to those reported by Battaglini et al. (2009) and Renna et al. (2014), who studied milk yield and milk composition of Valdostana cows. The contents of fat, protein and CN in the Va milk were lower than those found in pure dairy breeds including Holstein-Friesian, Brown Swiss and Simmental cows (Penasa et al. 2014). When compared with milk from other local and dual purpose cow breeds reared in the Italian mountain region, such as Alpine Grey, Rendena and Burlina, the milk from the Va cows had greater protein content, a lower fat content and lower SCS (De Marchi et al. 2007; Niero et al. 2016b). Concerning MCP, among 236 rennet treated samples, only 164 samples (about 70%) were able to reach k_{20} (Table II.2.). The Va's results were at the average of other Italian local breeds kept in mountain areas; they had a more favourable MCP than Burlina cows (Niero et al. 2016b) but less favourable technological traits than Alpine Grey and Rendena breeds (De Marchi et al. 2007).

Pearson correlation coefficients between traits of milk chemical composition, pH and MCP are presented in Table II.3. Rennet coagulation time was only weakly correlated with milk protein and CN content and a_{30} also had only a moderate correlation with the same traits. This rather weak relationship might at least be partly explained by observations that the curd of the slow coagulating milks had not enough time to develop, thus providing only a limited description of the potential gel firmness. The three MCP traits were closely correlated. Rennet coagulation time had a medium and positive correlation with k_{20} and was strongly negatively correlated with a_{30}. Milk pH showed weak but significant correlations with RCT, k_{20} and a_{30} in a way that a lower milk pH was associated with more favourable MCP. This is consistent with the results of previous studies (Ikonen et al. 2004; Toffanin et al. 2015). Negative low correlations were found between RCT and milk protein content and between RCT and CN content which was unexpected because a higher casein content may increase RCT due to the higher ratio of substrate to enzyme. Among the MCP traits measured, k_{20} showed the highest (negative) correlations with milk protein and CN contents, while correlations between a_{30} and milk protein and CN contents were slightly lower and positive. These relationships, different from those with contents of fat, lactose, MUN and SCS, were expected and in agreement with previous studies (Visentin et al. 2015a). Indeed, CN is the only milk constituent reacting to rennet addition and curd formation occurs because of the aggregation of para-CN micelles, which subsequently enclose other milk constituents (Visentin et al. 2015a).

3.3. Effect of highland grazing and parity on milk yield and composition

In HI compared to LO grazing period MY was lower in both primiparous and multiparous cows (Table II.4.). A small part of this effect could have resulted from the concomitantly progressing lactation. The finding of a depression in MY in HI compared to LO is in agreement findings from a number of studies with pure dairy breed cows with their typically higher initial MY (Christen et al. 1996; Leiber et al. 2005b). Consistently with previous observations by Leiber et al. (2004, 2006), the higher milk fat content at the HI site could be explained by the increase in body fat mobilization of the animals in order to cope with mountain conditions and by the higher content of fibre – the main nutrient transformed to milk fatty acids by *de novo* synthesis from acetate – of the HI compared to the LO pasture (Table II.1.) (Leiber et al. 2006). The Va cows of the present study showed no depression in milk protein content which was repeatedly found when moving pure dairy breed cows to higher altitude pastures (Christen et al. 1996; Leiber et al. 2005b). Milk lactose content was lower on HI than on LO ($P < 0.001$), but only in the multiparous cows (interaction, $P < 0.001$). However, this decline was without practical relevance from its magnitude (Tiezzi et al. 2013). There was also a large decrease ($P < 0.001$) in MUN from LO to HI, which can be explained by excessive dietary CP content of the LO pasture compared to the HI pasture. Like described before (Berry et al. 2001; Leiber et al. 2006), there was an increase by HI grazing in SCS, suggesting an increased incidence of subclinical mastitis. This increase was more pronounced in the primiparous (+200 %) compared to the multiparous cows (+82 %) where the LO level was lower ($P < 0.05$) in the primiparous cows. Possible reasons include carry-over effects from disturbed milk let-down during transport and walking, a higher frequency of injuries of the mammary gland when climbing the steep slopes and a lower hygiene standard when milking on pasture especially during times of intensive precipitation (Berry et al. 2001).

3.4. Effect of highland grazing and parity on milk coagulation traits

Parity had only weak effects on milk coagulation traits. In addition, as could have been expected from the lack of adverse effect on milk protein and CN content, curd firmness (k_{20} and a_{30}) remained unaffected by moving cows from LO to HI (Table II.4). Rennet coagulation time increased from LO to HI, in both primiparous and multiparous cows and concomitantly pH declined. A longer RCT of milk produced on HI pastures was also found by others authors (Leiber et al. 2006) who measured MCP with lactodynamographic tool. Different from that, Zendri et al. (2016b) reported an improvement in MCP predicted from MIRS data in HI compared to LO sites. Leiber et al. (2006) attributed the increase in RCT mainly to the lower protein and CN content of HI milk compared to that of LO, which was not the case in the present study. Thus, the unfavourable effect of HI on RCT found in the present study could be partially due to the concomitantly higher SCS, because a high density of somatic cells is antagonistic in this respect (Politis & Ng-Kwai-Hang, 1988).

A sufficiently high Ca content of the milk is also important for a favourable MCP (Franzoi et al. 2017). The grass from LO pasture was richer in Ca than that from the HI pasture (Table II.1.), but cows always received a Ca containing mineral feed and Ca contents of the milk are difficult to influence by diet anyway as the cow tries to keep milk composition stable for their offspring (Gaucheron 2005). Milk Ca content was not measured in the present study. Overall, it is established that MCP are influenced also by other effects that have not been taken into account, such as laboratory procedures, detailed milk protein and mineral composition (Niero et al. 2016a; Visentin et al. 2016) and genetics of cows (Ikonen et al. 2004). Nevertheless, since the cows used in the present study were the same on LO and HI, the latter factor was not contributing in the present study.

Table II.3. Pearson correlation coefficients between milk composition, milk pH and milk coagulation traits.

Item	Protein	Casein	Lactose	MUN	SCS	pH	RCT	k_{20}	a_{30}
Fat	0.32***	0.29***	-0.16**	-0.33***	0.26***	-0.28***	0.00	-0.32***	0.11
Protein		0.98***	0.03	-0.06	0.20***	-0.06	-0.07*	-0.51***	0.36***
Casein			0.03	0.02	0.21***	-0.23***	-0.06*	-0.52***	0.35***
Lactose				0.37***	-0.50***	0.51***	-0.15*	-0.03	0.16*
MUN					-0.40***	0.26***	-0.08	-0.01	0.09*
SCS						-0.16*	0.22***	-0.12	-0.13*
pH							0.16*	0.27***	-0.17***
RCT								0.46***	-0.84***
k_{20}									-0.84***

MUN, milk urea nitrogen; SCS, somatic cell score; RCT, rennet coagulation time; k_{20}, curd firming time; $a_{30,}$ curd firmness.
**P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Table II. 4. Least squares means of cows of different parity status at different sites on milk yield and milk related traits.

Partity status	Primiparous		Multiparous			*p* values		
Site	Lowland	Highland	Lowland	Highland	SEM	Parity	Site	Parity × Site
No of cows	10	10	37	37				
No of samples	40	40	74	74				
Milk yield (kg/day)	13.3^{bc}	10.8^{c}	19.6^{a}	14.2^{b}	1.10	<0.001	<0.001	0.018
Milk composition								
Fat, %	3.33^{b}	4.19^{a}	3.44^{b}	3.99^{a}	0.183	0.749	<0.001	0.264
Protein, %	3.28	3.34	3.28	3.31	0.070	0.842	0.317	0.702
Casein, %	2.57	2.65	2.54	2.58	0.060	0.437	0.101	0.598
Lactose, %	4.87^{a}	4.80^{a}	4.81^{a}	4.57^{b}	0.041	<0.001	<0.001	<0.001
Urea nitrogen, mg/dL	22.2^{a}	15.5^{b}	22.2^{a}	16.1^{b}	0.82	0.629	<0.001	0.669
Somatic cell score	0.96^{c}	2.88^{ab}	2.15^{bc}	3.91^{a}	0.404	0.008	<0.001	0.704
pH	6.63^{ab}	6.59^{b}	6.64^{a}	6.59^{b}	0.013	0.559	<0.001	0.526
Milk coagulation traits								
Rennet coagulation time, min	16.4^{b}	18.5^{ab}	17.5^{b}	21.1^{a}	1.17	0.083	<0.001	0.339
k_{20}, min	5.63	4.98	5.45	5.15	0.431	0.985	0.136	0.567
a_{30}, mm	30.1	30.1	29.3	30.0	3.25	0.125	0.119	0.134

k_{20}, curd firming time; $a_{30,}$ curd firmness. $^{a\text{-}c}$ Least squares means with different superscripts within a row are significantly different (*P* <0.05).

3.5. Differences between morning vs. evening milk

The effect of daytime of milking was investigated as it has a specific relevance since Fontina cheese has to be manufactured within 2 h after every single respective milking, as stated in the official procedural disciplinary manual (Giannino et al. 2009). Due to the longer nocturnal milking interval (13 h) than that during daytime (11 h), the milk amount was higher ($P < 0.001$) in the morning than in the evening, but this mainly in LO (interaction, $P < 0.001$) (Table II.5.). There were also interactions ($P < 0.01$ to 0.001) between milking time and site in contents of fat, protein and CN as well as SCS resulted affected by milking daytime. In LO, contents of fat, protein and CN were higher ($P < 0.05$) in the evening milk, whereas this difference disappeared in HI. The same was true for SCS. One likely reason for that was that this was mainly a dilution effect in LO where morning and evening milk amount clearly differed. The different milk composition did not result in differences between morning and evening milk in pH, RCT, k_{20} and a_{30}. There was a milking time × site interaction ($P < 0.001$) in k_{20} which decreased in the evening in LO and increased in the evening in HI.

3.6. Non coagulating samples

In the present study, 13.2 % of milk samples did not coagulate within 30 min from rennet addition. This proportion was close to 12.9 % reported by Toffanin et al. (2015), who studied MCP through lactodynamographic tool reference analyses in milk from Holstein Friesian cows kept in an intensive system. Conversely, the proportion found in the present study was considerably greater than the 2.5 % reported by Penasa et al. (2014) for milk from Holstein-Friesian, Brown Swiss and Simmental cows reared in multi-breed herds. Actually, it is quite difficult to compare the results, because Penasa et al. (2014) predicted MCP from MIRS spectra in the time-span from 5 to 30 min and considering samples that showed values out of this range as non-coagulating (NC) milk. In the present study, the proportion of NC milk samples increased from 8.5 % at LO to 15.0 % at HI. On average, the NC milk samples had slightly lower contents of fat, protein and CN (3.69, 3.24 and 2.53 % vs. 3.90, 3.45 and 2.72 %, respectively), but considerably greater SCS (3.35 vs. 2.42) compared with means obtained by the coagulating samples. In the lowland, the NC samples showed a similar fat, protein and CN content (data not shown), but considerably greater SCS (3.24) when compared with mean obtained for coagulating LO milk samples (2.33). Finally, also the highland NC samples had a similar protein and CN content (data not shown), but again a greater SCS (3.60) with respect to the average value obtained for coagulating milk samples of HI (2.50). Overall, these findings suggest that SCS plays a major role in impairing the coagulation aptitude of cow's milk. This relationship was recently confirmed by a study of Summer et al. (2015) when relating SCS in bulk milk and milk coagulation properties.

Table II.5. Least squares means of milking time at different sites on milk yield and milk related traits.

Milking time	Morning		Evening			*p* Value		
Site	Lowland	Highland	Lowland	Highland	SEM	Time	Site	Time × Site
No of cows	7	7	7	7				
No of samples	35	35	35	35				
Milk yield (kg/milking)	8.72^{a}	6.49^{b}	6.96^{b}	6.35^{b}	0.341	<0.001	<0.001	<0.001
Milk composition								
Fat, %	3.31^{b}	3.82^{a}	4.09^{a}	3.85^{a}	0.169	<0.001	0.276	0.002
Protein, %	3.34^{b}	3.42^{b}	3.57^{a}	3.39^{b}	0.050	<0.001	0.150	<0.001
Casein, %	2.61^{c}	2.72^{ab}	2.78^{a}	2.68^{bc}	0.043	<0.001	0.927	<0.001
Lactose, %	4.86^{a}	4.79^{ab}	4.83^{ab}	4.76^{b}	0.041	0.079	0.019	0.929
Urea nitrogen, mg/dL	22.2^{ab}	19.5^{b}	23.7^{a}	19.7^{b}	0.868	0.155	<0.001	0.261
Somatic cell score	1.32^{c}	2.78^{a}	2.07^{b}	2.62^{ab}	0.305	0.022	<0.001	<0.001
pH	6.64	6.64	6.65	6.65	0.012	0.083	0.909	0.212
Milk coagulation traits								
Rennet coagulation time, min	17.7	19.4	18.3	19.8	1.22	0.458	0.070	0.869
k_{20}, min	5.33	4.95	4.67	5.45	0.389	0.705	0.363	0.008
a_{30}, mm	28.5	28.7	29.2	26.2	3.49	0.643	0.525	0.430

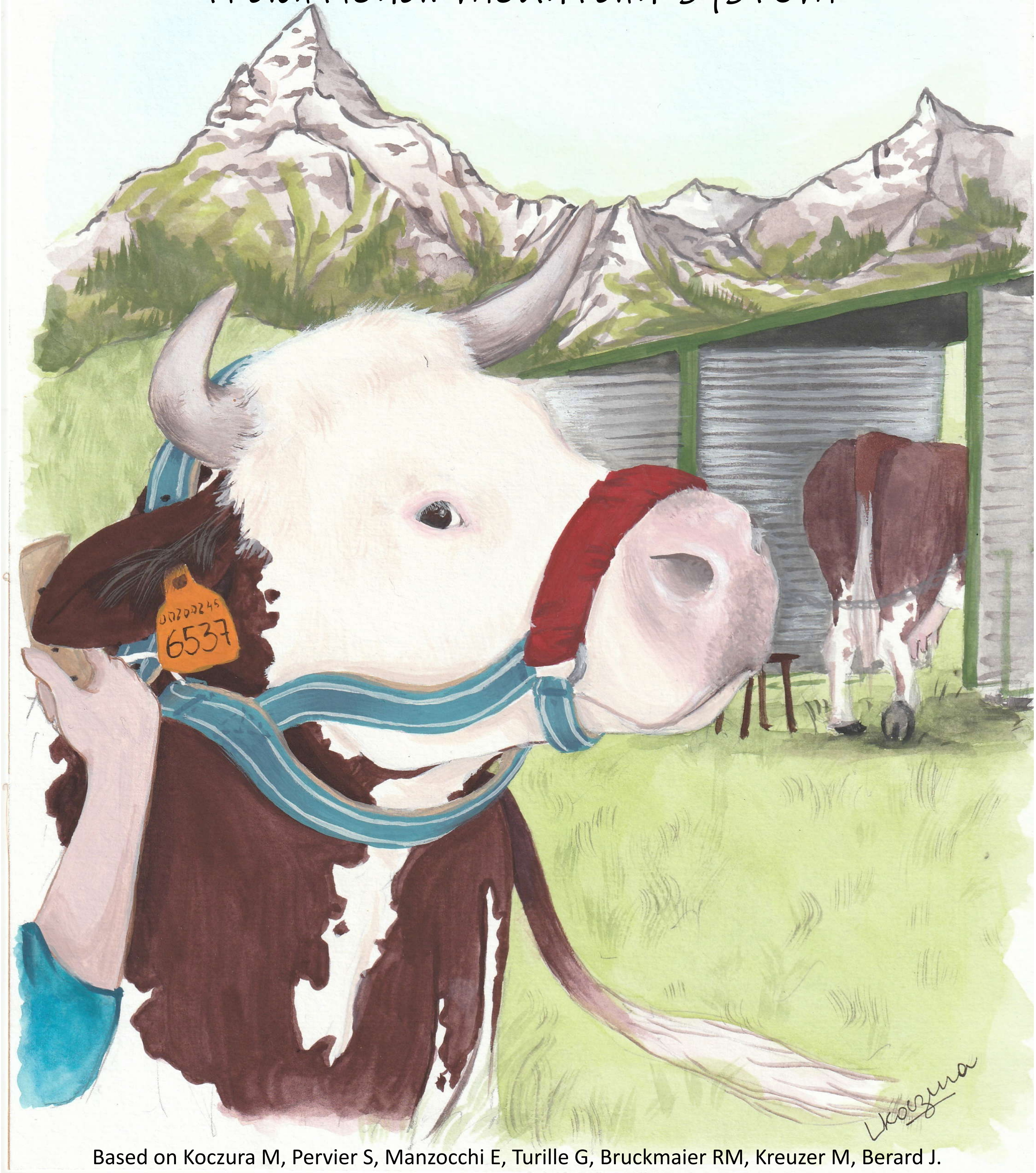
k_{20}, curd firming time; a_{30}, curd firmness 30 min after rennet addition. $^{a\text{-}c}$ Least squares means with different superscripts within a row are significantly different (P <0.05).

4. Conclusion

In the present study, Valdostana Red Pied cows were used as a model to investigate the effect of mountainous farming and grazing on milk coagulation properties of dual purpose cattle. The highland sojourn indeed compromised these properties in terms of rennet coagulation time, while rennet firming and firmness remained unaffected by the transhumance. The latter was different from effects found in the more susceptible specialized dairy breed cows. It was interesting to note that Valdostana Red Pied cows familiar with the transhumance system and the specific mountain pasture areas from previous years (multiparous cows) obviously had no advantage over the primiparous cows. A much undesired phenomenon of the highland conditions was that, along with a substantial increase in somatic cell score, the percentage of non-coagulating milk samples considerably increased compared to the lowland conditions. This indicates that also adapted cow types like the Valdostana Red Pied cattle need special care under highland conditions, where milking hygienic standards are more difficult to maintain and cows are more exposed to situations resulting in mammary gland injuries. As the results show, this not only is important for maintaining cow health but also to produce raw milk with favourable cheese-making properties. Finally, the results show that morning and evening milk appear similarly suitable for cheese-making.

CHAPTER III

Previous mountain grazing experience of cows has little medium-term effect on feeding behaviour, milk yield and composition in a traditional mountain system



Based on Koczura M, Pervier S, Manzocchi E, Turille G, Bruckmaier RM, Kreuzer M, Berard J.
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ABSTRACT

Previous grazing experience on high mountain pastures may help cows to optimise feed selection and minimise impairments in milk production. Eight inexperienced and eight experienced Valdostana Red Pied cows were compared (primiparous:multiparous=1:1). Measurements were performed when cows grazed sites at 600, 1800 and 2100 m a.s.l. The statistical model included experience, parity and site as fixed effects. The longest ingestion time was recorded for primiparous inexperienced cows at 1800 m a.s.l. Milk yield remained unaffected by experience, but main milking time was shorter in inexperienced cows in the lowlands. Milk of inexperienced cows had a higher urea content than experienced cows. Somatic cell score remained unaffected by experience, but inexperienced cows had more incidences of clinical mastitis on mountain pastures. Compared with experienced cows, proportions of the fatty acids ΣC16:1 and ΣC17:1 in milk fat of inexperienced cows were higher and proportions of C18:1 t9 and C18:1 t6–8 lower. Overall, mountain grazing had substantial effects on milk yield and composition coinciding with results from previous studies. There were interactions between experience and site in milking characteristics and milk composition, and for the latter also between experience and parity. In conclusion, experience had effects on several of the variables tested, effects which were sometimes exhibited already on lowland pasture. Still, the effects of experience were much lower than those of the transhumance system.

HIGHLIGHTS

- The advantages of previous site-specific and age experience of cows were distinguished.
- Lack of experience had mostly minor effects on economically relevant traits.
- Experience helped to maintain udder health in this mountain environment.

1. Introduction

Transhumance for exploiting high-altitude pastures for milk production during summer is a traditional practice in mountain regions. Effects of mountain conditions on the cow's physiological status and links to milk and cheese properties were investigated in various previous studies (e.g., Leiber et al. 2006; Sturaro et al. 2013; Farruggia et al. 2014; Zendri et al. 2016b). Conditions in the hypoxic environment of mountain pastures differ from those in the lowlands, and therefore require metabolic and behavioural adaptation of cows. In addition, mountain grazing regularly results in a milk yield decline as a consequence of moving to and staying on these pastures (Kreuzer et al. 1998; Leiber et al. 2006; Zendri et al. 2016a).

Experience affects feeding behaviour and physiological status of ruminants. Accordingly, grazing experience acquired at a juvenile age affects diet selection years later in cows (Lopes et al. 2013). Early exposure of lambs to dietary diversity affected their acceptance of new flavours in new environments and reduced stress (Villalba et al. 2012). Primiparous cows, exposed to a high-quality pasture early in life, exhibited higher milk yields during the first days on pasture than cows unfamiliar with grazing (Lopes et al. 2013). Mountain animal caretakers also report about behavioural differences between cows experienced with the specific conditions of a distinct alp and others arriving there for the first time (Silbernagel 2002). This included that experienced cows remembered the places on mountain pastures where the most palatable feed grows from the previous year. However, it remains unclear whether this knowledge is helpful for feed selection and maintaining milk yield over the whole season. A different feeding behaviour might also affect the mountain-specific fatty acid (FA) profile of the milk, as cows might ingest herbage with a different botanical composition (Elgersma 2015). Low-input systems practiced on biodiverse mountain pastures allow such selection and, concomitantly, affect amount and profile of plant secondary compounds (PSC) ingested (Willems et al. 2014). Accordingly, body lipids of lambs and milk fatty acid profiles of goats varied between different mountain pastures (Willems et al. 2014; Iussig et al. 2015).

The aim of the present study was to test the possible advantages of previous site-specific mountain grazing experience on feeding behaviour, performance, milking characteristics and milk composition. It was further tested if multiparous cows, due to the experience obtained as lactating cows before, had an advantage over primiparous cows. The investigation was performed in an established transhumant mountain system in the Aosta Valley (Northwestern Italy) with autochthonous Valdostana Red Pied (Va) cows.

2. Materials and methods

2.1. Experimental design

All animal-related procedures were in compliance with EU Directive 2010/63/E.U. Supplementary Table S1 gives an overview of the experimental schedule. Sixteen Va cows were used where half were 'experienced' and originated from the herd of the Institut Agricole Régional (IAR, Aosta, Italy). They had experience on the experimental mountain pastures (> 800 m a.s.l.) as cows and on comparable plots in the same valley as heifers before. The other half, obtained from a private herd (Pollein, Aosta Valley, Italy), had never experienced mountain pastures on comparable sites, either as heifers or cows. Animals were randomly selected from these two herds in a way that in both groups, (1) half of the cows were primiparous and half multiparous, and (2) mean values for DIM, milk yield, fat and protein contents were similar during the pre-experimental period. Accordingly, pre-experimental milk yields were 16.3±3.5 (mean±standard deviation) and 15.3±3.2 kg/day in experienced and inexperienced cows, respectively. The corresponding milk composition was 32.2±3.2 and 32.4±2.8 g fat/kg, and 31.5±1.4 and 35.6±1.9 g protein/kg, and cows weighed 514±25 and 522±21 kg. Experienced and inexperienced cows were 121±33 and 114±30 days in milk (DIM), respectively. Individual milk yield, fat and protein contents had been obtained from milk control (one sample/cow/month during 5 months) in their respective home-tied stalls, where they had received hay from their respective lowland pastures and 3 kg/day of concentrate (Mangime Settebello Ma. Co. Pa., Mareine & Cie, Bosconero, Italy) containing, per kg of original substance, 925 g organic matter, 208 g crude protein, 85 g crude fibre and 38 g ether extract. Data on feeding behaviour and milking characteristics were individually collected two times during the weeks before the start of the experiment for their use as covariates.

At the beginning of the grazing season (calendar week 16), the 16 experimental cows were integrated in the IAR herd and grazed on lowland pastures. Cows were transported by truck from 600 m (Montfleury, Aosta) to 1800 m a.s.l. (Alp Chaudanne), and later walked on a steep track to Alp Entrelor (2100 m a.s.l.). On each site, samples were collected 9 and 28 days after moving cows to the respective pasture (equivalent to calendar weeks 17 and 20, 25 and 27, and 29 and 32 on the three pastures, Supplementary Table III.S1). We focused on medium-term effects (9 days after arrival) of experience in grazing on mountain pastures and therefore sampling started at a time where there were no longer carry-over effects of the transhumance. In the lowlands, cows started grazing outside during daytime, were tethered at night and milked at 0500 and 1600 h (AfiMilk, TDM, Brescia, Italy). They received local hay and 3 kg/day of the same concentrate as that used in the pre-experimental period, and had free access to water. At high altitude, cows had permanent access to pasture and water resources. Throughout the grazing period, a new portion of the pasture was offered daily to the animals (strip grazing technique). Milking was accomplished outside with a mobile milking parlour (Eliar 4, Eli IAR, equipped with AfiMilk machines) at 0400 and 1600 h, where cows received exclusively 2 kg/day of the same concentrate manually.

2.2. Sampling protocols

Botanical composition of the pastures was recorded using the point-quadrat method (Daget & Poissonet, 1971). Samples of fresh grass were collected two times per sampling period from 10 cm × 10 m strips dispersed randomly across the area offered to the cows. Compositional analysis was performed with NIRSystems 5000 (Foss, Hillerød, Denmark; Niero et al. 2018). Feeding behaviour of the cows was recorded using chewing sensors and a data analysis software (MSR Electronics, Henggart, Switzerland) (Nydegger et al. 2011). Each of the 16 cows was equipped with these sensors during 2 × 24 h on each site.

Individual milk-related measurements were conducted during two morning and evening milkings per period. A Lactocorder® (WMB AG, Balgach, Switzerland) provided data on milk yield, milking characteristics and electrical conductivity. Milking time was separated into time to reach the plateau (incline phase, flow < 0.500 kg/min), durations of plateau and decline phases, and main milking time (start to a decline to 0.200 kg/min). Two 50–ml milk samples/day were taken with the Lactocorder equipment. The morning samples were stored at 4°C, and the evening samples at -20°C. For the baseline winter barn period, an average between data on milk yield and composition during the 5 months before the experiment were obtained along with the official milk control analyses of the breeders' association of Aosta. Milk samples then were obtained with the sampling device MPC (AfiMilk, TDM, Brescia, Italy).

In the morning milk, mid-infrared spectroscopy (MilkoScan FT6000, Foss Electric A/S, Hillerød, Denmark) was used to determine milk gross composition. Somatic cells were counted by a fluorimetric method (Fossomatic 5000, Foss Electric, Hillerød, Denmark) and converted into somatic cell scores (SCS; Ali and Shook 1980). All devices were calibrated monthly using reference samples (Regione Autonoma Valle d'Aosta 2006). Clinical mastitis was assumed when milk conductivity was > 9 µS/cm and milk reacted positively with Leucocytest solution (Sacco System, Cadorago, Italy). The percentage of cows with clinical mastitis was calculated. Milk compositional data of these cows were removed from the dataset. The milk FA profile was determined by gas chromatography (Hewlett-Packard HP, Waldbronn, Germany) following Kälber et al. (2011), but with a CP7421 column (200 m × 0.25 mm × 0.25 µm, Varian Inc., Darmstadt, Germany). Individual FA were grouped into saturated FA (SFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), n-3 FA, n-6 FA and conjugated linoleic acids (CLA), and the *iso*-to-*anteiso* FA. The C14:1 c9 to C14:0 ratio was calculated.

2.3. Statistical analysis

After the normality of the data was checked using Shapiro-Wilk's test, Repeated Time Mixed Procedure was run with SAS version 9.4 (SAS Institute, Cary, NC, USA). Individual cow data obtained in the pre-experimental lowland barn period were used as a parity-centred covariate, as statistical differences were observed for milk yield and protein content already in the barn. As no significant differences between sampling days occurred within the period (9 and 28), these data were averaged. The model included previous site-specific mountain grazing experience (Yes vs. No), parity (1 vs. > 1), site (600, 1800 vs. 2100 m a.s.l.) and all their interactions as fixed effects. Site was used as repeated factor with a compound symmetry covariance structure, with the cow (experimental unit) being the subject nested within group. For milk yield, DIM was used as covariate to differentiate between DIM and site effect. Multiple comparisons among means were adjusted with Tukey's method. Effects were considered significant at $p<.05$ and as trend at $.05 \leq p<.10$. The tables give least square means and standard errors of the mean.

3. Results

3.1. Composition of the grass

Grass from mountain pastures at 1800 and 2100 m a.s.l. was generally richer in acid detergent fibre and lignin than lowland grass (Table III.1.). All other constituents analysed were quite similar at all three sites, with generally moderate contents of crude protein. Details of the botanical composition of the experimental pastures are listed in Supplementary Table S2.

Table III.1. Chemical composition of the pasture grass (g/kg DM)

Site (m a.s.l.)	600	1800	2100
Dry matter (g/kg grass)	168±7*	179±7	184±1
Crude protein	119±10	125±1	128±6
Ether extract	23.5±0.8	25.1±1.3	27.2±0.8
Neutral detergent fibre	442±7	452±5	455±18
Acid detergent fibre	284±3	335±11	327±11
Acid detergent lignin	41.6±0.1	62.5±1.8	61.3±1.2
Calcium	7.23±1.38	7.68±0.46	6.18±0.81
Phosphorus	2.97±0.11	2.60±0.00	2.57±0.04

*n=2; means±standard deviation.

3.2. Feeding behaviour

Inexperienced cows tended (P < 0.10) towards a longer rumination time (342±21.7 min/day) compared to experienced cows (286±18.7 min/day) (Table III.2.). Ingestion time (min/day) increased (P < 0.05) from lowland (287±28.3) to 1800 m a.s.l. (416±33.7) and declined thereafter to 335±34.6. Ingestion time doubled for inexperienced primiparous cows (P < 0.05) between lowland and 1800 m a.s.l. (P < 0.05), but not in experienced primiparous cows (three-way interaction, (P < 0.10)). The ingestion-to-rumination ratio changed like ingestion time. The multiple comparison among means using Tukey's method showed that the total number of ingestion chews (× 10^3/day) was higher (P < 0.05) at 1800 m (27.6±2.43) than at 600 m (20.1±2.07) and at 2100 m a.s.l. (21.5±2.50), independent of parity and experience, even though the three-way interaction was significant. The number of rumination boli/day was higher (P < 0.05) at 2100 m (470±21.0) than at 600 m a.s.l. (403±15.5).

3.3. Milking characteristics and milk properties

The DIM-adjusted milk yield was not affected by the transfer from lowland to 1800 m a.s.l., but declined ((P < 0.05), Table III.3) from 12.5 to 11.3 kg/day at 2100 m a.s.l. Milking characteristics were mainly influenced by experience and site; changes within sites occurred for experienced cows only. Main milking time declined between lowland (7.27±0.292 min) and mountain sites (5.65±0.284 and 5.39±0.284 min for 1800 and 2100 m a.s.l., respectively) only for experienced cows. The milking plateau phase of experienced cows did not decline at 1800 m a.s.l. (3.27±0.284 min) whereas it was lower (P < 0.05) at 2100 m a.s.l. (2.57±0.284 min) than at 600 m a.s.l. (3.78±0.292 min). However, the experienced cows' decline phase was already reduced at 1800 m a.s.l. (2.06±0.284 min) compared to 600 m a.s.l. (3.19±0.292 min) and did not decline between 1800 and 2100 m a.s.l. (2.55 min±0.284 min) (Figure III.1.). Maximum milk flow decreased (P < 0.05) from 1.70±0.101 kg/min to 1.53±0.103 min when cows went from 1800 m to 2100 m a.s.l. Multiparous cows had a longer (P < 0.05) decline phase and a reduced (P < 0.05)maximal milk conductivity compared to primiparous cows.

Table III.2. Effect of experience, site and parity on feeding behaviour

Variable	Experience (E)[1]	Site (m a.s.l.) (S) and parity (P): 600		1800		2100		SEM	*p*-values						
		1	>1	1	>1	1	>1		E	P	S	E×P	E×S	P×S	E×P×S
Ingestion time (min/day)	Yes	258^{ab}	316^{ab}	342^{ab}	450^{ab}	259^{ab}	341^{ab}	84.2	0.520	0.946	0.001	0.174	0.558	0.229	0.063
	No	245^{b}	327^{ab}	516^{a}	357^{ab}	443^{ab}	294^{ab}								
Rumination time (min/day)	Yes	267	325	205	324	298	299	69.9	0.097	0.702	0.560	0.128	0.848	0.206	0.999
	No	355	312	318	338	411	317								
Idling time (min/day)	Yes	876	793	888	661	879	794	132.7	0.462	0.646	0.242	0.210	0.673	0.487	0.369
	No	863	798	660	742	649	826								
Ratio of ingestion to rumination	Yes	0.94	0.98	1.73	1.39	0.95	1.23	0.259	0.520	0.584	<0.001	0.620	0.373	0.056	0.153
	No	0.79	1.10	1.49	1.08	1.28	0.94								
Chews during total ingestion (10^3/day)	Yes	17.8	22.5	20.3	31.3	14.9	23.2	6.07	0.499	0.662	0.007	0.143	0.514	0.417	0.046
	No	16.8	23.0	34.5	24.1	28.6	19.4								
Chews during total rumination (10^3/day)	Yes	16.5	21.4	14.8	22.7	20.7	20.1	4.70	0.541	0.734	0.748	0.128	0.949	0.386	0.892
	No	20.9	18.1	20.9	20.3	23.9	18.9								
Number of rumination boli/day	Yes	372	429	379	433	492	445	52.2	0.747	0.497	0.048	0.134	0.986	0.172	0.967
	No	411	401	436	401	525	418								

Values are Least Square means, standard errors of the means (SEM) and *p*-values.

[1]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures.

[ab] Within the same variable (rows Yes and No experience), least square means without a common superscript differ (*P* <0.05).

Table III.3. Effect of experience, site and parity on milk yield, milking characteristics and milk properties.

Variable	Experience (E)[1]	600: 1	600: >1	1800: 1	1800: >1	2100: 1	2100: >1	SEM	E	P	S	E×P	E×S	P×S	E×P×S
		Site (m a.s.l.) (S) and parity (P)							*p*-values						
Milk yield (kg/day)	Yes	12.5	13.8	12.6	13.9	12.0	12.3	1.93	0.192	0.801	0.013	0.626	0.848	0.187	0.567
	No	10.8	11.6	12.1	11.4	11.0	10.1								
Milking characteristics															
Main milking time (min)	Yes	7.18	7.96	5.82	5.39	5.19	5.77	0.621	0.004	0.248	<0.001	0.644	0.012	0.269	0.636
	No	4.41	5.40	4.09	4.65	3.80	4.45								
Incline phase (min)	Yes	0.25	0.23	0.19	0.21	0.27	0.22	0.028	0.299	0.139	0.067	0.912	0.810	0.710	0.232
	No	0.28	0.24	0.24	0.21	0.24	0.25								
Plateau phase (min)	Yes	3.69	3.19	3.16	2.83	2.12	2.14	0.459	0.120	0.125	0.005	0.454	0.028	0.734	0.908
	No	2.83	1.97	2.53	1.84	2.58	1.90								
Decline phase (min)	Yes	3.24	4.02	2.40	1.95	2.74	3.15	0.605	0.065	0.046	0.014	0.147	0.080	0.270	0.810
	No	1.46	3.35	1.54	2.76	1.14	2.47								
Maximum milk flow (kg/min)	Yes	1.32	1.78	1.37	1.92	1.37	1.67	0.223	0.777	0.389	0.008	0.202	0.690	0.502	0.264
	No	1.71	1.52	1.79	1.73	1.55	1.53								
Electrical conductivity (µS/cm)															
	Yes	5.84	6.20	5.75	6.10	5.77	6.21	0.158	0.275	0.001	0.990	0.730	0.466	0.981	0.856
	No	5.82	6.27	5.88	6.39	5.87	6.31								

Values are Least Square means, standard errors of the means (SEM) and *p*-values. DIM, days in milk.

[1]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures

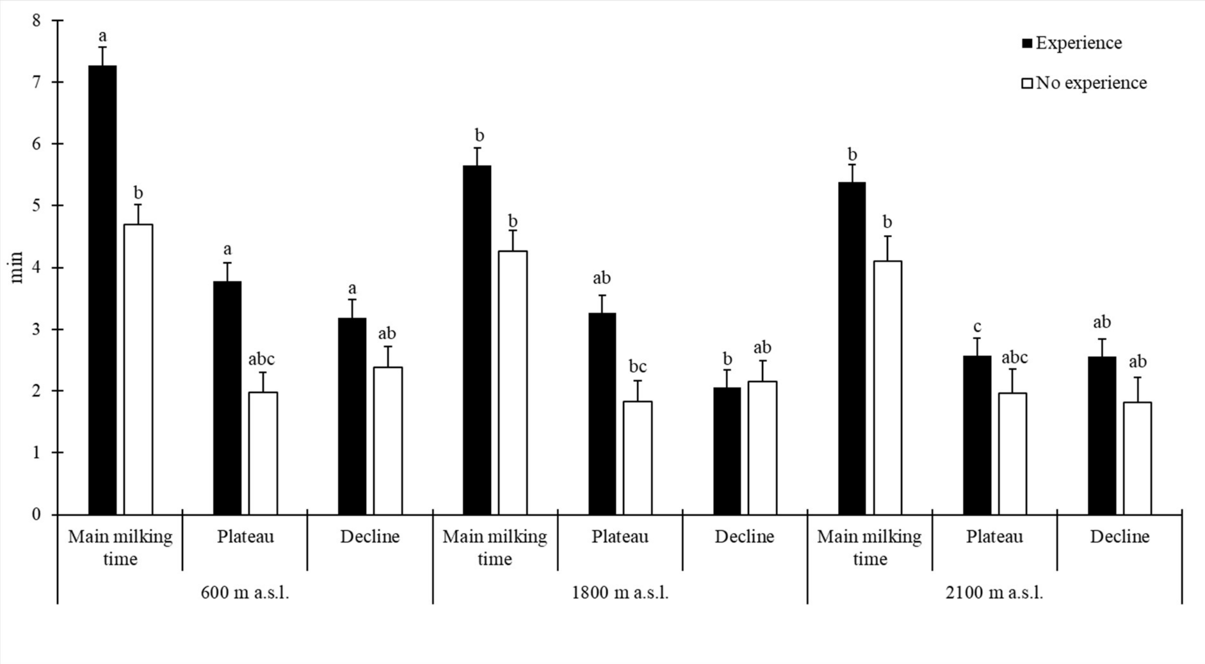

Figure III.1. Effect of experience at the sites on durations of main milking time, plateau and decline phase of milking (interactions of experience × site, $P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively). Error bars reflect standard errors. Within the same variable, columns (Least Square means) marked without a common superscript differ ($P < 0.05$).

There were some effects of experience (without interaction with site or parity) on milk composition, and a number of significant interactions, but not always the same in different traits (Table III.4.). Milk casein content decreased ($P < 0.05$) in inexperienced cows at 1800 m a.s.l. but remained unchanged in experienced cows. Milk urea content was 1.4 times higher ($P < 0.05$) and, along with a general increase in SCS ($P < 0.05$) from lowland to mountain pastures, SCS was 2.4 times higher ($P < 0.05$) in inexperienced cows compared to experienced cows, respectively. The latter difference was numerically highest at 600 m a.s.l. and declined thereafter (experience × site, $P < 0.001$). More inexperienced than experienced cows exhibited clinical mastitis on mountain pastures (37.5 and 12.5% vs. 12.5 and 0% of inexperienced and experienced cows at 1800 m and 2100 m a.s.l., respectively; data not shown). Milk protein content was higher in primiparous than multiparous experienced cows (36.7±0.76 and 33.5±0.92 g/kg, respectively, $p<.05$). Milk fat increased ($p<.05$) from 29.0±1.42 g/kg at 600 m a.s.l. to 34.9±1.56 g/kg at 1800 m a.s.l. and 40.0±1.85 g/kg at 2100 m a.s.l. Milk lactose declined ($P < 0.05$) from 600 to 1800 m a.s.l. Site effects on contents of fat were recovered in total solids and on lactose in solids-non-fat.

Table III.4. Effect of experience, site and parity on milk composition.

Variable	Experi-ence (E)[1]	Site (m a.s.l.) (S) and parity (P) 600		1800		2100		SEM	p-values E	P	S	E×P	E×S	P×S	E×P×S
		1	>1	1	>1	1	>1								
Total solids (g/kg)	Yes	123	111	132	120	132	126	7.2	0.476	0.074	0.007	0.261	0.050	0.765	0.366
	No	125	127	125	122	134	128								
Solids non-fat (g/kg)	Yes	94.0[ab]	88.3[bc]	92.5[abc]	87.4[c]	92.9[abc]	86.9[c]	2.18	0.356	0.056	<0.001	0.060	0.040	0.171	0.094
	No	92.7[abc]	95.4[a]	90.8[abc]	88.9[bc]	91.3[abc]	90.3[abc]								
Fat (g/kg)	Yes	28.5	22.3	38.9	31.8	38.1	38.6	5.56	0.165	0.257	<0.001	0.438	0.133	0.813	0.414
	No	31.7	33.2	35.0	34.1	43.0	40.1								
Protein (g/kg)	Yes	36.2	32.7	36.7	33.6	37.3	34.2	1.71	0.722	0.163	0.335	0.029	0.026	0.629	0.285
	No	35.2	37.4	34.8	35.1	35.6	35.9								
Casein (g/kg)	Yes	28.4	25.1	28.6	25.6	29.1	26.2	1.02	0.019	0.109	0.130	0.032	0.008	0.346	0.135
	No	28.9	30.8	28.2	28.5	29.2	28.7								
Casein (g/kg total protein)	Yes	798	794	792	785	795	790	7.2	0.147	0.176	<0.001	0.805	0.484	0.351	0.828
	No	792	787	785	773	790	785								
Lactose (g/kg)	Yes	50.3	48.8	48.4	46.9	48.2	45.9	0.99	0.152	0.108	<0.001	0.980	0.325	0.228	0.227
	No	48.6	48.1	46.8	43.9	46.6	44.5								
Urea (mg/kg)	Yes	167	143	192	176	177	161	32.5	0.009	0.267	0.207	0.922	0.252	0.895	0.728
	No	232	219	244	209	252	234								
Somatic cell score[2]	Yes	0.69[c]	1.47[bc]	2.66[ab]	2.08[abc]	2.53[ab]	2.91[abc]	0.642	0.038	0.229	0.001	0.433	0.073	0.305	0.013
	No	3.03[abc]	2.42[abc]	2.85[abc]	4.25[a]	1.90[abc]	3.74[abc]								

Values are Least Square means, standard errors of the means (SEM) and *p*-values.

[1]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures.

[2]Somatic cell score=log2 (somatic cell count/100)+3.

[abc] Within the same variable (rows Yes and No experience), least square means without a common superscript differ ($P < 0.05$)

Proportions of some C18:1 *trans* isomers were influenced by experience, whereas this did not apply for C18:0, C18:1 n-9, C18:2 n-6, C18:2 c9t11 and C18:3 n-3 (Table III.5.). Proportions of C18:1 t6–8&t9 were higher ($P < 0.05$) by 18.2 and 24.3%, respectively, in milk of experienced compared to inexperienced cows. For C18:1 t10 & t12, the experience effect was less systematic. C18:1 t10 proportion was higher (p<.05) in experienced than inexperienced primiparous cows, but only at 600 m a.s.l., and higher ($P < 0.05$) than in experienced multiparous cows, but only at 2100 m a.s.l. ($P < 0.05$). Apart from an interaction with site for C18:2 c9t11 and C18:1 t11, parity had no effect on C18 FA proportions. Site increased ($P < 0.05$) proportions of C18:0, C18:1 t6–8, C18:1 t9, C18:1 t11, C18:2 n-6, C18:2 c9t11 and C18:3 n-3 by 13.9, 14.5, 17.9, 38.1, 19.6, 27.5 and 52.2%, respectively, between 600 and 1800 m a.s.l.

Among the short- and medium-chain-length FA (C4 to C17) only C8:0, ΣC16:1 and ΣC17:1 were affected ($P < 0.05$) by experience and only one interaction (C15:1 c10; with site, P < 0.01) was found (Supplementary Table III.S3). Experience enhanced proportions of C8:0 and reduced those of ΣC16:1 and ΣC17:1. Almost every short- and medium-chain-length FA was affected by site. C4:0 proportion increased ($P < 0.05$) by 9.4 and 12.6% at 1800 and 2100 m a.s.l. compared to 600 m a.s.l. Proportions of C8:0, C10:0, C12:0, C15:0, C16:0, ΣC16:1 and ΣC17:1 decreased ($P < 0.05$) by mountain grazing by 8.8, 15.5, 17.2, 6.3, 9.0, 17.3 and 17.4%, respectively. Proportions of C14:0 and C14:1 c9 decreased by 15.6 and 10.3%, respectively, at 1800 m a.s.l., then increased at 2100 m a.s.l. ($P < 0.05$). Experience and parity did not affect proportions of FA > C18 (Supplementary Table III.S4). Proportions of C20:0, ΣC20:1, C20:2 n-6, C20:4 n-6, C20:4 n-3, C22:0 and C22:1 c13 increased (p<.05) by 12.9, 20.7, 13.7, 76.2, 65.9, 18.8 and 38.9% between 600 and 1800 m a.s.l., respectively. C20:3 n-3 proportion decreased ($P < 0.05$) between 1800 and 2100 m a.s.l. by 82.7%.

Site significantly affected every group of FA (Table III.6). Interactions found with total CLA resembled those in C18:2 c9t11. The *iso*-to-*anteiso* FA ratio was higher ($P < 0.05$) for experienced than inexperienced multiparous cows at 2100 m a.s.l. Moreover, it increased ($P < 0.05$) in experienced multiparous cows and inexperienced primiparous cows from 600 to 1800 m a.s.l. The SFA proportion ($P < 0.05$) decreased by 5.2% from lowland to mountain pastures. Proportions of PUFA, total CLA, n-3 and n-6 FA increased ($P < 0.05$) by 28.8, 23.2, 44.5 and 24.1% between 600 and 1800 m a.s.l., respectively, and returned to an intermediate level from 1800 to 2100 m a.s.l. for PUFA and total CLA ($P < 0.05$). Between 1800 and 2100 m a.s.l., n-6 FA proportion returned to its previous level at 600 m a.s.l. The n-6–to-n-3 FA ratio decreased ($P < 0.05$) by 32.4% between 600 and 2100 m a.s.l. The ratio C14:1 c9 to C14:0 was higher in milk of inexperienced cows (0.119±0.002) than in that of experienced cows (0.111±0.003, $P < 0.05$).

Table III.5. Effect of experience, site and parity on selected individual C18 fatty acids in total milk fat (g/100 g).

		Site (m a.s.l.) (S) and parity (P)													
	Experi-	600		1800		2100			*p*-values						
Variable	ence (E)[2]	1	>1	1	>1	1	>1	SEM	E	P	S	E×P	E×S	P×S	E×P×S
C18:0	Yes	10.7	10.3	12.6	11.5	12.3	11.8	0.822	0.331	0.298	<0.001	0.979	0.905	0.564	0.956
	No	10.2	9.79	11.8	10.9	11.8	11.0								
C18:1	Yes	23.8	21.7	22.7	22.0	23.3	21.6	1.55	0.374	0.622	0.989	0.509	0.834	0.365	0.952
n-9	No	23.9	23.4	23.5	24.3	23.6	24.0								
C18:	Yes	0.293	0.279	0.315	0.317	0.330	0.334	0.020	0.009	0.405	<0.001	0.551	0.252	0.293	0.142
1 t6–8[1]	No	0.238	0.238	0.279	0.287	0.295	0.241								
C18:1	Yes	0.297	0.271	0.329	0.331	0.335	0.338	0.024	0.008	0.167	0.001	0.507	0.618	0.455	0.402
t9	No	0.239	0.221	0.273	0.277	0.285	0.237								
C18:1	Yes	0.445[a]	0.374[abcd]	0.429[a]	0.464[a]	0.410[abc]	0.427[ab]	0.034	<0.001	0.004	0.046	0.034	0.883	0.347	0.026
t10	No	0.318[cd]	0.319[bcd]	0.376[abcd]	0.352[abcd]	0.372[abcd]	0.263[d]								
C18:1	Yes	2.78	3.07	4.13	4.06	4.02	3.68	0.367	0.685	0.818	<0.001	0.937	0.900	0.037	0.577
t11	No	3.09	3.15	4.19	4.31	4.16	3.73								
C18:1	Yes	0.385[abc]	0.369[bc]	0.419[abc]	0.471[a]	0.377[abc]	0.397[abc]	0.037	0.063	0.241	<0.001	0.068	0.877	0.704	0.019
t12	No	0.308[c]	0.277[c]	0.418[ab]	0.324[abc]	0.364[abc]	0.263[c]								
C18:2	Yes	2.23[abc]	2.36[abc]	2.56[abc]	2.78[ab]	2.36[abc]	2.61[abc]	0.208	0.179	0.102	<0.001	0.793	0.223	0.265	0.053
n-6	No	1.70[c]	2.27[abc]	2.31[ab]	2.58[a]	2.02[abc]	2.01[bc]								
C18:2	Yes	1.43	1.47	1.67	1.91	1.76	1.68	0.143	0.464	0.982	<0.001	0.538	0.258	0.006	0.890
c9t11	No	1.52	1.47	1.92	2.02	1.88	1.60								
C18:3	Yes	0.843	0.957	1.40	1.32	1.29	1.39	0.090	0.531	0.165	<0.001	0.868	0.307	0.901	0.639
n-3	No	0.783	0.878	1.27	1.40	1.24	1.29								

Values are Least Square means, standard errors of the means (SEM) and *p*-values.
[1]t6, t7 and t8 C18:1 are presented summed here, as they could not be differentiated by the applied chromatographic conditions.
[2]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures.
[abcd] Within the same variable (rows Yes and No experience), least square means without a common superscript differ ($P < 0.05$).

Table III.6. Effect of experience, site and parity on the groups of fatty acids (FA) in total milk fat (g/100 g).

Variable	Experience (E)[1]	Site (m a.s.l.) (S) and parity (P): 600, 1	600, >1	1800, 1	1800, >1	2100, 1	2100, >1	SEM	*p*-values: E	P	S	E×P	E×S	P×S	E×P×S
Saturated FA	Yes	60.4	61.1	57.7	57.7	58.1	59.0	2.30	0.355	0.484	<0.001	0.665	0.862	0.464	0.934
	No	57.3	59.7	55.0	56.2	55.4	58.4								
Monounsaturated FA	Yes	32.4	31.5	33.1	33.1	33.4	32.2	2.04	0.261	0.428	0.060	0.685	0.975	0.400	0.922
	No	35.4	32.6	35.8	34.7	35.9	33.7								
Polyunsaturated FA	Yes	5.54	5.82	7.49	7.45	6.86	7.07	0.475	0.994	0.852	<0.001	0.783	0.535	0.431	0.501
	No	5.74	6.06	7.42	7.49	7.02	6.53								
Conjugated linoleic acids[2]	Yes	1.55	1.57	1.83	2.05	1.91	1.81	0.204	0.882	0.780	<0.001	0.950	0.852	0.036	0.672
	No	1.62	1.55	1.74	2.12	1.89	1.67								
n-6 FA	Yes	2.37	2.40	3.32	2.88	2.59	2.72	0.374	0.706	0.701	0.003	0.440	0.638	0.424	0.563
	No	2.17	2.72	2.79	2.95	2.44	2.49								
n-3 FA	Yes	0.916	1.07	1.35	1.46	1.36	1.51	0.104	0.598	0.127	<0.001	0.815	0.356	0.702	0.966
	No	0.964	1.11	1.47	1.54	1.36	1.46								
n-6 FA/ n-3 FA	Yes	2.50	2.22	2.45	1.96	1.86	1.80	0.303	0.865	0.313	0.001	0.350	0.482	0.793	0.663
	No	2.40	2.54	1.97	1.96	1.87	1.76								
Iso/anteiso[3]	Yes	1.64^{abcd}	1.62^{cde}	1.66^{abcd}	1.77^{ab}	1.65^{abcd}	1.69^{abc}	0.051	0.187	0.424	<0.001	0.075	0.002	0.171	0.002
	No	1.60^{bde}	1.61^{abc}	1.77^{ac}	1.64^{abc}	1.62^{bde}	1.44^{d}								
C14:1 c9/ C14:0	Yes	0.104	0.107	0.102	0.118	0.114	0.121	0.006	0.046	0.112	0.007	0.394	0.124	0.029	0.921
	No	0.115	0.114	0.118	0.129	0.120	0.118								

Values are Least Square means, standard errors of the means (SEM) and *p*-values.

[1]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures.

[2]CLA include C18:2 c9t11, C18:2 c9c11, C18:2 t9t11.

[3]C12:0 iso, C13:0 iso, C14:0 iso, C14:0 aiso, C15:0 iso, C16:1 iso, C16:1 aiso, C17:1 iso, C17:1 aiso.

[abcde] Within the same variable (rows Yes and No experience), least square means without a common superscript differ (P < 0.05).

4. Discussion

4.1. Effects of mountain grazing

The present study involves a common type of mountain transhumance system, where cows are moved to progressively higher altitudes. This scheme offers grass of sufficient quality throughout the season due to the delayed vegetation growth at higher altitude. The nutrient composition of the forages was quite similar at the three sites except that the mountain grass contained more lignified fibre, consistent with the botanical composition found. The phenology of the plants may also have contributed, but this was not recorded in the present study. Indeed, highly digestible grasses make up a smaller proportion in mountain swards (Jeangros et al. 1999).

Most variables measured in the cows changed between sites. Ingestion time was lower than previously observed by O'Driscoll et al. (2010) with Holstein-Friesian cows, which most probably had higher intake capacity and energy requirements than Vadostana Red Pied in the present study. Its increase when cows were moved from 600 to 1800 m a.s.l. likely resulted from the extension of the time on pasture, as rearing system changed between these sites. Indeed, with cows milked once a day, the same authors showed that the extra time allowed on pasture resulted in an increased grazing time. Romanzin et al. (2018) showed that a low level of supplementation increased the grazing time on low-energetic heterogeneous pastures, which could be another possible explanation. The decline in ingestion time at 2100 m a.s.l. might indicate that cows spent less time searching for the more palatable grasses. Moreover, the cows' energy requirements declined with the advancing stage of lactation, which probably diminished their ingestion time, too. Differences between pastures in lignified fibre were obviously too small to cause rumination time to differ. Cows with higher milk yield typically express a substantial decline in milk yield when moved to high altitude (Leiber et al. 2006). Causes are the limited nutrient supply by the grass and the extra energy needed to cope with hypoxia and climbing on steep slopes (Leiber et al. 2006). Also in the present study, milk yield declined to some extent from 1800 to 2100 m a.s.l. However, this decrease was smaller than that reported from other studies with Va cows (Gorlier et al. 2012; Renna et al. 2010). The transfer from 600 and 1800 m a.s.l. was tolerated by this robust breed, even without milk yield decline going beyond that caused by the progressing lactation, as DIM-adjusted values show. At 2100 m a.s.l. the harsher conditions (higher temperature difference between night and day, hypoxic environment, topography) may have prevented the full exhibition of the milk yield potential. Under stress like changing the milking environment, shorter milking times and changes in milk fat content were expected, as milk ejection may be inhibited (Wellnitz and Bruckmaier 2001). In the present study, this was only the case for inexperienced cows at the lowland site. A confounding effect by changing milking equipment with different properties (e.g., vacuum strength) when moving from lowland to mountain pastures could not be excluded.

In the lowland, milk fat content was unexpectedly low compared to Renna et al. (2014). However, to our knowledge, previous measurements only featured hay. In the present study, cows in the lowland grazed on young and less fibrous grass, with fibre being the substrate for milk fat synthesis (Chilliard et al. 2001). The milk composition found on mountain pastures was consistent with previous studies using Va cows (Battaglini et al. 2005; Renna et al. 2010), even though grazing system and location were not the same. The milk fat content continuously increased during the mountain sojourn, because of the increasing need for body fat mobilisation (Kreuzer et al. 1998) and the elevated fibre content of the grass. The continuous decrease in lactose was likely only an effect of the progressive lactation (Dillon et al. 2003). Different from previous studies (Leiber et al. 2005, 2006), protein and urea contents remained unaffected by the mountain sojourn in the present experiment. This may be related to the comparably low requirements of the Va cows for energy, the most limiting factor for milk protein synthesis. The effect of change from lowland to mountain grazing in FA profile was the subject of several previous studies (e.g. Bugaud et al. 2001; Leiber et al. 2005), and, more specifically, studies with Va cows (Battaglini et al. 2003, 2005; Renna et al. 2010). Consistent with that, the mountain milk fat was characterised by low SFA and high MUFA, PUFA and CLA proportions. The n-6–to-n-3 FA ratio was also changed towards n-3 FA as expected (Leiber et al. 2005), although long-chain n-3 FA were present in minutes amounts. Elevated CLA, *trans* C18:1 FA (especially C18:1 t11) and n-3 FA, and less C18:0 suggest that PSC in mountain forage might have inhibited ruminal biohydrogenation of part of the PUFA (Buccioni et al. 2012). Proportions of branched-chain *iso*- and *anteiso*-FA, indicative of rumen microbial activity, were higher in milk fat in the mountain period, a shift which is further enhanced by diets poor in starch and rich in fibre (Zhang et al. 2017).

4.2. Effect of experience

Cows start learning how to graze as young animals, mostly by observing older or more experienced animals (Costa et al. 2016). However, this opportunity was not given to calves in the current Va husbandry system. Cows have a long memory, leading them to remember where older animals were grazing and on what (Fraser and Broom 1997). Touching the grass and exploring are important for feeding behaviour, too (Distel and Provenza 1991; Currie 1995). It was reported that older cattle spend less time grazing than younger ones because of their eating experience, but still ingest for a longer time when arriving on a new pasture because they spend more time exploring (Krysl and Hess 1993). However, this was not the case in the present study. Eventually, feeding behaviour depends on prior bad eating experience and recognition of the presentation of plants as forages (Distel et al. 1995; Provenza et al. 2015). Moreover, cows have cognitive and physiologic abilities to determine whether they achieved their nutritional and energetic needs (Provenza et al. 2015). This can also affect their grazing behaviour.

Taken together, previous experience on specific mountain pastures could therefore result in more efficient feeding behaviour. Animals originated from two different farms, but their genetic background was similar. Thus, differences in milk yield and composition can be attributed to the experience with experimental conditions and associated behavioural differences. Cows of both groups were found to be similar in feeding behaviour and milk yield on mountain pastures. The only direct effect of experience was a tendency towards a reduced rumination time. This could indicate the ability of experienced cows to select plants with higher digestibility. However, this needs to be confirmed by a more in-depth analysis of the feeding behaviour. Finally, the results indicate that when using robust breeds in extensive mountain farming systems, previous experience would not provide a general medium-term advantage. Experience could be more important directly after transhumance, but its effect would be confounded with that of the walk or transport to the highland.

Milking dynamics were affected by experience, but this was already the case in the lowlands. Since the inexperienced cows, different from the experienced cows, did not know milking equipment and staff in the farming system investigated, the effects observed were likely due to the novel milking procedure and not to the lack of pasture-related experience, even though this was determined after an adaptation period. Overall, milk flow data indicate that milking time of experienced cows was longer compared to inexperienced cows at the lowland site. As all milking machines were left on each cow for the same length of time, this could have led to overmilking of the inexperienced cows. The breeder of inexperienced animals may have selected cows with short milking time, which might have intensified the problem. Overmilking is detrimental to udder health (Edwards et al. 2013). This could also explain why SCS and incidence of clinical mastitis were subsequently higher in inexperienced cows in both mountain periods. Mastitis and high SCS can also be linked to the stress of animals arriving on a new pasture, as mountain grazing promotes somatic cell count (Lamarche et al. 2000; Leiber et al. 2005). Furthermore, Coulon et al. (1998) demonstrated that physical motion increases SCC in milk of untrained cows. However, as strip grazing was applied, only small effects of the latter were expected.

Data on milk composition indicate that the feed of inexperienced and experienced cows differed in composition. The lower casein content in milk of inexperienced compared to experienced cows at 1800 m a.s.l. points towards a lower energy supply and, thus, metabolisable protein (Leiber et al. 2005). The higher urea content in milk of inexperienced cows was unexpected. Possibly their previous grazing experience could have had an effect, as they were used to grazing lowland pastures rich in protein-rich plants. Although the strip grazing technique reduces the possibility to select among plant species, cows still may have been able to recognise and select species rich in protein through 'feed hedonics' (Villalba et al. 2015). Further studies on ingested feed composition would be needed to confirm this assumption. Effects of experience on milk FA profile consisted of three categories: experience enhanced proportions of some short- and medium-chain FA, reduced proportions of mono-unsaturated medium-chain FA, and enhanced those of several C18:1 *trans* isomers. The latter points towards a specific selection by experienced cows towards forages rich in PSC. Relationships between phenolic compounds and ruminal biohydrogenation were repeatedly shown (Vasta et al. 2009; Kälber et al. 2011; Willems et al. 2014). However, herb selection was not monitored in the present experiment. Apart from that, higher ΣC16:1 and ΣC17:1 proportions in milk fat of inexperienced cows, together with the higher C14:1 c9–to-C14:0 ratio, point towards a higher Δ9–desaturase activity in the mammary gland (Rutkowska et al. 2012).

4.3. Distinction between site-specific and age experience

Cows are able to learn through observation of older animals (Costa et al. 2016), which is a different experience from that acquired by grazing a specific site. In the present study, primiparous cows could be considered less experienced than multiparous cows because of their young age and the fact that calves and heifers are not kept together with more experienced animals before first calving. We aimed at testing if the age experience was more important than the site-specific one. Interactions of experience with parity (and together with site) were rare, suggesting that the site-specific experience obtained before first calving was equally valuable as that obtained as a dairy cow. One exception was that only inexperienced primiparous cows responded to the new situation at 1800 m a.s.l. They increased their ingestion time in response to the new pasture. However, it is unclear if they increased it more than what would have been needed to cover the extra energy and nutrients necessary to maintain milk yield at this altitude.

5. Conclusion

No clear advantage of previous experience with distinct mountain pastures used in a traditional extensive transhumance system and those associated with age (multiparous vs. primiparous cows) was found on feeding behaviour. Several medium-term effects on milking characteristics and milk composition were observed. However, these effects were much lower in general than those caused by the transhumance to mountain pastures. In addition, the new farming environment rather than the lack of habituating to the specific mountain pastures caused part of the stress for inexperienced cows. As the milking characteristics demonstrate, familiarisation to new staff and milking equipment already on lowland pastures could be effective. In the transhumance system investigated, the dispersal of feed across inhomogeneous mountain pastures had been minimised by strip grazing. In all other approaches, experience may have a greater importance. Future studies are needed to determine the importance of experience during transport and the actual days of transition and to compare autochthonous breeds with high-yielding genotypes.

Supplementary Table III.S1. Schedule applied in the experiment in 2014.

Calendar week	Day	Sampling	Feeding	Milking system
14	–	**Pre-experimental period**		
14	–	Experienced cows in their barn for obtaining covariate data	Local hay from Montfleury + 3 kg/day concentrate	Barn equipment in Montfleury
15	–	Inexperienced cows in their barn for obtaining covariate data	Similar local hay + 3 kg/day concentrate	Barn equipment in Pollein
16	0	**Start at 600 m a.s.l. (Montfleury)**		
16	0	Start of the grazing season, all cows grouped together on Montfleury pasture for adaptation	Pasture at 600 m a.s.l. during the day, local hay + 3 kg/day of concentrate	Barn equipment in Montfleury
17	9	1st sampling session 600 m a.s.l.		
20	28	2nd sampling session 600 m a.s.l.		
24	0	**Move to the mountain pasture at 1800 m a.s.l. (Alp Chaudanne)**		
25	9	1st sampling session 1800 m a.s.l.	Pasture 24 h/day + 2 kg/day of the same concentrate	Mobile milking parlour on the mountain pasture
27	28	2nd sampling session 1800 m a.s.l.		
28	0	**Move to the mountain pasture at 2100 m a.s.l. (Alp Entrelor)**		
29	9	1st sampling session 2100 m a.s.l.	Pasture 24 h/day + 2 kg/day of the same concentrate	Mobile milking parlour on the mountain pasture
32	28	2nd sampling session 2100 m a.s.l.		

Supplementary Table III.S2. Botanical composition of the pastures.

600 m a.s.l.		1800 m a.s.l.		22100 m a.s.l.	
Species	%	Species	%	Species	%
Trifolium repens	20.5	*Alchemilla xanthochlora*	16.0	*Phleum pratense*	11.7
Dactylis glomerata	19.0	*Agrostis stolonifera*	9.6	*Alchemilla xantochlora*	10.8
Poa pratensis	16.4	*Poa pratensis*	9.6	*Trisetum flavescens*	10.0
Taraxacum officinale	16.0	*Anthriscus sylvestris*	7.4	*Poa vulgaris*	8.8
Lolium multiflorum	13.6	*Dactylis glomerata*	7.4	*Plantago atrata*	6.3
Plantago lanceolata	5.9	*Trifolium pratense*	6.9	*Polygonum bistorta*	5.4
Capsella bursa-pastoris	2.2	*Polygonum bistorta*	5.9	*Festuca rubrens*	5.0
Ranunculus bulbosus	1.6	*Phleum pratense*	5.9	*Achillea millefolium*	4.6
Rumex obtusifolius	1.6	*Geranium sylvaticum*	4.8	*Poa alpina*	3.8
Lolium perenne	1.0	*Veratrum album*	3.7	*Phleum alpinum*	3.8
Poa annua	0.5	*Arrhenatherum elatius*	3.2	*Crocus vernus*	3.8
Polygonum aviculare	0.5	*Ranunculus acris*	2.7	*Ranunculus pyrenaeus*	3.3
Rumex acetosa	0.5	*Vicia sativa*	2.7	*Trifolium badium*	3.3
Silene alba	0.5	*Trisetum flavescens*	2.1	*Poa pratensis*	2.5
		Campanula rotundifolia	2.1	*Lotus corniculatus*	2.5
		Rumex acetosa	2.1	*Geranium sylvestris*	2.1
		Elytrigia repens	1.6	*Trifolium repens*	2.1
		Heracleum sphondylium	1.1	*Ranunculus acris*	2.1
		Poa trivialis	1.1	*Gentiana acaulis*	1.7
		Taraxacum officinale	1.1	*Anthyllis montana*	1.7
		Silene vulgaris	1.1	*Leontodon sp.*	1.3
		Pimpinella major	0.5	*Geum alpinum*	1.3
		Anthoxanthum odoratum	0.5	*Potentilla repens*	0.8
		Phleum alpinum	0.5	*Taraxacum officinale*	0.4
		Leucanthemum vulgare	0.5	*Veronica chamaedrys*	0.4
		Epilobium angustifolium	<0.1	*Vicia cracca*	0.4
		Phyteuma ovatum	<0.1	*Silene vulgaris*	0.4
		Veronica chamaedrys	<0.1	*Veratrum album*	<0.1
				Cirsium canovirens	<0.1
				Rumex acetosa	<0.1
				Rhinanthus alectorolophus	<0.1
				Myosotis alpestris	<0.1
				Campanula rotundifolia	<0.1

Supplementary Table III.S3. Effect of mountain grazing experience, site and parity on the proportions of selected short- and medium-chain length fatty acids in total milk fat (g/100 g)

		Site (m a.s.l.) (S) and parity (P)													
	Experi-	600		1800		2100			*p*-values						
Variable	ence (E)[1]	1	>1	1	>1	1	>1	SEM	E	P	S	E×P	E×S	P×S	E×P×S
C4:0	Yes	1.23	1.34	1.35	1.44	1.44	1.46	0.081	0.729	0.911	<0.001	0.284	0.828	0.827	0.197
	No	1.29	1.21	1.45	1.33	1.41	1.42								
C6:0	Yes	1.16	1.26	1.16	1.28	1.23	1.27	0.064	0.957	0.534	0.056	0.079	0.938	0.553	0.298
	No	1.28	1.15	1.29	1.17	1.33	1.30								
C8:0	Yes	1.39	1.50	1.25	1.43	1.25	1.38	0.090	0.019	0.597	0.004	0.129	0.934	0.700	0.917
	No	1.32	1.21	1.19	1.13	1.19	1.14								
C10:0	Yes	2.48	2.65	2.13	2.37	2.14	2.40	0.205	0.051	0.645	<0.001	0.390	0.989	0.799	0.828
	No	2.24	2.18	1.98	1.82	1.94	1.94								
C12:0	Yes	3.05	3.15	2.57	2.82	2.57	2.87	0.261	0.104	0.658	<0.001	0.577	0.911	0.770	0.562
	No	2.75	2.79	2.38	2.21	2.34	2.37								
C14:0	Yes	11.06	11.19	9.44	10.04	9.86	10.41	0.715	0.438	0.593	<0.001	0.872	0.666	0.757	0.524
	No	10.44	10.76	9.14	8.97	9.62	10.15								
C14:1 c9	Yes	1.13	1.13	0.951	1.12	1.11	1.20	0.079	0.235	0.124	0.002	0.940	0.549	0.250	0.534
	No	1.21	1.25	1.07	1.15	1.13	1.23								
C15:0	Yes	1.55	1.56	1.37	1.45	1.42	1.40	0.063	0.363	0.549	0.001	0.139	0.165	0.282	0.297
	No	1.45	1.48	1.47	1.39	1.41	1.28								
C15:1 c10	Yes	0.410	0.355	0.338	0.340	0.366	0.354	0.018	0.568	0.057	<0.001	0.702	0.002	0.118	0.164
	No	0.416	0.408	0.366	0.343	0.337	0.294								
C16:0	Yes	25.3	26.4	23.8	23.6	23.4	24.1	1.19	0.430	0.385	<0.001	0.808	0.864	0.523	0.766
	No	26.3	27.0	24.1	24.7	24.2	25.7								
ΣC16:1[2]	Yes	1.69	1.63	1.42	1.34	1.46	1.40	0.097	0.003	0.892	<0.001	0.526	0.607	0.325	0.180
	No	2.03	1.96	1.65	1.80	1.68	1.73								
C17:0	Yes	0.709	0.772	0.713	0.676	0.753	0.733	0.048	0.622	0.584	0.195	0.614	0.310	0.497	0.119
	No	0.715	0.675	0.701	0.698	0.731	0.695								
ΣC17:1[2]	Yes	0.278^{abc}	0.264^{ab}	0.235^{abc}	0.194^{c}	0.249^{abc}	0.214^{bc}	0.029	0.025	0.685	<0.001	0.332	0.445	0.578	0.051
	No	0.335^{a}	0.312^{abc}	0.269^{abc}	0.314^{abc}	0.286^{abc}	0.304^{abc}								

[1]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures. [2]ΣC16:1 includes C16:1 c7, C16:1 t9 and C16:1 c9; ΣC17:1 includes C17:1 c8 and C17:1 c9 ^{abc} Within the same variable (rows Yes and No experience), least square means without a common superscript differ ($P < 0.05$).

Supplementary Table III.S4. Effect of mountain grazing experience, site and parity on the proportions of selected fatty acids >C18 in total milk fat (g/100 g)[1]

		Site (m a.s.l.) (S) and parity (P)													
	Experi-	600		1800		2100			*p*-values						
Variable	ence (E)[2]	1	>1	1	>1	1	>1	SEM	E	P	S	E×P	E×S	P×S	E×P×S
C20:0	Yes	0.173	0.163	0.199	0.192	0.233	0.220	0.014	0.762	0.096	<0.001	0.566	0.300	0.219	0.284
	No	0.176	0.169	0.202	0.177	0.235	0.198								
ΣC20:1[3]	Yes	0.282	0.300	0.355	0.370	0.377	0.379	0.021	0.252	0.952	<0.001	0.479	0.201	0.210	0.776
	No	0.324	0.328	0.388	0.378	0.402	0.368								
C20:2n-6	Yes	0.073	0.071	0.081	0.081	0.086	0.083	0.005	0.993	0.316	<0.001	0.596	0.307	0.038	0.146
	No	0.072	0.074	0.084	0.083	0.089	0.072								
C20:3n-6	Yes	0.086[abc]	0.076[cd]	0.086[abc]	0.104[ab]	0.079[abc]	0.091[abc]	0.009	0.309	0.500	<0.001	0.635	0.112	0.769	<0.001
	No	0.070[bd]	0.085[abc]	0.092[ac]	0.085[abc]	0.072[abc]	0.066[abc]								
C20:3n-3	Yes	0.099	0.087	0.098	0.084	0.063	0.053	0.018	0.785	0.675	<0.001	0.118	0.660	0.443	0.523
	No	0.096	0.099	0.084	0.097	0.022	0.069								
C20:4n-6	Yes	0.012	0.024	0.033	0.045	0.055	0.069	0.010	0.976	0.928	<0.001	0.026	0.617	0.561	0.402
	No	0.025	0.023	0.040	0.030	0.073	0.045								
C20:4n-3	Yes	0.047	0.043	0.072	0.073	0.069	0.072	0.006	0.164	0.734	<0.001	0.744	0.109	0.203	0.486
	No	0.044	0.044	0.076	0.070	0.052	0.064								
C22:0	Yes	0.087	0.080	0.098	0.104	0.113	0.110	0.009	0.845	0.164	<0.001	0.275	0.980	0.621	0.239
	No	0.089	0.082	0.111	0.093	0.123	0.101								
C22:1 c13	Yes	0.022	0.017	0.027	0.033	0.027	0.026	0.005	0.385	0.669	0.001	0.723	0.232	0.395	0.325
	No	0.015	0.016	0.021	0.021	0.029	0.022								
C22:4n-6	Yes	0.033	0.036	0.034	0.036	0.035	0.045	0.008	0.336	0.765	0.482	0.345	0.173	0.942	0.323
	No	0.041	0.041	0.047	0.049	0.046	0.036								
C22:5n-6	Yes	0.048	0.051	0.046	0.049	0.043	0.046	0.007	0.972	0.243	0.422	0.057	0.660	0.705	0.677
	No	0.050	0.043	0.056	0.044	0.054	0.037								

[1]C20:5n-3, C22:5n-3; C22:6n-3 not detected. [2]Yes: n=8 cows that already experienced mountain pastures; No: n=8 cows without previous experience in mountain pastures. [3]ΣC20:1 includes C20:1 t9, C20:1 c5, C20:1 c9 and C20:1 c11. [abcd] Within the same variable (rows Yes and No experience), least square means without a common superscript differ ($P < 0.05$)

CHAPTER IV

Grazing behaviour of dairy cows on biodiverse mountain pastures is more influenced by slope than cow breed

Based on Koczura M, Martin B, Bouchon M, Turille G, Berard J, Farruggia A, Kreuzer M, Coppa M.
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ABSTRACT

The aim of this study was to determine how cows with different genetic merit behave and perform when grazing biodiverse and heterogeneous mountain pastures with different slopes. Three groups of 12 cows in late lactation, each composed of four Holstein, four Montbéliarde and four Valdostana Red Pied cows, breeds of increasing presumed robustness and decreasing milk yield potential. Cows grazed without concentrate either on a low diversity flat pasture or on two species-rich mountainous pastures having slopes of either 7° or 22°. Milk yield, BW and grazing behaviour were monitored two times in the first and once in the second grazing cycle. Cows of different breeds had similar behaviour on all pastures. The Montbéliarde cows performed close to their production potential; Holstein and Valdostana cows produced less milk than anticipated. No breed difference in terms of BW loss was found. The Valdostana cows exhibited the least selective behaviour with respect to plant species and plant growth stage. Still, all cows searched for the most palatable vegetation regardless of pasture diversity. On the steep pasture, cows optimised the trade-off between ingesting and saving energy to obtain feed. They remained longer at the lowest zone and selected forbs whereas cows on the flatter pasture went to the upper zone to select grasses. The present study gave no evidence for a superior short-term adaptation to harsh grazing conditions through an optimised feeding behaviour of the Valdostana breed compared to Montbéliarde and Holstein cows.

HIGHLIGHTS

- The milk yield decreased by the same percentage along season, regardless of breed.
- Montbéliardes were the ones who performed closest to their potential milk yield.
- Only few differences between breeds were observed in diet selection on all pastures.
- Holstein showed the highest preference for grasses.
- Valdostana were indifferent to forbs.
- On a steep slope, cows stayed lower and selected forbs, whereas on a low slope, they went up and selected grasses.

1. Introduction

The presence of ruminants using mesotrophic natural mountain grasslands is essential to maintain biodiversity and landscapes (Santini et al. 2013). However, these pastures are heterogeneous, steep and often provide a low forage quality (Tamburini et al. 2005). In mountain dairy systems, autochthonous dairy cattle are often preferred to exploit mountain pastures linked with the production of region-specific dairy products (Sturaro et al. 2013). Their robustness, which includes numerous traits that allow carrying on various activities in the face of environmental constraints (Friggens et al. 2017), is helpful in harsh mountain conditions. Due to the multi-trait selection applied (including lower BW and limited milk yield (**MY**) compared to high genetic merit breeds), autochthonous cows may tolerate low forage quality and hesitate less to climb steep slopes. Indeed, under severe nutritional restriction, Coulon et al. (1994) found that primiparous Tarentaise cows decreased their MY, whereas high genetic merit Holstein cows maintained it at the expense of BW and reproductive performance. On pasture, these results may be linked to the grazing behaviour. McCarthy et al. (2007) found that less productive cows graze longer than high genetic merit cows. In other studies, the grazing time of both cow types was similar, but the cows of lower genetic merit spent more time walking and playing on pasture (Saether et al. 2006), selected herbage of greater quality in spring (Aharoni et al. 2009) and had a lower grass DM intake per bite and per unit of BW (Prendiville et al. 2010). However, in some other studies, differences in the diet choices made by traditional and improved cattle were small (Dumont et al. 2007a; Coppa et al. 2015). In summary, results are available for high-yielding cows (mainly Holstein) in comparison to lower yielding breeds, but are contradictory. Concerning autochthonous cows, it is difficult to find information about grazing behaviour and selection as they are often rarely investigated local breeds. Therefore, a comprehensive comparison of the behaviour of different breeds under difficult grazing conditions is needed.

Other factors such as seasonal evolution of the pasture or topography are likely to interact with breed concerning the animal's diet choices. Indeed, large seasonal variations in behaviour and performance of cows along with herbage growth and availability were found (Kohler et al. 2006, Farruggia et al. 2014). Space use on pasture was found to change with season due to pasture morphology. Accordingly, cows initially grazed the entrance of large plots and flat fertile areas before gradually exploring the entire pasture (Farruggia et al. 2014). In beef calves, the use of steep slopes increased lying time even though feeding time was apparently not affected (Gangnat et al. 2016). However, there is no report about the effect of steep slopes on actual diet selection and space use of lactating cows of different breeds on pastures.

Therefore, in the present study, the following hypotheses were tested through a controlled grazing experiment involving three breeds of cows with a gradient in genetic merit. With increasing genetic merit, dairy cows adapt their (i) diet selection according to the biodiversity and (ii) grazing behaviour according to the slope, in order to match their lactation requirements.

2. Materials and Methods

2.1. Animals and pastures

The experiment was performed in 2017 at Marcenat, INRA's experimental farm (Herbipôle, 45◦15'N, 2◦55'E; 1135 to 1215 m a.s.l.), which is an approved animal experimental unit (Certificate of Authorization to Experiment on Living Animals N° D 15-114-01). Thirty-six late-lactating multiparous dairy cows with different experience backgrounds and increasing genetic merit for MY were monitored: 12 Valdostana Red Pied (Va, 173±32 days in milk (DIM)), 12 Montbéliarde (Mo, 219±33 DIM) and 12 Holstein (Ho, 199±23 DIM). The Ho and Mo cows originated from Marcenat had experienced rotational grazing on moderately biodiverse pastures. The Va cows had been transferred to Marcenat by truck from the Institut Agricole Régional (IAR), Aosta, Italy 1 month before the experiment started. In Italy, Va cows had strip grazed on lowland and biodiverse mountain pastures. Cows were milked at 0700 h and 1600 h. Three weeks after the arrival of the Va cows, a herd of 12 cows per breed was formed and no concentrate was provided anymore. In calendar week 22, cows were divided in a randomised way into three equivalent groups balanced by breed (4 cows per breed), MY (within breed) and stage of lactation. At that time, Ho cows produced 22.9 ± 3.9 kg milk/day with fat and protein contents of 37.6 ± 5.6 g/kg and 31.6 ± 2.2 g/kg, respectively. The corresponding values of the Mo cows were 24.1 ± 3.3 kg milk/day, 38.4 ± 4.3 g fat/kg and 32.8 ± 2.3 g protein/kg, those of the Va cows were 14.7 ± 2.3 kg milk/day, 35.4 ± 4.5 g fat/kg and 32.7 ± 1.7 g protein/kg. Cows of the three groups were grazing (0.3 ha/cow) on i) a flat grass-dominated control pasture with low botanical diversity (13 species) (L, 2°), comprising mainly *Poa pratensis* (32%), *Trifolium repens* (21%) and *Dactylis glomerata* (19%), and ii) two adjacent semi-natural pastures both with a very similar botanical composition (39 species in average) but with different slopes (7° and 22°; H_7 and H_{22}). The latter pastures were composed of three zones differing in slope and biodiversity (Supplementary Figure F1). Zone Z1 was at the lower end of the slope, near the water supply and less diverse (26 plant species), was dominated by *Dactilys glomerata* (30%), *Agrostis capillaris* (20%) and *Poa pratensis* (15%). Zone Z2 at mid slope (36 species) was dominated by *Agrostis capillaris* (22%), *Festuca* gr. *rubra* (19%) and *Dactylis glomerata* (15%). Zone Z3, the upper and most biodiverse zone of the pasture (56 species), was dominated by *Thymus* gr. *serpyllum* (16%), *Festuca* gr. *rubra* (14%) and *Agrostis capillaris* (10%). Botanical composition was determined using the vertical point-quadrat method (Daget & Poissonet 1971). Because of its homogeneity, no zones were distinguished on L.

2.2. Experimental design

The experiment lasted for 8 weeks (from calendar weeks 22 to 30), with measurements on swards and animals performed after 2 weeks of grazing in calendar weeks 24 (early in 1st grazing cycle) and 26 (late in 1st grazing cycle), and in week 30 (early in 2nd grazing cycle). Aiming to maximise grazing selection, extensive rotational grazing with long duration of paddock utilisation was applied as described in Coppa et al. (2015) with cows being moved off pastures between weeks 26 and 30 to allow a minimal regrowth. In each of the measurement weeks, five grass samples of 10 cm × 1 m per zone were taken on H pastures, and five samples for the entire pasture on L plot. These five samples were pooled by zone (H_7 and H_{22}) or pasture (L), oven-dried at 60°C during 72 h and then analysed as described by Coppa *et al.* (2015) for proximate composition in order to describe the nutritional value of the herbage. Contents of net energy for lactation (NEL) of the samples were estimated using the calculation module of the official Swiss feeding recommendations for ruminants (Agroscope 2018).

Feeding behaviour was measured in the 3 weeks during two consecutive days by scan-sampling of the cow's bites at 5 min intervals (Dumont et al. 2007a). Eighteen cows (two per breed per group) were observed for 3 h after the morning milking and for 3 h in the afternoon (1.30 h before and 1.30 h after milking). Observers were recording activities first, which were distinguished into resting (lying, standing still, ruminating), grazing and other activities (walking, exploring, drinking, etc.). Bite type was characterised by botanical group and vegetation stage (Dumont et al. 2007b). Vegetation was distinguished into short vegetative (height <10 cm, leaf development), tall vegetative (>10 cm, stem elongation) and mature vegetation (regrouping inflorescence emergence, heading and dead materials) (Coppa et al. 2015). In addition, grasses, legumes and forbs were defined as main botanical groups. On H_7 and H_{22}, the zones the cow grazed on were recorded. During each measurement week, the available herbage on the plot was characterised through 30 cm^2 random samples (100 per zone) on the day before the behavioural observations were performed. These samples were described for vegetation stage and botanical groups similarly to cow's bites. Diet selection, defined as the proportion of a bite's type in the diet in relation to its available proportion in the plot, was quantified by calculating the indices of selectivity (**IS**) using Jacobs' (1974) modification of Ivlev's selectivity index (Dumont et al. 2007b). These indices range from -1 (aversion) to +1 (preference), with 0 meaning indifference. Two days after observing behaviour, faeces samples from the 36 cows were collected after morning and evening milking. They were analysed for CP and ADF according to Farruggia et al. (2014). Organic matter digestibility (**OMD**) was estimated as described by Mesquita et al. (2016) with the following equation: OMD = 0.980 - 2.474/faecal CP (% of organic matter) – 0.00276 × faecal ADF (% of organic matter). In the measurement weeks and, additionally, in the pre-experimental period, MY and BW were determined at each milking and averaged per week. The potential MY (**MY_{pot}**) was calculated by the model of Coulon and Pérochon (2000), with **ΔMY** being the difference between MY and MY_{pot}. On the days of behaviour observation, individual 20 mL samples from four consecutive milkings were sampled and conserved at +4°C until being analysed for fat and protein contents (MIRS, NF ISO 9622).

2.3. Statistical analysis

Data on MY, MYpot and milk composition collected in the pre-experimental period were used as a breed-centred covariate. For IS, means were weighted by the number of observations per zone. All data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC). Normality was checked using Shapiro-Wilk test. The following variables underwent a Box-Cox transformation in order to reach normal distribution: IS, proportion of other activities, time spent in Z3. All variables were analysed by ANOVA using a repeated measures mixed model considering grazing period, animal breed, pasture type and their interactions as fixed effects. Grazing period was defined as repeated factor, and cow as subject. The random factor was the cow nested within its pasture type. Student t-tests were performed on the IS to assess aversion (IS < 0), indifference (IS = 0) or preference (IS > 0). For transformed variables, standard error of the mean was calculated using the non-transformed data.

Table IV.1. Characterisation of the pastures (low/high diversity (div.) and slopes), grazing period and zone within the two high diversity pastures (arithmetic means and standard error of the mean).

	Pasture type			Grazing period			Zone (high div. pastures)			
	Low div. (L)	High div. (H_7)	High div. (H_{22})	Early 1st grazing	Late 1st grazing	Early 2nd grazing	Z1	Z2	Z3	SEM
Vegetation theoretically available for bites (%)										
Short vegetation	35	39	41	21	43	54	30	36	54	5.7
Tall vegetation	58	43	41	60	46	28	43	48	36	5.5
Mature vegetation	7	17	18	19	11	18	27	16	10	3.3
Grasses	64	58	56	65	61	48	66	56	49	2.8
Legumes	22	6	8	10	8	10	12	5	4	2.0
Forbs	14	36	36	25	31	42	22	38	47	4.1
Sward composition (g/kg DM)										
Organic matter	901	928	919	928	919	914	924	924	923	3.1
CP	144	92	97	116	88	100	92	97	93	4.8
NDF	593	616	631	600	620	638	645	628	597	7.4
ADF	296	328	325	296	335	334	333	325	321	5.5
Nutritional value										
Digestibility	0.666	0.592	0.601	0.652	0.593	0.576	0.592	0.601	0.598	0.013
NE_L (MJ/kg DM)	5.25	4.64	4.68	5.24	4.58	4.41	4.60	4.70	4.67	0.109
PDIE (g/kg DM)	87.3	72.8	73.4	82.3	71.3	71.9	72.2	74.3	72.8	1.70
PDIN (g/kg DM)	95.3	60.8	64.0	76.6	58.6	66.1	60.8	64.5	61.8	3.27

NE_L: net energy for lactation; PDIE: absorbable protein at the duodenum according to supply with fermentable energy and rumen undegradable protein; PDIN: absorbable protein at the duodenum according to supply with rumen degradable protein

3. Results

3.1. Qualitative description of vegetation characteristics

At the beginning of the experiment, L-pasture was dominated by tall vegetative patches (Supplementary Table VI.S1.). On H-pastures, a decreasing gradient in vegetation stage was observed along zones: Z1 was rich in tall vegetative and mature patches, Z2 in tall vegetative patches and Z3 in short and tall vegetative patches (Supplementary Table VI.S2.). Zones Z1 and Z2 were richer in grasses than Z3. Zone Z1 had the highest proportion of legumes, and forbs increased in proportion from Z1 to Z2 and Z3. The forage from the L-pasture contained more NEL than that of the H-pastures, and this across all season (Table IV.1.). Zones on H-pastures did not differ in NEL content. The L-pasture was richer in CP and lower in ADF than the H-pastures, accompanied by a lower OMD of the latter; and this was similar in all zones. Throughout the experiment, a decrease in the proportion of grasses was observed on all pastures (Table IV.1.). This was compensated by an increase in legume proportion on L-pasture and in forb proportion on H-pastures (Supplementary Table IV.S1.). With progressing season, the proportion of short vegetative patches increased, replacing the tall vegetative patches. In Z1 and Z2, these changes took place at late 1st grazing cycle, whereas in Z3 it happened at early 2nd grazing cycle (Supplementary Table IV.S2.). During the latter period, the nutritional quality of forage on the H-pastures decreased in Z1 and Z2, but not Z3. The OMD also decreased with progressing season in all zones, but during the 2nd grazing cycle, it was higher in Z3 than in Z2 and Z1.

3.2. Breed differences

There was no breed difference in the time allocated to the main activities and times spent in the zones (Table IV.2.). The Ho preferentially selected grasses (IS > 0) more than Va and were found to have a higher proportion of grasses in their bites as compared to Mo and Va. The Va were indifferent to forbs and mature vegetation (IS ≤ 0), whereas Ho and Mo cows avoided them (IS < 0). In Va bites, the proportion of mature vegetation was higher than in those of Mo. The Va faeces contained more CP and less ADF than that of Ho and more CP than that of Mo. The estimated OMD was higher in Va and Mo compared to Ho. The MY was lower in Va compared to Ho and Mo but MY loss, as compared to the calculated MY_{pot}, was the same in Ho and Va. Loss of MY was on average by 40% less severe in Mo than Ho and Va. The Mo had the highest yield of milk fat and protein and BW, always followed by Ho, then Va.

Table IV.2. Effects of cow breed (B), pasture type (P), grazing period (G) and their interactions on cow's behaviour, diet selection, faecal composition and performance.*

	Cow breed				P-value						
	Ho	Mo	Va	SEM	B	P	G	B×P	B×G	P×G	B×P×G
Time allocated to activities (%)											
Resting time	36	37	30	2.0	0.071	0.274	<0.001	0.010	0.920	0.112	0.423
Grazing time	54	55	60	2.0	0.152	0.099	<0.001	0.019	0.872	0.333	0.728
Other activities	10	8	10	1.9	0.316	<0.001	0.739	0.338	0.539	0.074	0.365
Time spent in Z1	32	34	35	1.6	0.236	<0.001	<0.001	0.817	0.030	0.335	0.935
Time spent in Z2	40	40	37	2.0	0.399	<0.001	<0.001	0.905	0.413	0.127	0.266
Time spent in Z3	28	26	28	5.1	0.332	0.274	<0.001	0.679	0.005	0.011	0.130
Vegetation types in the bites (%)											
Short vegetation	48	55	50	6.1	0.059	<0.001	<0.001	0.050	0.986	0.005	0.291
Tall vegetation	43	39	37	2.0	0.108	0.001	<0.001	0.210	0.598	0.433	0.521
Mature vegetation	8[ab]	6[b]	13[a]	2.1	0.006	0.004	<0.001	0.170	0.215	<0.001	0.212
Grasses	75[a]	72[b]	68[b]	1.4	0.017	<0.001	0.143	0.583	0.711	0.009	0.861
Legumes	6	8	7	1.6	0.106	<0.001	0.017	0.048	0.244	<0.001	0.170
Forbs	19	20	24	3.1	0.183	<0.001	0.239	0.676	0.643	<0.001	0.722
Jacob's index of selectivity (-1 < IS < 1)											
Short vegetation	0.29	0.44	0.33	0.090	0.087	0.025	<0.001	0.118	0.992	0.003	0.581
Tall vegetation	-0.14	-0.25	-0.24	0.045	0.160	0.007	0.531	0.157	0.412	0.001	0.366
Mature vegetation	-0.36[ab]	-0.46[b]	-0.15[a]	0.145	0.006	<0.001	<0.001	0.103	0.181	0.000	0.205
Grasses	0.33[a]	0.25[ab]	0.18[b]	0.032	0.008	0.060	<0.001	0.648	0.717	<0.001	0.909
Legumes	-0.46[b]	-0.28[a]	-0.30[a]	0.070	0.005	0.003	0.058	0.138	0.094	<0.001	0.288
Forbs	-0.33[b]	-0.26[b]	-0.10[a]	0.036	0.001	<0.001	<0.001	0.170	0.435	<0.001	0.331
Faeces composition (g/kg DM)											
CP	126[b]	129[b]	133[a]	2.1	0.001	<0.001	<0.001	0.783	0.999	0.001	0.787
ADF	349[a]	338[b]	340[b]	4.7	0.006	<0.001	<0.001	0.480	0.053	0.065	0.793
Calculated OMD†	0.697[b]	0.705[a]	0.710[a]	0.003	<0.001	<0.001	<0.001	0.928	0.592	0.001	0.964
Yield (per day per cow)											
Milk (kg)	14.3[a]	15.1[a]	10.3[b]	0.28	<0.001	<0.001	<0.001	0.099	0.130	0.079	0.957
Δ Milk yield (kg)	-3.2[b]	-1.9[a]	-3.2[b]	0.29	0.002	0.001	<0.001	0.131	0.519	0.105	0.992
Milk fat (g)	564[b]	611[a]	396[c]	12.7	<0.001	<0.001	<0.001	0.093	0.177	0.060	0.934
Milk protein (g)	445[b]	482[a]	338[c]	18.4	<0.001	<0.001	<0.001	0.391	0.183	0.018	0.956
BW (kg)	653[b]	681[a]	507[c]	4.8	<0.001	0.112	0.006	0.904	0.968	0.954	0.952

* Ho: Holstein; Mo: Montbéliarde; Va: Valdostana. a-c Within same trait and effect, values without common superscripts differ. †Organic matter digestibility.

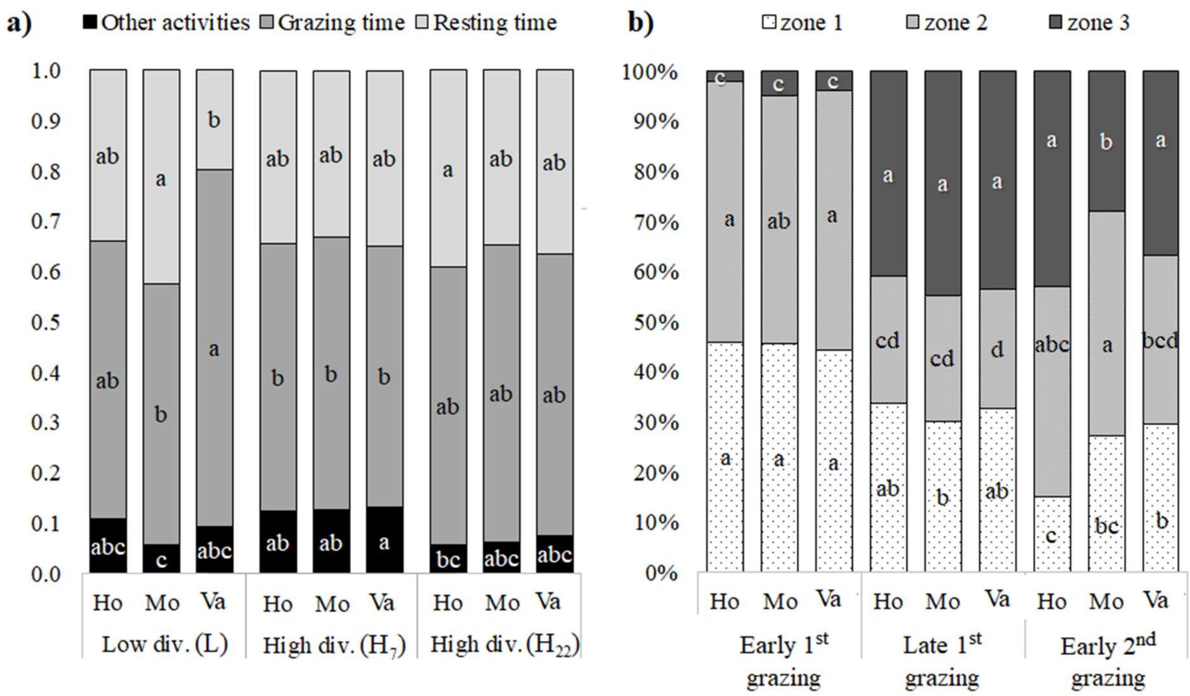

Figure IV.1. Proportion of time allocated to a) grazing, resting and other activities on the three pasture types (breed × pasture type, P < 0.05) and b) the different zones of the high diversity pastures at the three grazing stages (breed × grazing stage, P < 0.05) by Holstein (Ho), Montbéliarde (Mo) and Valdostana (Va) cows. Within variable, bars without common superscript differ with P < 0.05.

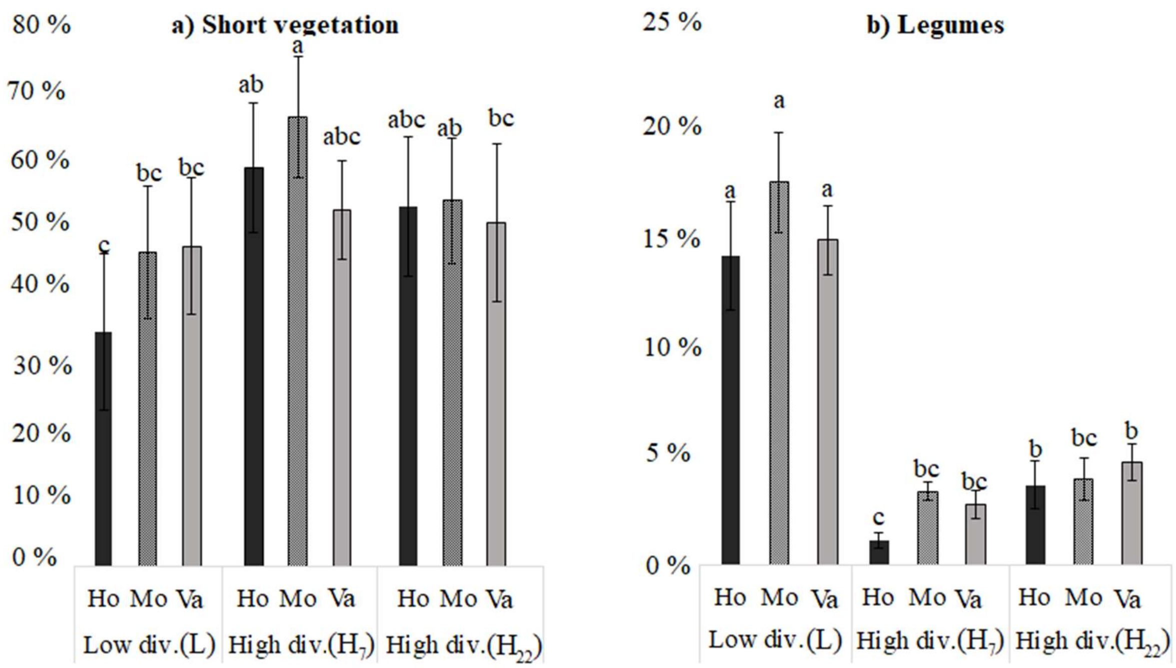

Figure IV.2. Proportion of a) short vegetation and b) legumes in the diet of Holstein (Ho), Montbéliarde (Mo) and Valdostana (Va) cows on the three pasture types (breed × pasture type, P < 0.05). Within variable, bars without common superscript differ.

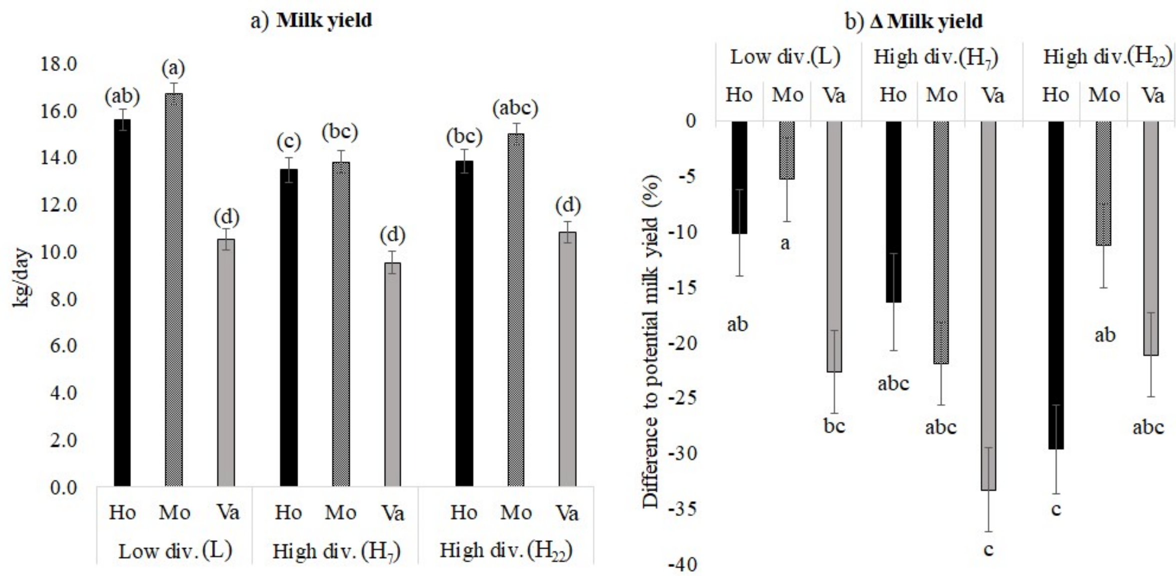

Figure IV.3. Milk yield (a) and milk yield change (b) of Holstein (Ho), Montbéliarde (Mo) and Valdostana (Va) cows on the three pastures types (breed × pasture type, $P < 0.05$). Within variable, bars without common letter differ at $P < 0.05$ or, when in brackets, tend to differ at $P < 0.10$.

There were only few interactions between breed and either pasture type or grazing period (Table IV.2.). The Va allocated more time for grazing than Mo at the expense of resting, but this only on the L-pasture (Figure IV.1a). The three breeds visited the zones similarly except for early 2nd grazing cycle, where Mo spent less time in Z3 than Ho and Va; this in favour of Z2, whereas Ho spent a shorter time on Z1 than Va (Figure IV.1b). The observed proportion of short vegetative patches was higher in the bites of Ho and Mo grazing the H_7- instead of the L-pasture, whereas this was not the case for Va (Figure IV.2a). On H_7 pasture, Ho had a smaller proportion of legumes in their bites than on H_{22} pasture (Figure IV.2b), whereas this was not the case for Mo and Va. The Ho and Mo tended to have a smaller MY on H_7- than on L-pasture, whereas Va had a similar MY on all three pastures (Figure IV.3a). The difference to MY_{pot} (ΔMY) expressed in % was similar in Mo and Va, except on L-pasture where ΔMY was only -5% in Mo and as high as -23% in the Va (Figure IV.3b). The Ho had a larger ΔMY on H_{22} than Mo.

3.3. Pasture type, grazing stage and their interaction

The interaction between pasture type and grazing period was significant for almost every variable (Table IV.2.). Globally, cows spent more time on other activities (*i.e.* walking, exploring, drinking, etc.) on H_7 than on H_{22} and L (Supplementary Table IV.S3.). Along with the progressing season, the time allocated for resting decreased and that for grazing increased (not significant at the transition from early to late 1st grazing cycle). These shifts were also found on the different pasture types (Table IV.3.). Cows stayed longer in Z1 and Z3 and shorter in Z2 on H_7 compared to H_{22} (Supplementary Table IV.S3.). With progressing season, Z1 and Z2 were less intensively visited than Z3. On a closer look, during early 1st grazing cycle, cows on H-pastures barely spent time in Z3 (Table IV.3.). With the advancement of the season they started to visit Z3 more frequently. During late 1st grazing cycle, cows spent less time in Z3 on H_{22} than on H_7. In early 2nd grazing cycle, cows spent the same time in Z3 on both H-pastures.

Table IV.3. Least Square means of the grazing period × pasture type interaction on cow's behaviour, diet selection, faecal composition and performance.*

Pasture type	Early 1st grazing			Late 1st grazing			Early 2nd grazing			
Pasture type	Low div. 2°	High div. 7°	High div. 22°	Low div. 2°	High div. 7°	High div. 22°	Low div. 2°	High div. 7°	High div. 22°	SEM
Time allocated to activities (%)										
Resting time	37^{abcd}	51^{a}	46^{ab}	37^{abcd}	34^{bcde}	39^{abc}	22de	17^{e}	25^{cde}	3.5
Grazing time	54^{bc}	40^{c}	47^{c}	54^{abc}	49^{c}	55^{abc}	70^{a}	70^{b}	68^{ab}	3.5
Other activities	9^{abc}	9^{abc}	7^{bc}	9^{abc}	17^{a}	5^{c}	8^{bc}	13^{ab}	7^{bc}	1.0
Time spent in Z1	–	53^{a}	37^{bc}	–	39^{b}	25^{d}	–	28^{cd}	19^{d}	2.3
Time spent in Z2	–	44^{bc}	58^{a}	–	12^{d}	38^{bc}	–	32^{c}	48^{ab}	2.8
Time spent in Z3	–	3^{c}	4^{c}	–	47^{a}	37^{b}	–	39 ^{b}	32^{b}	2.1
Vegetation types in the bites (%)										
Short vegetation	17^{f}	41^{d}	21^{ef}	40de	52^{cd}	62^{bc}	70^{b}	83^{a}	73^{ab}	4.1
Tall vegetation	64^{a}	53^{ab}	67^{a}	40^{bc}	31^{c}	33^{c}	28^{cd}	15^{d}	24^{cd}	3.4
Mature vegetation	18^{a}	5^{bcd}	12^{abc}	20^{a}	17^{ab}	5^{cd}	2^{d}	2^{d}	2^{d}	3.1
Grasses	76^{ab}	70^{abc}	75^{ab}	82	72^{abc}	62^{c}	74^{ab}	70^{abc}	65^{bc}	2.5
Legumes	13^{a}	2^{d}	6^{b}	14^{a}	3^{cd}	2^{d}	2^{a}	3^{cd}	4^{bc}	1.6
Forbs	11de	28^{abc}	19^{cd}	5^{e}	26^{bc}	37^{a}	7^{e}	27^{abc}	31^{ab}	4.0
Jacob's index of selectivity (-1 < IS < 1)										
Short vegetation	0.57^{ab}	0.64^{a}	0.28^{bc}	-0.17^{c}	0.25^{bc}	0.26^{bc}	0.35^{ab}	0.48^{ab}	0.53^{ab}	0.084
Tall vegetation	-0.53^{b}	-0.04^{a}	0.07^{a}	-0.16^{ab}	-0.30^{ab}	-0.24^{ab}	-0.26^{ab}	-0.29^{ab}	-0.12^{a}	0.078
Mature vegetation	0.36^{a}	-0.80^{d}	-0.42^{bc}	0.66^{a}	-0.03^{ab}	-0.36^{bc}	-0.62^{cd}	-0.78^{d}	-0.87^{d}	0.118
Grasses	0.19^{bc}	0.04^{c}	0.18^{bc}	0.37^{ab}	0.35^{ab}	-0.06^{c}	0.36^{ab}	0.40^{ab}	0.46^{a}	0.056
Legumes	-0.16^{a}	-0.69^{c}	-0.21^{a}	-0.29^{a}	-0.31^{ab}	-0.62^{bc}	-0.21^{a}	-0.24^{a}	-0.36^{ab}	0.088
Forbs	-0.19^{cd}	0.13^{ab}	-0.13^{bc}	-0.46^{d}	-0.33^{cd}	0.16^{a}	-0.48^{d}	-0.38^{cd}	-0.40^{cd}	0.062
Faeces composition (g/kg DM)										
CP	154^{a}	140^{b}	141^{b}	117^{cd}	117^{cd}	115^{d}	136^{b}	118^{cd}	125^{c}	2.9
ADF	321^{b}	344^{a}	350^{a}	343^{a}	351^{a}	360^{a}	320^{b}	351^{a}	342^{a}	6.2
Calculated OMD†	0.741^{a}	0.718^{b}	0.717^{b}	0.688^{cd}	0.685^{cd}	0.677^{d}	0.722^{b}	0.688^{cd}	0.699^{c}	0.005
Yield (per cow per day)										
Milk (kg)	19.0^{a}	15.5^{b}	17.1^{ab}	13.0^{c}	12.1^{cd}	12.7^{cd}	10.7^{de}	9.1^{e}	9.8^{e}	0.50
Δ Milk yield (kg)	1.8^{a}	-1.1^{b}	-0.3^{ab}	-3.7^{c}	-3.9^{c}	-4.1^{c}	-3.8^{c}	-5.0^{c}	-5.0^{c}	0.54
Milk fat (g)	755^{a}	601^{b}	685^{ab}	506^{c}	461^{cde}	487^{cd}	452^{cde}	385de	378^{e}	24.1
Milk protein (g)	626^{a}	511^{b}	562^{ab}	399^{c}	378^{c}	383^{c}	348^{cd}	301^{d}	288^{d}	22.4
BW (kg)	615	623	615	616	617	611	607	610	603	8.3

**P*-values for interaction of pasture type and grazing period (P×G) are reported in Table 3.

$^{a-e}$ Within the same trait and effect, values without common superscripts differ. †Organic matter digestibility; for calculation see Materials and Methods.

The proportion of short vegetation in the bites increased with progressing season (Supplementary Table IV.S3.) at the expense of tall vegetative patches, especially on L. This occurred earlier on H_{22} (Table IV.3., late 1st grazing cycle) than on H_7 (early 2nd grazing cycle). During the 1st grazing cycle, IS for mature vegetation was higher on L, whereas it decreased during the 2nd grazing cycle. On H-pastures, it was already low in early 1st grazing cycle and lower on H_7 than on H_{22} (Table IV.3.). The IS for mature vegetation increased on H_7 during the late 1st grazing cycle but decreased again during the 2nd grazing cycle. The botanical composition of the vegetation consumed stayed quite constant across the season, with some differences in the legume proportion. A preference for grasses was observed on all pastures (IS > 0), except in the late 1st grazing cycle on H_{22} (IS ≥ 0) (Table IV.3.). In that period, H_{22} cows did not climb the steep slope to Z3 to select grasses but selected forbs in the lower parts instead (IS > 0). This resulted in a higher proportion of forbs in the cows' bites at that time on H_{22}. In the early 2nd grazing cycle, no differences between H_7 and H_{22} were observed anymore.

The estimated OMD was higher on L than on H-pastures, except during the late 1st grazing cycle where it was equivalent in all pastures (Table IV.3.). Yield of total milk, fat and protein across all breeds was higher and ΔMY was lower on L- than on H-pastures (Supplementary Table IV.S3.). The MY substantially decreased with progressing season, leading to a high ΔMY in late 1st and early 2nd grazing cycles. Pasture type had no influence on BW, but BW declined from early 1st to 2nd grazing cycle. A closer look at this data shows that the difference in MY and in ΔMY between L- and H-pastures mainly originated from early 1st grazing cycle, whereas pasture type was less important in the two later grazing periods (Table IV.3.).

4. Discussion

4.1. Breed differences according to pasture biodiversity

Unlike our hypotheses, differences in diet selection and performance between cow breeds and interactions between breed and pasture type during the season were minimal. The Va were expected to exhibit a less selective grazing behaviour and maintain BW despite MY, as anticipated for alpine breeds (Coulon et al. 1994). However, even though the difference to MY_{pot} was higher in percentage in Va than in Ho and especially Mo, no breed difference in BW loss along the season occurred. Still, Va were generally a little less selective towards vegetation stage and botanical composition, and this not only on high biodiversity pastures. This could also be related to their previous early experience, as suggested by Lopes et al. (2013). Indeed, the strip-grazing method traditionally used for this breed in their native environment (Coppa et al. 2012) aims at optimising the consumption of mature and high size swards, reducing opportunities for plant selection. Still, the lower faecal ADF content of Va compared to Ho could suggest that Va might have ingested the best digestible parts of forbs and mature vegetation. The differences shown in diet selection by Va highlighted a certain independence of the latter in the other two breeds, even though cows from all breeds were kept together.

An increasing gradient from Va to Mo and to Ho was also expected in the selection of vegetative patches, which should provide most energy to cover requirements for the prioritised milk production. Actually, the selection behaviour towards grasses differed only in Ho. It seems though that this was not sufficient to maintain a higher MY than Mo, and also not to cover the extra energy required by grazing without concentrate, as supported by the low milk protein yield. McCarthy et al. (2007) already suggested that cows selected for high MY are not able to achieve their full potential under exclusively grazing conditions. The Ho appeared instead to increase their total daily grazing time, as previously observed by Heublein et al. (2017) and Romanzin et al. (2018). Mesquita et al. (2016) found minor differences between Ho and Mo on the same type of pasture, but these authors faced difficulties to link it to a more selective behaviour.

The low ΔMY of the Mo could be partly explained by their tendency to select especially short vegetative patches, and most of all their ability to avoid less digestible herbage such as forbs and mature vegetation. Moreover, cows from the three breeds showed a strong apparent aversion to legumes, which was probably due to their low height, making them difficult to access and choose without consuming tall vegetation at the same time (Coppa et al. 2015). Out of the three breeds, Mo avoided legumes the least. The lower faecal ADF content of Mo compared to Ho also points towards avoidance of low-quality fibrous stems and other plants. The Mo are widespread in French mountainous regions, and thus may have well adapted to this kind of pasture. Other resilience indicators such as a high fertility and body condition score (Hazel et al. 2017) as well as a high technological quality of the milk (Puppel et al. 2018) are reasons for using this breed in mountainous regions and considering it to improve other cattle breeds in lowland systems.

4.2. Breed differences according to slope and slope effect

Both high biodiversity pastures became more heterogeneous and lost nutritional value when the season progressed, as is typical for extensive grazing systems (Farruggia et al. 2014). The Va were expected to explore the upper zones from H-pastures sooner than Mo and especially Ho, because they are lighter and had already experienced steep slopes on mountainous pastures. However, even with their assumed better agility and lower energy requirements for maintenance, just like the other breeds Va also switched to the previously avoided zones only when the feed available was getting scarce, a phenomenon also observed by Putfarken et al. (2008).

When abundantly available, grasses were preferred and forbs were avoided by all cows, regardless of breed, as previously shown by Dumont et al. (2007b) and Farruggia et al. (2014). In addition, cows generally preferred short and tall vegetative grasses, and therefore re-grazed the lower and flat zones first rather than selecting mature vegetation. This behaviour, called 'patch grazing' (Adler et al. 2001) was also reported by Farruggia et al. (2014). Along with the decrease of the nutritional value of the grass, and of the grass abundance on the preferred patches, cows, regardless of breed, progressively explored the further parts of the plot, as observed by Dumont et al. (2007b) and Coppa et al. (2011). It seems that a higher nutritional value was maintained in the upper zone due to its more biodiverse botanical composition, different from the lower zones. However, during late 1st grazing cycle, even when the nutritional value of the herbage was not different between zones, cows grazing on pasture H_7 went up to the upper zone and selected grasses which were still abundant there. On the pasture with the steepest slope, cows had to make a decision for a trade-off between having access to the most palatable feed and the physical strain to get there. They seem to have chosen to avoid the extra physical effort, as they stayed longer in the lower zone where they selected forbs. During the 2nd grazing cycle, when the nutritive value of the sward was higher on the upper zone than on the lower zone, cows chose to go up despite the physical effort needed, regardless of breed. In their own way, cows have to choose where to put priority between lactation, reproduction or ability to survive, and then adapt their behaviour and physiology accordingly (Blanc et al. 2006). In the present experiment, in late lactation and on mountain pastures, cows might have avoided the extra physical effort of climbing slope in order to maintain their BW which was barely affected along the grazing season. Regardless of breed, their grazing behaviour was determined by their choices in the trade-off between milk production and body condition for reproduction or coping with the environmental conditions. According to Friggens et al. (2017), repeated measurements over time have a high potential for quantification of the animal's ability to cope with environmental challenges. Therefore, on mountain pastures, a deeper investigation on the long term, which takes into account the main life functions, is necessary.

5. Conclusion

In our study, only small differences in grazing behaviour and performance were observed between cows with increasing gradient of genetic merit for MY late in the lactation period. The Va were a little less selective than Mo and especially Ho, regardless of the pasture biodiversity. In the end, all breeds exhibited similar grazing behaviour by selecting preferentially vegetative grasses on all pasture types. This resulted in a similar MY decrease for all cows, regardless of pasture type and breed, even when having different previous grazing experiences. The cows' grazing behaviour was actually more influenced by the steepness of the slope than the breed. When the season progressed, all cows looked for the best trade-off between grazing the usually preferred vegetation patches or the physical effort of climbing the steep slope to get grass with a higher nutritional value. Grazing behaviour may thus not be a trait contributing to a possibly higher short-term resilience of performance of low genetic merit cows on complex mountain pastures, compared to adapted high genetic merit cows. Therefore, the breed choice on such grasslands has to be based criteria on a system scale and system scale such as longevity, fertility and possibility for local valorisation of the milk. Concerning the best use of steep mountainous pastures, the trade-off of the cows in order to save energy has to be taken into account.

Supplementary Table IV.S1. Characterisation of the swards by pasture type (low/high diversity (div.) and average slopes) during each grazing period (arithmetic means and standard error of the mean).

Grazing period	Early 1st grazing			Late 1st grazing			Early 2nd grazing			
Pasture type	Low div. 2°	High div. 7°	High div. 22°	Low div. 2°	High div. 7°	High div. 22°	Low div. 2°	High div. 7°	High div. 22°	SEM
Vegetation types theoretically available for bites (%)										
Short vegetative	5	18	29	48	36	48	53	63	45	18.6
Tall vegetative	86	59	51	48	48	44	40	24	29	13.5
Mature vegetation	9	22	20	4	16	8	7	13	26	10.1
Grasses	68	66	62	67	55	65	57	52	42	9.1
Legumes	17	9	8	22	5	7	27	5	9	3.7
Forbs	15	25	30	10	41	28	16	43	49	11.8
Sward composition (g/kg DM)										
Organic matter	900	939	926	902	925	920	901	922	910	3.7
CP	156	103	115	123	83	82	153	89	93	7.0
NDF	581	601	606	609	602	641	590	645	647	25.5
ADF	274	307	293	312	335	342	301	341	339	9.2
Nutritional value										
Digestibility	0.702	0.635	0.651	0.645	0.583	0.585	0.649	0.559	0.567	0.013
NEL (MJ/kg DM)	5.6	5.1	5.2	5.0	4.5	4.5	5.1	4.3	4.3	0.11
PDIE (g/kg DM)	92.0	79.3	82.0	82.0	70.0	69.0	88.0	69.0	69.3	3.26
PDIN (g/kg DM)	103.0	68.0	76.3	81.0	55.3	54.3	102.0	59.0	61.3	6.43

Supplementary Table IV.S2. Characterisation of the zones of high diversity pastures during each grazing period (arithmetic means and standard error of the mean).

Grazing period	Early 1st grazing			Late 1st grazing			Early 2nd grazing			
Zone	Z1 (n=2)	Z2 (n=2)	Z3 (n=2)	Z1 (n=2)	Z2 (n=2)	Z3 (n=2)	Z1 (n=2)	Z2 (n=2)	Z3 (n=2)	SEM
Vegetation types theoretically available for bites (%)										
Short vegetative	9	14	48	38	42	46	42	53	68	18.7
Tall vegetative	52	67	47	47	46	45	32	30	16	19.5
Mature vegetation	39	19	6	15	11	9	26	17	16	9.9
Grasses	71	67	54	67	54	58	59	48	34	9.4
Legumes	14	5	8	10	4	3	11	7	2	3.8
Forbs	15	28	38	23	42	39	29	45	64	10.8
Sward composition (g/kg DM)										
Organic matter	935	930	932	920	923	924	916	919	914	11.6
CP	108	114	106	80	88	80	88	88	95	9.4
NDF	614	602	594	643	624	597	679	657	602	39.9
ADF	304	293	303	340	338	338	355	343	321	13.7
Nutritional value										
Digestibility	0.640	0.653	0.636	0.585	0.587	0.580	0.551	0.562	0.578	0.027
NEL (MJ/kg DM)	5.2	5.3	5.1	4.5	4.6	4.5	4.2	4.3	4.5	0.2
PDIE (g/kg DM)	80	83	79	69	71	68.5	67.5	69	71	4.0
PDIN (g/kg DM)	71	76	69.5	53	59	52.5	58.5	58.5	63.5	6.5

Supplementary Table IV.S3. Least Square means for overall effects on cow's behaviour, diet selection, faecal composition and performance.*

	Pasture type			Grazing period			
	Low div. (L)	High div. (H_7)	High div. (H_{22})	Early 1st grazing	Late 1st grazing	Early 2nd grazing	SEM
Time allocated to activities (%)							
Resting time	32	34	37	45^a	37^b	21^c	2.0
Grazing time	59	53	57	47^b	53^b	69^a	2.0
Other activities	9^b	13^a	6^b	8	10	9	1.9
Time spent in Z1	–	40^a	27^b	45^a	32^b	24^c	1.6
Time spent in Z2	–	29^b	48^a	51^a	25^c	40^b	2.0
Time spent in Z3	–	30^a	24^b	3^c	43^a	36^b	5.1
Vegetation types in the bites (%)							
Short vegetation	42^c	59^a	52^b	27^c	51^b	75^a	6.1
Tall vegetation	44^a	33^b	41^a	62^a	35^b	22^c	2.0
Mature vegetation	13^a	8^b	6^b	12^a	14^a	2^b	2.1
Grasses	77^a	71^b	67^b	74	72	70	1.4
Legumes	16^a	2^c	4^b	7^{ab}	6^b	9^a	1.6
Forbs	8^b	27^a	29^a	19	22	22	3.1
Jacob's index of selectivity (-1 < IS < 1)							
Short vegetation	0.25^b	0.46^a	0.36^{ab}	0.50^a	0.12^b	0.45^a	0.090
Tall vegetation	-0.32^b	-0.21^{ab}	-0.10^a	-0.17	-0.23	-0.23	0.045
Mature vegetation	0.13^a	-0.54^b	-0.55^b	-0.29^b	0.09^a	-0.76^c	0.145
Grasses	0.31	0.26	0.19	0.14^b	0.22^b	0.40^a	0.032
Legumes	-0.22^a	-0.41^b	-0.40^b	-0.35^{ab}	-0.41^b	-0.27^a	0.070
Forbs	-0.38^b	-0.19^a	-0.12^a	-0.06^a	-0.21^b	-0.42^c	0.036
Faeces composition (g/kg DM)							
CP	136^a	125^b	127^b	145^a	116^c	126^b	2.1
ADF	328^b	349^a	351^a	338^b	351^a	338^b	4.7
Calculated OMD†	0.717^a	0.697^b	0.698^b	0.725^a	0.683^c	0.703^b	0.003
Milk (kg)	14.3^a	12.3^b	13.2^b	17.2^a	12.6^b	9.9^c	0.28
Δ Milk yield (kg)	-1.9^a	-3.3^b	-3.1^b	0.1^a	-3.9^b	-4.6^b	0.29
Milk fat (g)	571^a	483^b	517^b	681^a	485^b	405^c	12.7
Milk protein (g)	457^a	397^b	411^b	567^a	386^b	312^c	18.4
BW (kg)	613	617	610	618^a	615^{ab}	608^b	4.8

**P*-values are reported in Table 2. Ho: Holstein; Mo: Montbéliarde; Va: Valdostana. $^{a\text{-}c}$ Within same trait and effect, values without common superscripts differ.

†Organic matter digestibility

Supplementary Figure IV.1. Map of the experimental pasture areas (2 cm = 100 m).

L

Low diversity, no slope

64 % Grasses
14 % *Forbs*
22 % *Legumes*

H7

High biodiversity, slope 7°

58 % *Grasses*
36 % Forbs
6 % *Legumes*

H22

High biodiversity, slope 22°

58 % *Grasses*
34 % Forbs
8 % *Legumes*

CHAPTER V

Milk composition is impaired the day after transhumance, but the properties of Fontina, a semi-hard cheese, are not affected

Based on Koczura M, Martin B, Turille G, De Marchi M, Kreuzer M, Berard J.
In preparation for the International Dairy Journal

ABSTRACT

Short-term effects of transhumance to high altitude were investigated in Valdostana cow milk and Fontina cheeses. Individual cows and, in addition, herds were observed around transhumance. Milk was collected and bulk milk was manufactured to cheese at each milking on days 1, 2 and 5 after transhumance compared to 5 days before. Somatic cell count and milk fat content increased the day right 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 fatter and easier melting cheeses in the evening. Our results furnish first indications allowing to better characterise the changes during this transition and will help farmers to tackle the difficulties to produce cheese during this period.

HIGHLIGHTS

- The day right after transhumance, bulk milk was fatter and richer in somatic cells.
- Changes in milk composition do not influence coagulation properties and only have a small effect on Fontina cheese.
- Fontina from the evening were fatter, brighter and easier melting, whereas Fontina from the morning were harder.

1. Introduction

In mountainous regions, transhumance is a common practice allowing an optimal valorisation of highland pastures during summer. 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 consist of mountains and a prospering agricultural sector in these areas supports other sectors such as tourism in economy (Santini et al. 2013). Moreover, the agricultural and food production linked with tradition and local know-how (the *terroir*) are of great importance to the local culture. Such products are labelled and protected by the European Union (Santini et al. 2013). In the Aosta Valley (Italy), a semi-hard cheese is traditionally produced with raw milk from autochthonous cows grazing high mountain pastures in summer, the Fontina PDO. With progressing season, these cows are grazing pastures of increasing altitude. For that, they are transported or have to walk or both to the upland pastures (transhumance). Farmers manufacturing the cheese directly in the buildings at the site of the mountain pastures, often report facing challenges in cheese production shortly after the transhumance. These difficulties, and defects noted in the cheeses, appear specifically 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 et al. 2006), which is playing an important role in cheese manufacturing. These effects are even pronounced under high altitude grazing conditions (Leiber et al. 2006). Proportions of unsaturated fatty acids and carotenoids in milk fat from cows grazing highland pastures strongly affect the cheese texture and colour (Nozière et al. 2006; Martin et al. 2005). However, diet changes concern the entire mountain grazing season and are mostly associated with positive cheese quality. Extensive studies on mountain systems with transhumance showed that the milk yield, milk protein content and rennet coagulation properties are impaired on highland pastures (Leiber et al. 2006; Bergamaschi et al. 2016; Zendri et al. 2016a) especially at very high altitude (Leiber et al, 2006; Koczura et al. 2018). In addition, cheese yield is thus reduced (Zendri et al. 2016b). There is also a higher percentage of milk samples that do not coagulate (Niero et al. 2018). These differences are partly linked to the higher somatic cell count (SCC) in milk (Lamarche et al. 2000; Bergamaschi et al. 2016; Niero et al. 2018). Milk with a 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 et al. 2004). These studies typically gave cows a sufficiently long adaptation period to the pastures before investigating milk properties.

To the knowledge of the authors, direct effects in the days around transhumance on milk and cheese quality were not yet 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 to a new environment. Indeed, it was previously demonstrated that milk had a higher fat content and SCC (D'Hour et al. 1994; Coulon et al. 1998). However, even transport to high mountain 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 involving 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 somatic cell count occurs right after the transhumance, (ii) the change in milk composition impairs milk coagulation properties and leads to fatter cheeses with taste and texture defects and (iii) these changes are no longer observed after an adaptation time of several 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. A first part of the study involved 16 autochtonous Valdostana Red Pied cows (172±30 days in milk, 8.9±2.3 kg milk per milking at the beginning of the experiment) which were randomly selected from one herd (Institut Agricole Régional d'Aoste, Aosta, Italy) in a way that half of the cows were primiparous and the other half multiparous. 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 am (morning) and 4.00 pm (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 milk flow were recorded and individual milk samples taken at two milkings each once 5 days before transhumance (Day -5), then on the evening and the following morning following transhumance (Day 1), then the next two milkings (Day 2) and then on the fifth day after transhumance (Day 5).

In the second part of the study, bulk milk was sampled during five walked transhumances to high altitude (between 200 and 400 m of difference in height) practiced in different farms all located in Aosta Valley (Table 1). Cows were either managed exclusively outside using a mobile milking parlour or milked and housed in tied-stall barns overnight, grazing outside during the day, receiving 2 kg.d^{-1} of the previously mentioned concentrate. The management system (outside or barn) did not change within transhumance. Transhumances lasted for between 1 and 1.5 h and were performed after morning milking. Bulk milk was collected at 7.00 am (morning) and 6.00 pm (evening) applying the same four-day sampling scheme as in the first study part. In this second project part, bulk milk samples from the respective entire herds were collected in amounts allowing the analysis of milk properties and to produce Fontina PDO cheeses.

Table V.1. Description of the transhumance events.

Trans-humance event	Date	Altitude range (m a.s.l.)	Location	Milking system	Size of the herd	Duration
1	10/06/2016	1500–1800	Rhêmes St George	Mobile	60 cows	1h30
2	14/06/2016	1400–1600	Valnontey	Barn	38 cows	1h30
3	05/07/2016	1800–2100	Entrelor	Mobile	55 cows	1h00
4	23/07/2016	1700–2100	Avise	Barn	90 cows	1h30
5	06/09/2016	1800–2100	Rhêmes Notre Dame	Mobile	50 cows	1h00

2.2. Milk analysis

The milk samples, preserved at +4°C with Bronopol (D&F Inc., Dublin, CA, USA), were analysed for contents 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). 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") 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 manufacturing (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 out of the fire to drain the chunks, and a 10 to 15 min resting phase was applied. Then, the drained curd was shaped and pressed for approximately 24 h, using a wooden mould with a diameter varying between 35 and 45 cm and a height of 7 to 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 × 4 × 5 cheeses). The pH of the fresh cheeses was measured thanks to 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 contents of DM, fat, protein, ash, calcium and NaCl using NIRSystems 5000 (Foss Electric A/S). 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, 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., USA). Force at failure, Young 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 tastes 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 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 grissini, if requested) were provided to each panellist in between the samples.

2.5. Statistical analyses

Data were analysed using SAS version 9.4 (SAS Inst. Inc., Cary, NC). Normality of the residuals was checked using the Shapiro-Wilk test. 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 individuals, with the fixed effects of the day in relation to transhumance (-5, 1, 2, or 5), time of milking (evening or morning), parity (primiparous or multiparous), and interactions day × time of milking and day × parity. All other interactions were excluded from the model as they turned out to be never significant. Day in relation to transhumance was considered as the repeated factor, with the cow nested as subject. 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 in order to compare the gross composition of NC samples with that of the coagulated (C) samples.

For bulk milk and cheeses there were five replicates (transition events; Table V.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 in relation to 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 to 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

All parameters were affected by the day of transhumance (Table V.2.). Individual MY dropped by 44% in the evening and 36% in the morning on day 1 (Figure V.1.). In the morning, it did not increase back to its previous level, while it did in the evening. The milk flow followed the same evolution, decreasing after the transhumance and never reaching back the level from Day -5. Fat content increased, especially on the morning of Day 2 (+9.2 g kg^{-1}, Figure V.2.), leading to an increase in fat-to-protein ratio in Day 2. Protein content increased by 1.2 g kg^{-1} on Day 1 and casein followed the same evolution, whereas 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. The SCC increased by 71 × 10^3 cells mL^{-1} on Day 1 and 45 × 10^3 cells mL^{-1} on Day 2. Transhumance also increased the BHB content of the milk on Day 1, by 0.039 mmol L^{-1} in the evening and 0.027 mmol L^{-1} in the morning. Compared to Day -5, urea content was higher on Day 1 (+40 mg kg^{-1}) and lower on Day 5 (-34 mg kg^{-1}). Compared to Day -5, RCT increased by 1.7 min in Day 5 but no differences were observed in Days 1 and 2. The a_{30} increased by 3.2 and 4.1 mm in Day 1 and 2, respectively, while the k_{20} decreased by 0.7 min in both Days 1 and 2. Milk pH was slightly lower in these days too: -0.02 and -0.04 compared to Day -5, respectively. The proportion of NC samples doubled in Day 5 compared to Day -5 (Figure V.2.). Globally, 18.6 % of the milk samples did not coagulate. These NC samples had a lower protein and casein content and a higher SCC and BHB content than C samples (Figure V.3.). The lactose content was 1.3 g kg^{-1} lower and the pH higher by 0.02 in NC samples, compared to C samples (data not shown, $P < 0.05$ an $P < 0.01$, respectively). Other parameters of milk composition did not differ between NC and C samples.

The time of milking also affected a few parameters: in average milk yield, flow, lactose content and casein-to-protein ratio were lower in the evening than in the morning, while milk fat, fat-to-protein ratio and urea content were higher in the evening. Lactose content of the milk of multiparous cows was lower than that of primiparous cows and it decreased in Day 1 compared to Day -5, whereas this did not happen in primiparous cows. The casein-to-protein ratio was higher in primiparous cows, and the SCC of multiparous cows' milk was 2.4 times higher than that of primiparous.

Table V.2. Effect of the day in relation to 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 (n = 16 per day and per milking).

	Day in relation to transhumance				Time of milking		Parity			*P*-values				
	-5	1	2	5	Evening	Morning	Primi-parous	Multi-parous	SEM	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

***, **, *, $^{(*)}$Values differ from those of Day -5 at $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.10$, respectively.

[a]Abbreviations are: SCC, somatic cell count, BHB, β-hydroxybutyrate; RCT, rennet coagulation time, a_{30}, curd firmness 30 min after rennet addition; k_{20}, curd-firming time until 20 mm firmness is reached.

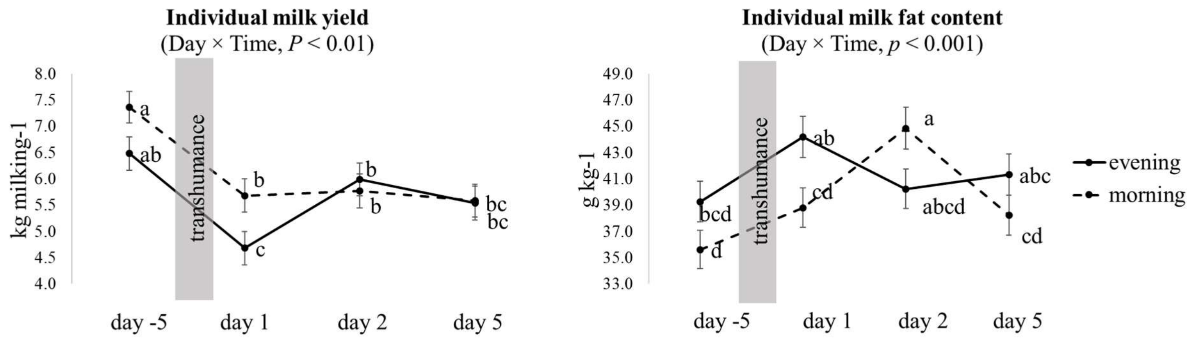

Figure V.1. Effect of the day in relation to transhumance on evening and morning individual milk yield and fat content.

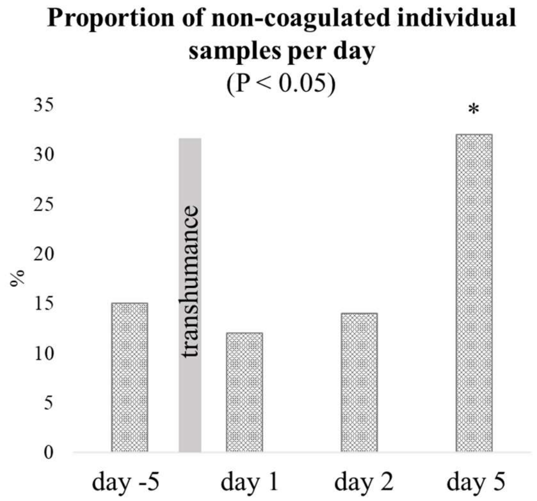

Figure V.2. Proportion of non-coagulating samples of individual milk, according to the day in relation to transhumance.

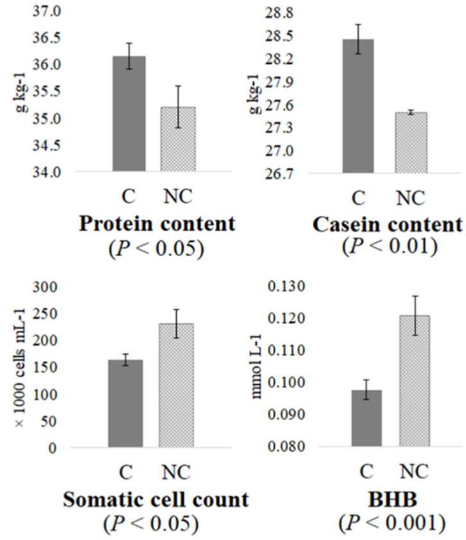

Figure V.3. Comparison of non-coagulating (NC) and coagulating (C) samples (individual milk).

3.2. Bulk milk

Milk fat and protein contents significantly increased the day after transhumance, by 4.2 and 0.9 g kg^{-1}, respectively (Table V.3.). While protein content came back to normal on Day 2, fat content tended to stay higher by 3.7 g kg^{-1} and the fat-to-protein ratio was affected in the same way. Casein content followed the evolution of protein and increased by 0.7 g kg^{-1}, without affecting the casein-to-protein ratio. On Day 1, the SCC increased by 161×10^3 cells mL^{-1}. All samples did coagulate, and the day of transhumance affected the curd firmness a_{30}: it was higher by 6.4 mm on Day 1 compared to Day 5, but not significantly different from Day -5. All milk composition traits that were affected by the transhumance went back to their previous level within 5 days. Moreover, the fat content was globally higher by 3.3 g kg^{-1} in the evening milk than in the morning, significantly increasing the fat-to-protein ratio likewise. Lactose content was higher by 0.4 g kg^{-1} in morning milk.

3.3. Cheese

3.3.1. Chemical composition, proteolysis, colorimetry and rheology

Cheeses' chemical composition, colorimetry and rheology were not significantly affected by the day of transhumance (Table V.4.). Only the WSN-to-N ratio was higher in the morning cheeses than in those of the evening in Days -5 and 5, whereas it was the contrary in Days 1 and 2 (Figure V.4.). The cheese manufacturing time, however, affected several parameters: the fat content was higher in the evening than in the morning by 2.1 % DM and protein content lower by 0.8 % in the evening. Cheeses from the evening had a higher index of brightness than those from the morning (+2.3). All rheology parameters were significantly lower in the evening cheeses than in the morning ones: the force at failure and Young module by 21 and 26%, respectively, and the resistance to compression (stress) at 20, 40 and 60% of the cheese's height was lower in the evening by 26, 28 and 25%, respectively.

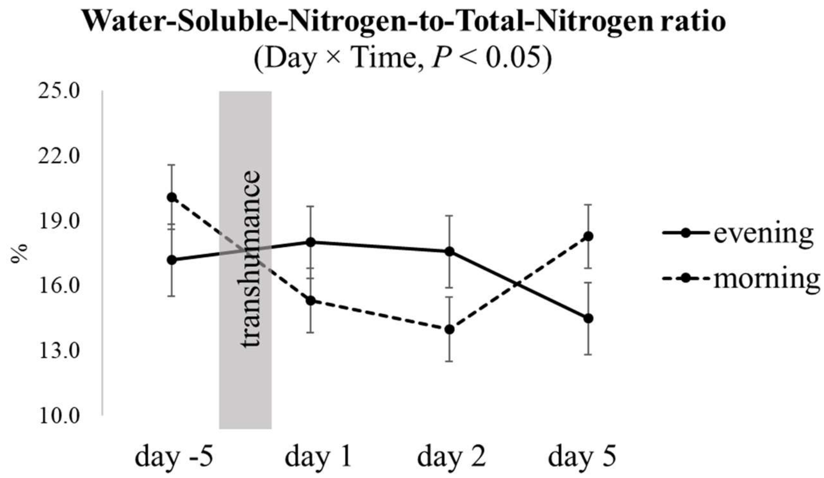

Figure V.4. Water-soluble-nitrogen-to-total-nitrogen ratio in cheeses from the morning and evening, according to the day in relation to transhumance.

Table V.3. Effect of the day in relation to transhumance, time of milking and their interaction on composition and coagulation properties of the bulk milk (n = 5 per day and per milking).

	Day in relation to transhumance				Time of milking			*P*-values		
	-5	1	2	5	Evening	Morning	SEM	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

**, *, $^{(*)}$Values differ from those of Day -5 at $P < 0.01$, $P < 0.05$ and $P < 0.10$, respectively.

[a]Abbreviations are: SCC, somatic cell count, BHB, β-hydroxybutyrate; RCT, rennet coagulation time, a_{30}, curd firmness 30 min after rennet addition; k_{20}, curd-firming time until 20 mm firmness is reached

Table V.4. Effect of the day in relation to transhumance, time of manufacturing and their interaction on composition, colour, proteolytic and rheological characteristics of the Fontina cheese (n = 5 per day and per milking).

	Day in relation to transhumance				Time of manufacturing			*P*-values		
	-5	1	2	5	Evening	Morning	SEM	Day	Time	Day × Time
pH 24 h	6.41	6.55(*)	6.38	6.46	6.46	6.45	0.172	0.058	0.843	0.286
Chemical composition										
Dry Matter (%)	59.7	59.9	60.3	60.1	60.2	59.8	0.97	0.675	0.387	0.846
Fat content (%)	30.6	31.3	31.1	30.2	31.6	30.1	3.40	0.431	0.038	0.228
Protein content (%)	24.7	24.3	24.6	25.2	24.3	25.1	0.47	0.370	0.030	0.767
Ashes (%)	3.68	3.57	3.58	3.47	3.55	3.60	0.116	0.307	0.569	0.097
NaCl (%)	1.87	1.87	1.75	1.62	1.84	1.71	0.125	0.147	0.143	0.850
Calcium (%)	0.828	0.799	0.821	0.847	0.805	0.842	0.024	0.364	0.056	0.715
Fat content (% DM)	51.2	52.6	51.9	50.6	52.6	50.5	0.95	0.248	0.007	0.124
NaCl (% DM)	3.13	3.15	2.95	2.72	3.08	2.90	0.234	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
Redness (a*)	1.91	1.65	2.13	1.94	1.97	1.85	0.486	0.681	0.637	0.723
Yellowness (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

(*)Values differ from those of Day -5 at $P < 0.10$.

[a]Abbreviations are: WSN, water soluble nitrogen; N, total nitrogen; PTSN, phosphotungstic acid-soluble nitrogen.

Table V.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 (n = 5 per day and per milking).[a]

	Day in relation to transhumance				Time of manufacturing			P-values		
	-5	1	2	5	Evening	Morning	SEM	Day	Time	Day × 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

**, *Values differ from those of Day -5 at $P < 0.01$ and $P < 0.05$, respectively.

[a]Grades from 1 to 7, 1 = low and 7 = high.

3.3.2. Sensory analyses

The sensory panel was able to detect few differences in the cheese taste and visual aspect according to the day of transhumance, in interaction with the time of manufacturing (Table V.5.). Cheeses from the Day 1 had a more sour taste than those from the Day 2, but were not significantly different from those from the Day -5. Appearance of the eyes of the cheeses globally improved on Day 2 after transhumance, and the same evolution was observed for the colour of the core (Figure V.5.). Morning cheeses were harder and less melting in average and on Day 1, the gap between evening and morning cheeses' texture increased. Eventually, the panel failed at characterising abnormal tastes or defects in the cheeses specific from the Day 1 after transhumance.

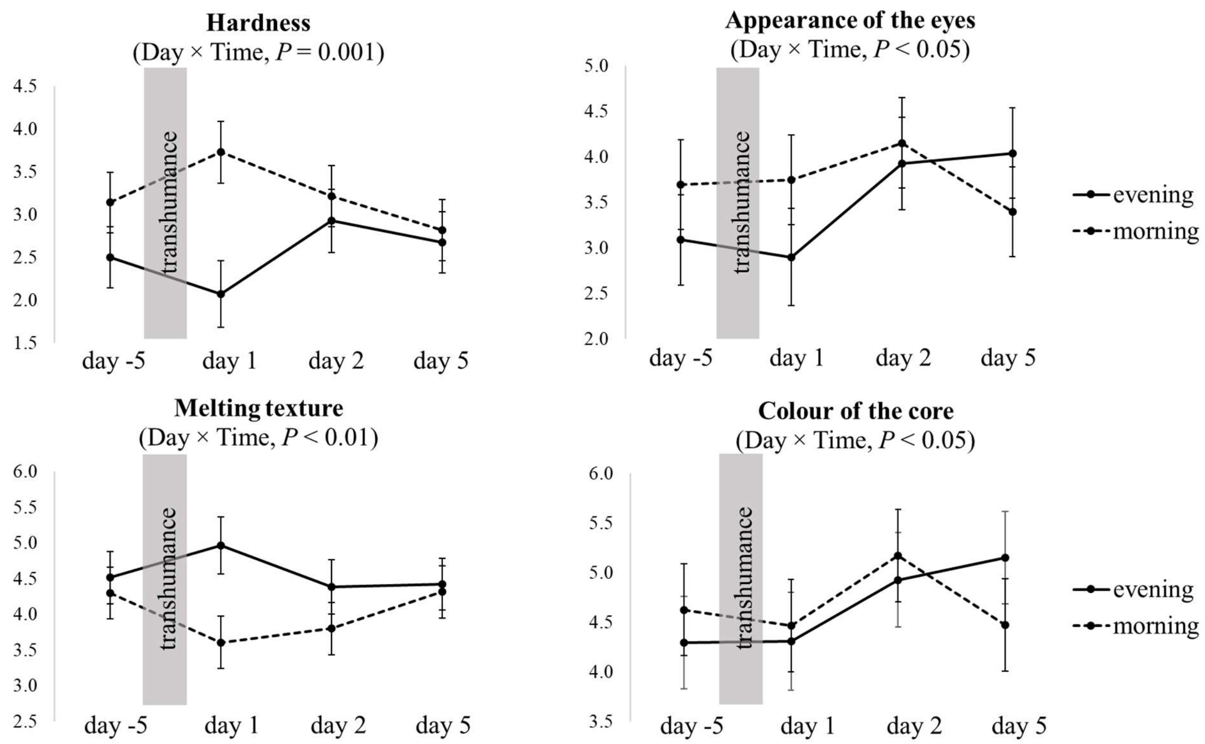

Figure V.5. Effect of the day in relation to transhumance on evening and morning cheeses' texture and visual aspect.

4. Discussion

4.1. Effects of the day of transhumance

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 walk of the cows, as previously shown by D'Hour et al. (1994) and Coulon & Pradel (1997). If the increase in fat content observed in individual milk in the present experiment was comparable to that found by these authors, it was slightly lower for bulk milk. Milk fat content increase can be explained by the drop in MY and especially the mobilisation of body fat by the animal to cope with the physical effort (Coulon & Pradel, 1997). The concomitant increase in BHB in milk right after transhumance confirms this, suggesting a lower energy supply (Ježek et al, 2017). In these low energy supply conditions, milk protein content should decrease (Coulon & Rémond, 1991). However, in the present study, the great drop in MY in Day 1 seems to counteract this effect and concentrate protein and urea (Coulon et al. 1998). The decreasing casein-to-protein ratio in the day right after transhumance means a higher proportion of soluble proteins that is linked to the higher SCC (Coulon et al. 2002). The increase in SCC was lower than previously observed during long walk conditions (Coulon & Pradel, 1997), probably because cows in the present experiment walked less time and were already experienced in walking as they were raised in mountain transhumant systems. Besides, 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 primiparous cows. Indeed, the increase in SCC in Day 1 was numerically lower for the latter. The udder of primiparous cows experienced less movement and milking events, and therefore probably presents less injuries and opportunities for primary infections (Martin et al. 2018). The lower level of SCC in individual milk compared to bulk milk could also be linked to the primiparous cows: indeed, in the first group half of the cows were multiparous, whereas in the herds this proportion usually do not goes up 30 % of the animals. Eventually, it is interesting to point out that except for protein and urea, all milk composition changes already disappeared after 5 days on the new mountain pasture. The increase in protein in Day 5 is probably due to the improved herbage nutritive value when arriving on the new pasture right after transhumance.

4.1.2. Milk coagulation properties

The proportion of individual milk samples that did not coagulate is coherent with previous studies on other breeds: 30 % of Finnish Ayrshire cows produced NC samples at least once in the lactation (Tyrisevä et al. 2004). Besides, 12.9% of the milk samples of Holstein cows in an intensive system (Toffanin et al. 2015) and 13.2 % of the samples from Valdostana cows managed in extensive conditions (Niero et al. 2018) did not coagulate. Individual milk does not always coagulate because of different parameters, among which casein genetic polymorphism (Coulon et al. 2004), which was not investigated here. Moreover, soluble proteins and enzymes found in milk include plasmin, which also hydrolyses casein and impairs coagulation through denaturation of the micelles (Bhatt et al. 2017). Differences in milk gross composition between NC and C samples in the present study confirmed that a higher SCC is related to a slow coagulation (Coulon et al. 2004, Niero et al. 2018). Only the link between coagulation ability and BHB content is unclear and may be indirect: Sundekilde et al. (2013) showed that BHB content is usually increased in milk with high SCC. A small increase in pH was observed for NC samples compared to C samples, which may have been sufficient to slow down coagulation as pH is inversely related to rennet coagulation time (Shalabi & Fox, 1982). Then, according to SCC and BHB content of the milk in Day 1, a higher proportion of NC samples and a longer RCT would have been expected right after transhumance. Unlike our hypothesis, this was not the case in the present study, probably because the moderate increase in SCC was counterbalanced by the lower pH in Day 1. The only difference observed in coagulation ability and RCT according to the day of transhumance occurred in Day 5 and is actually hard to interpret. In Day 1, the curd firmness a_{30} was however impacted: as previously observed in other studies (Bittante et al. 2012), its increase on Day 1 was concomitant with the increase in protein (including casein) content. As already observed in the same breed, parity did not affect milk coagulation properties (Niero et al. 2018).

4.1.3. Cheese

Unlike our hypothesis, day of transhumance hardly affected cheese chemical and sensory properties. First, unlike milk fat, fat content in the cheese dry matter did not significantly increase the day right after transhumance. It actually followed the evolution of the fat-to-protein ratio, which is coherent with previous observations (Walstra et al. 2006, Coppa et al. 2011b). Surprisingly, the high level of somatic cells in the milk in Day 1 is not associated with a higher proteolysis of the cheese as expected. Indeed, a high SCC usually induces 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). The presence of plasmin among the soluble enzymes and the increase moisture of the cheese made out of 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 Day 1 and 2 is coherent with the concomitant decreased melting texture and increased hardness highlighted by the sensory panel, and so is the opposite evolution of these parameters in the evening cheeses. Indeed, higher proteolysis (i.e. degradation of the protein matrix) induces a weaker cheese structure (Bugaud et al. 2001). Actually, the only direct effects of the days of transhumance in cheese taste in the present study were observed by the sensory panel and not measured 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. Sensations of panellists depend on a combination of different chemical compounds that may not directly influence the cheese when being taken into account individually. In the present study, panellists found a higher sour taste in the cheeses of Day 1, which could be partly explained by the numerically lower proteolysis at that time, but might also result from the acidification process or other combined effects. The tendentially higher pH 24h in Day 1 suggests a slower acidification in mould, which could results from poorly drained cheeses. The arrival to 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, therefore more acid ripened cheeses. When moving to the upland pastures, the change in the microbial environment may also play a role. Some other defects in taste were globally observed by the sensory panel but could not be linked to the day of transhumance and were more probably related to the different farms' typical environments, as previously underlined by Giannino, Marzotto, Dellaglio & Feligini (2009) in Fontina production. Eventually, the global improved visual aspect of the cheese after 2 and 5 days with a globally yellower core paste may be due to the younger phenological stage of the new pasture, leading to a higher carotenoid content in milk and cheese (Nozière et al. 2006).

4.2. Effects of the milking/manufacturing time

In the present study, milk fat content and SCC decreased when the preceding milking interval increased, as previously shown by Rémond et al. (2009). Moreover, the similarity of morning and evening milk's coagulation properties was confirmed (Niero et al. 2018). If the difference between morning and evening milk are well-documented, the consequences on cheeses have received little attention so far. Martin et al. (2009) showed that the milking frequency (once or twice daily) had an extremely low effect on cheeses properties, but no other studies to our knowledge compared morning and evening cheeses. It seems that the higher fat content and fat-to-protein ratio of the evening milk led to fatter corresponding cheeses. Accordingly, evening cheeses were less hard and meltier, which is coherent with a lower fracture stress (Bugaud et al. 2001). This lower mechanical resistance of the evening cheeses was not related to the other important factors influencing texture, namely dry matter and primary proteolysis. It could be partly due to milk fat composition. Indeed, milk from the evening has a lower content of de novo synthesised fatty acids than that from the morning (Bergamaschi & Bittante, 2017). Unsaturated fatty acids increase fat fluidity, leading to less hard cheeses (Hurtaud et al. 2001). Milk from the evening also is richer in C18:1 cis 9 and poorer in C16:0, which also increases fat fluidity, due to their respective melting point (Ferlay et al. 2010, Lerch et al. 2015). Moreover, fatter cheeses with a more fluid fat content may have a more pronounced oiling-off during processing and thus reflect light (Lerch et al. 2015), which explains the brighter aspect of the evening cheeses in the present study. Eventually, it seems that transhumance only enhanced the global morning-evening difference in cheese texture.

5. Conclusion

The present study confirmed that mountain transhumance clearly influences milk quality on the day after walking to the new mountain pasture, and our results provide first indications that allow quantifying the short-term changes in milk during this transition. All changes disappeared after five days. Results on cheese properties were not so distinct: it seems that apart from sour taste, the time of manufacturing (morning or evening) is more influencing cheeses' properties 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, the farmers could then adapt their transformation process during the days following transhumance for an optimised production. Further studies should investigate the role of the changing production areas and microbial environments in the development of unfavourable cheese's flavours during transhumance.

CHAPTER VI

Consequences of walk or transport by truck for milk composition, coagulation properties and blood metabolites of Holstein, Montbéliarde and Valdostana cows

Based on Koczura M, Coppa M, Bouchon M, Turille G, De Marchi M, Kreuzer M, Berard J, Martin B.
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ABSTRACT

In the mountains, the traditional practice of transhumance is common for dairy production systems to valorise the high altitude summer pastures. Although the effects of highland grazing were intensively studied with respect to performance of cows, milk and cheese quality, the actual moving of the animals to the highlands and the consequences of this stressor for performance and milk quality in the directly following days was poorly investigated yet. The aim of the present study was to compare the effects of a 6–km long walk or truck transport of 10.5 km (duration of 1 h) to accomplish transhumance in three dairy cow breeds contrasting in genetic merit. In a control treatment cows were not moved around. Each of the 12 late-lactating cows per breed was subjected to all three treatments in a Latin Square approach. Cows of the three breeds responded to both moving treatments in similar ways. Walk decreased MY by 1 kg/milking, whereas truck transport did not affect it. Both treatments led to an increase in plasma NEFA and milk SCC compared to control. Truck transportation increased milk fat content. Milk coagulation properties were better for Va and Mo than Ho, but were not affected by walk or truck transport. Further studies aiming at comparing the three breeds should include broader criteria (such as reproduction) on a longer term with repeated transhumance, and production of cheese in controlled conditions after moving the cows.

HIGHLIGHTS

- No clear difference in performance was observed according to the breed in late-lactating cows.
- Walk decreased the milk yield, whereas truck transportation did not affect it.
- Milk fat content was higher the evening after truck transportation.
- Milk SCC increased after both walk and truck treatments.

1. Introduction

Transhumance is a traditional practice used to valorise high summer pastures in mountain dairy systems. Several previous studies already highlighted the effects of highland grazing on milk and cheese quality. Milk produced on mountain pastures has a higher content of fat which is particularly rich in beneficial functional fatty acids (FA) but a lower protein content and, sometimes, less favourable cheese-making properties (Collomb et al. 2004; Hauswirth et al. 2004; Leiber et al. 2005; Koczura et al. 2018). Nozière et al. (2006) showed that the β-carotene and α-tocopherol concentrations were higher in the cheese fat of pasture-based diets, compared to preserved forage-based diets, regardless of cow breed. Studies involving the effect of transhumance on milk and cheese quality at the season scale showed that MY decreases and somatic cell count (SCC) increases in highland pastures (Bergamaschi et al. 2016; Zendri et al. 2016; Niero et al. 2018). Mountain conditions are thus strongly related to changes in milk in the medium term*.* *i.e.* a few weeks after arriving on the pasture. In the short term, it was demonstrated on farm that in the days directly after transhumance, milk composition was impaired (Koczura et al. unpublished), with high fat content and SCC. However, transhumance involves a number of factors including moving cows to different sites, the short-term effects of arriving on a new pasture with young grass, change in altitude, but also changes in milking and cheese manufacturing environments. Effects of altitude as such were already investigated. Higher altitude means hypoxic environment, inducing higher levels of erythrocytes, leucocytes and haemoglobin in blood, coupled with increased respiration and heart rates (Zemp et al. 1989a). Leiber et al. (2004) suggested that these changes cause anorexia and thus explain the decreased feed intake at the beginning of the alpine season. Movement of the cows by practicing transhumance is an important stressor. This was traditionally accomplished by letting the cows walk to the respective pasture. In case the infrastructure allows it, transporting them by truck is now common as well. Indeed, long-distance walks (more than 12 km) increase milk fat content and SCC (D'Hour et al. 1994; Coulon et al. 1998), it decreases the cow's feed intake, and milk yield (MY) declines as well (Coulon and Pradel, 1997). However, these results were obtained under experimental conditions, in stall-feeding conditions and with much longer walks than what could be observed on farm by Koczura et al. (unpublished). Besides, little is known about the effects of truck transportation of dairy cows. It was previously demonstrated that cows had higher level of stress indicators in blood (cortisol, lactate and NEFA) during several hours of transport by truck (Kreuzer et al. 1998). After transportation, thyroid hormone and BHB levels were still high, suggesting continuing energy shortage whereas cortisol levels rapidly declined after unloading the cows (Kreuzer et al. 1998). However, the effects on milk quality coagulation properties were never investigated.

The aim of the present study was to evaluate the effects of moving cows between sites on performance and milk quality directly after arrival. It was the goal to exclude all confounding effects of transhumance and focus exclusively on the effect of the movement of dairy cows between sites during transhumance. It was hypothesised that 1) movement of cows between sites apart by several kilometres will affect the composition of the milk and its rennet coagulation properties, 2) walk of cows will have more severe effects than truck transportation, and 3) the effects of transportation will be enhanced in cows less experienced with walking and truck transport and in cows with a higher genetic merit for milk production.

2. Materials and methods

2.1. Animals and experimental design

The experiment was performed in 2017 at INRA's experimental farm of Marcenat (Herbipôle, 45◦15'N, 2◦55'E; between 1135 and 1215 m a.s.l.), which is accredited to carry out experimentations on living animals (Certificate of Authorization to Experiment on Living Animals N° D 15–114–01). Thirty-six late-lactating multiparous dairy cows with different experience backgrounds and genetic merit for MY were monitored: 12 Valdostana Red Pied (Va, MY potential 4000 kg/lactation, 192±33 days in milk (DIM) at the beginning of the experiment), 12 Montbéliarde (Mo, 5900 kg/lactation, 218±23 DIM) and 12 Holstein (Ho, 6700 kg/lactation, 238±33 DIM). The Ho and Mo cows originated from the experimental farm of Marcenat and already experienced walk and truck transportation in their life. The Va cows had been transferred to Marcenat by truck from the Institut Agricole Régional (IAR, Aosta, Italy) 2 months before the experiment started, and they recovered their initial MY from before the transfer within a few days after arrival (data not shown). In Italy, Va cows were moved by truck and walked to high alpine pastures every year in summer. Seven weeks after the arrival of the Va cows, three groups balanced by breed (4 cows per breed) and milk yield (MY) were created in a complete randomised design. Each group was grazing separately (0.3 ha/cow) 24 h/day on flat grass-dominated pastures without concentrate. At the time the experiment started, Va cows produced 9.9 ± 2.0 kg milk/day, Mo 14.3 ± 1.7 kg/day and Ho 13.6 ± 2.4 kg/day.

Both transport treatment (walk and truck) consisted in a loop circuit leaving from the barn where the milking took place and coming back there. The 'walk' treatment consisted of a 6–km long walk on a flat path. This treatment was intended to last 1 h. Cows were guided by one person in front and followed by two persons encouraging the backmarkers to walk. During the 'truck' treatment, cows were transported for 1 h in a truck that followed a 10.5–km-long winding mountainous road. Cows were moved into the truck directly at the exit of the milking parlour, where they were kept tight. For the 'control' treatment, cows were let in an already grazed paddock near the barn where the milking took place, in order to minimise the walking efforts. After all treatments, cows came back to the barn for the sampling session, then returned on the same pastures they were before, which was 5 min away from the barn and paddock of the control cows. Cows were milked at 07:30 in the morning and 16:00 in the afternoon, and MY was automatically recorded at every milking (Herringbone System, DeLaval, Tumba, Sweden).

The experiment started on the 1st of July and lasted for 3 weeks. Following a Latin square design (Figure VI.1.), the three groups of cows successively underwent the three treatments (walk, truck or control) between 10:00 and 12:00 on the 2nd day of each week (1, 2 and 3), after being confined on a paddock near the milking parlour. Namely, in week 1, 12 cows were walking, 12 cows were transported by truck in parallel and 12 cows were simply grazing near the barn at the same time, and each group of 12 changed to the next treatment in the following 2 weeks.

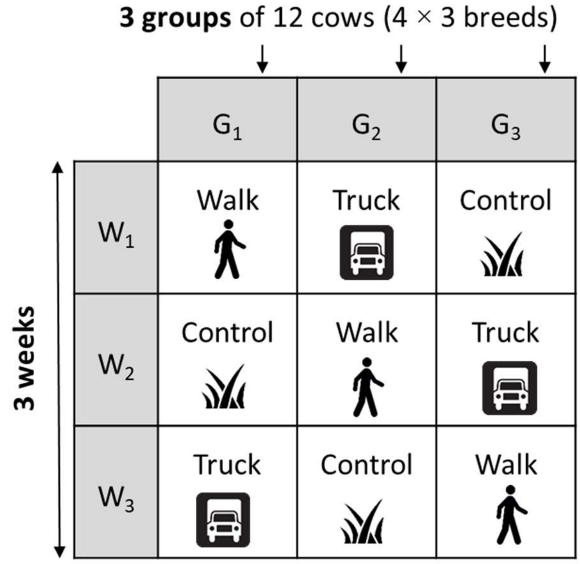


Figure V.I. Experimental design.

2.2. Milk chemical composition, lipolysis and coagulation properties

On the day before treatment (D-1), on the day of treatment (D0) and on the 2 days after (D1 and D2), individual milk samples were collected during evening milking. They were conserved with 2–bromo-2–nitropropane-1,3–diol (Bronopol®, D&F Inc., Dublin, CA, USA) at 4 °C until later analysis of contents of fat, protein, lactose, casein, urea as well as lipolysis by infrared spectroscopy according to NF ISO 9622 (LIAL, Aurillac, France, accredited by Cofrac Nr. 1–0196). Somatic cell count (SCC) in 1 mL of milk was measured by epifluorescence according to ISO 13366–2 (LIAL). Samples of 50 mL were stored at -20°C and later analysed for milk coagulation properties (rennet coagulation time (RCT) and curd firmness (a30)) using a Formagraph (Foss Electronics, Hillerød, Denmark). Samples that did not coagulate within 30 min were considered as non-coagulating (NC) samples and treated as missing values in the statistical evaluation.

2.3. Blood metabolites

Individual blood samples were taken from the tail vein of the cows 12 ± 1 h before the treatment and 6 ± 1 h afterwards in EDTA Vacutainers. Blood samples were additionally collected within half an hour right after the treatment (later defined as 0.25 h): first the control cows, then truck transported cows right after their arrival, then walked cows arriving latest. This was done in a randomised order with respect to breed representatives. Afterwards, blood samples were immediately centrifuged for 20 min at 1200 × g and 4°C. Blood plasma was then stored at -20°C until further analysis of concentrations of non-esterified fatty acids (NEFA, R1 (W1W434–91795) and R2 set (W1W436–91995), Thermo Scientific Arena 20 XT Chemistry System), β-hydroxybutyrate (BHB, Kit Thermo Scientific ref.nr. 984325), glucose (Kit Thermo Scientific ref.nr. 981379) and urea (Kit Thermo Scientific 981818).

2.4. Statistical analysis

Data where SCC was higher than 6000 ×103 cells/mL were considered as missing values. Normality of the data and residues were checked using the Shapiro-Wilk test. Data were subjected to analysis of variance applying a repeated mixed model on SAS version 9.4 (SAS Institute Inc., Cary, NC). Breed (Ho, Mo or Va), treatment (walk, truck or control), day (-1, 0, 1, 2) or hour (-12, 0.25 and 6) for the blood metabolites, and the interactions treatment × time and breed × treatment × time were considered as fixed effects. The repeated factor was day within week (1, 2 or 3), with the subject being the individual cow. Week, group and breed within group were used as random factors. The covariance structure was compound symmetry. Least square means were compared using the adjustment of Tukey for multiple comparisons. The percentages of NC and coagulating (C) samples were compared using a Chi square test. The composition of NC and C samples were compared using a two-sided T-test. For this comparison, the samples with SCC > 6000 ×103 cells/mL were also taken into account. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. General breed type differences

A few global differences between breed types under the conditions investigated and regardless of treatments were observed (Table VI.1.). As expected, Va had the lowest MY during the experiment. The Va's MY was, however, lower than that observed by Niero et al. (2018) and Koczura et al. (2018) in cows in late lactation fed 2 kg concentrate/day. In the present study, cows were also in late lactation, but were not fed any concentrate which might explain the difference. A decreasing gradient in protein and casein content was observed from Va to Mo and Ho. The milk of Va and Mo had +3.4 and +1.7 g/kg more protein than that of Ho, respectively. The lower protein content of Ho milk could be breed-type specific, but also point towards a negative energy balance (Coulon and Rémond, 1991) during the experiment. The lack of difference in blood metabolites between the three breed types sets aside the latter hypothesis. The Va had the lowest and Ho the highest milk pH (+0.09 compared to Va and +0.06 compared to Mo). This is coherent with the longer RCT of the milk observed for Ho compared to Mo (+6.2 min) and Va (+5.8 min), as milk coagulates faster at a low pH (Shalabi and Fox, 1982; De Marchi et al. 2009). The curd firmness (a_{30}) was two times lower for Ho milk than for milk of the two other breeds, which is explained by its lower RCT and casein content. Even though they were not investigated in the present study, the known low B allele frequency of κ-casein in Ho milk (20 %, A.Na.Bo.Ra.Va, 2010) compared to Mo (55 %, OS Montbéliarde, 2014) and Va (70 %, A.Na.Bo.Ra.Va., 2010) could explain the poorer ability to coagulate of the milk. Indeed, cows with BB genotype show better coagulation properties (Delacroix-Buchet et al. 1993).

In total, 20 % of the individual milk samples did not coagulate within 30 min, which is a slightly higher proportion than that observed by Niero et al. (2018). Actually, this is consistent with the generally poor ability of the milk of the Ho to coagulate (Table VI.2.). Indeed, 42 % of Ho samples did not coagulate, whereas this was only 6 % in the Va samples. Compared to C samples, NC samples had lower fat-to-protein and casein-to-protein ratios, a twice higher SCC, a lower lactose content (-4 g/kg) and an increased pH (+ 0.17). The increased pH and SCC content of NC samples are in line with the inability of the milk to coagulate within 30 min, as high SCC and pH are usually associated with slow coagulation (Coulon et al. 2004).

Table VI.1. Breed differences in milk yield, milk composition, lipolysis, pH, coagulation properties and blood metabolites.

	Breed (B)				P-values				
	Va	Mo	Ho	SEM	Breed (B)	Treatment (T)	Day (D)	T × D	B × T × D
Milk yield (kg/milking)	3.55^{b}	5.14^{a}	4.77^{a}	0.412	<0.001	0.080	0.100	<0.001	0.520
Milk composition									
Fat (g/kg)	46.1	42.8	42.7	2.04	0.217	0.603	<0.001	0.020	0.719
Protein (g/kg)	32.6^{a}	30.9^{ab}	29.2^{b}	0.93	0.016	<0.001	<0.001	0.172	0.869
Fat-to-protein ratio	1.42	1.39	1.46	0.042	0.444	0.101	<0.001	0.059	0.858
Casein (g/kg)	28.1^{a}	26.7^{ab}	25.1^{b}	0.791	0.010	<0.001	<0.001	0.217	0.383
Casein-to-protein ratio	0.860	0.864	0.857	0.004	0.300	0.061	0.003	0.021	0.409
Lactose (g/kg)	47.6	47.6	46.5	0.82	0.548	0.001	0.003	<0.001	0.043
Urea (mg/L)	286	318	267	17.6	0.096	<0.001	<0.001	<0.001	0.621
SCC (× 10^3/mL)	516	461	505	272.8	0.303	0.597	<0.001	0.018	0.143
Lipolysis (Meq oleic acid/100 g fat)	0.82	1.10	1.31	0.209	0.144	0.071	0.057	0.041	0.340
Milk pH	6.66^{b}	6.69^{ab}	6.75^{a}	0.021	0.010	0.052	<0.001	0.236	0.504
Coagulation properties									
RCT (min)	16.9^{ab}	16.5^{b}	22.7^{a}	1.42	0.037	0.002	0.011	0.744	0.347
a_{30} (mm)	25.9^{a}	26.1^{a}	10.1^{b}	3.31	0.016	0.006	0.264	0.838	0.075
Blood metabolites (mmol/L)									
NEFA	0.212	0.181	0.177	0.033	0.329	<0.001	<0.001	<0.001	0.618
BHB	0.410	0.465	0.353	0.046	0.128	0.272	<0.001	<0.001	0.793
Glucose	0.519	0.476	0.449	0.033	0.306	<0.001	0.002	<0.001	0.947
Urea	0.230	0.244	0.187	0.020	0.082	0.101	<0.001	0.005	0.726

RCT: Rennet coagulation time, a30: curd firmness, NEFA: Non-esterified fatty acids, BHB: β-hydroxybutyrate.

$^{a\text{-}b}$Values without common superscript differ at P<0.05

Table VI.2. Proportions of coagulating and non-coagulating milk samples and their composition and pH.

	Coagulating	Non-coagulating	SEM	P-value
Proportion of samples (%)	80	20		Chi^2
Truck	85	15	-	0.224
Walk	78	22	-	
Control	77	23	-	
Va	94	6	-	<0.001
Mo	88	13	-	
Ho	58	42	-	
Milk composition				T-test
Fat (g/kg)	44.8	43.0	1.06	0.109
Protein (g/kg)	31.7	32.8	0.68	0.128
Fat-to-protein ratio	1.42	1.33	0.029	0.005
Casein (g/kg)	27.2	27.7	0.54	0.467
Casein-to-protein ratio	0.861	0.845	0.002	<0.001
Lactose (g/kg)	47.7	43.7	0.44	<0.001
Urea	292	288	5.3	0.559
SCC ($x10^3$/mL)	580	1813	285.6	<0.001
Milk pH	6.68	6.85	0.017	<0.001

3.2. Effect of the moving treatment on milk yield and blood metabolites

The MY decreased directly after the walk treatment (day 0) (Table VI.3.), as already reported by Coulon and Pradel (1997). The decrease by 1 kg/milking observed in our study was similar to that observed by the latter authors (-1.2 and -1.3 kg in the two subsequent milkings), even though in the present study cows walked for a shorter time. Investigations of walked transhumance in real on-farm conditions also was accompanied by a similar decrease (-1.5 kg/milking) (Koczura et al. unpublished). Our study thus confirms that the temporary decrease in MY observed in the latter study on the day after transhumance in the Aosta Valley is mostly due to the walk of cows. Unlike the findings of Coulon and Pradel (1997), but similar to observations of Koczura et al. (unpublished), the decline in MY was not recovered after 2 days in the present study. Under real farming conditions, seasonal calving is performed during winter in order to avoid calving on mountain pastures. Therefore, cows investigated under experimental conditions by Coulon and Pradel (1997) were in an earlier stage of lactation than the cows investigated by Koczura et al. (unpublished) and in the present study, where the actual conditions of transhumance were simulated. This may explain the persistence of the effect of the walk on the MY after 2 days. The decreased MY after walking is most probably related to the increased energy expenditure to cope with the physical effort. Supporting this hypothesis, NEFA content in blood was three times higher 0.25 h after the walk (Table VI.4.). The BHB content decreased simultaneously, by the same proportion, showing that the time of deficient energy was too short for the occurrence of ketone bodies from fatty acid overload of the metabolism. It therefore points towards using BHB as short-term energy source as well. All blood metabolites concentrations went back to their previous level 6 h after the treatment.

Unlike walk, truck treatment did not affect the MY (Table VI.3.) although a certain impairment could be expected. Truck transportation, associated with vibrations, noise and proximity between animals, induces high levels of stress (Kreuzer et al. 1998). Maybe the duration of transport was not long enough to cause a clear MY decrease. Moreover, the physical effort to keep balance during transport may involve less fat mobilisation than walk. However, the latter hypothesis is contradicted by the increased levels of NEFA and glucose found in plasma 0.25 h after the transport (they were 4 and 1.3 times higher, respectively, Table VI.4.). Kreuzer et al. (1998) also observed an increase in plasma NEFA due to transport, but to a much higher level. However, the duration of the transport in that study was of 5 h, whereas in the present study, cows spent only 1 h in the truck.

3.3. Effect of the moving treatment on milk composition

The milk fat content significantly increased by 6.4 g/kg on day 0 after the truck transport, and numerically increased by 5.1 g/kg after the walk. Consequently, as protein content was not affected, the fat-to-protein ratio was also increased in both treatments on the same day (by 0.24 for truck and 0.20 for walk, respectively). An increase in milk fat content (+4.1 g/kg) on the day after walked transhumance was observed by Koczura et al. (unpublished). Coulon and Pradel (1997) found an increase by 6 g/kg after a longer walk. At least part of this increase is due to the concentration effect resulting from the concomitant decrease of MY. Still, body fat mobilisation linked to the physical effort (confirmed by the increase in plasma NEFA) also helps explaining the increase in milk fat content, especially with truck transport where MY was not affected compared to day -1 values.

A significant increase in SCC was also observed for both moving treatments on day 0 in the order of + 264 × 10^3 and + 246 × 10^3 cells/mL for truck and walk, respectively. Also Coulon and Pradel (1997) observed a similar increase in SCC on the day after walking, whereas the increase was 3 times lower in Koczura et al. (unpublished). Actually, this might also be linked to the initial level of somatic cells on the day before the walk, that was higher in the present study than in Koczura et al. (unpublished). Again the decrease in MY might explain part of the increase in concentration of somatic cells in milk of walked but not in truck transported cows. Besides, the motion of the mammary gland and inflammation caused by walking or truck transport could trigger a transfer of somatic cells from blood to milk (Coulon et al. 2004). The latter explanation is most probably explaining the big increase in SCC after truck transportation. Milk pH also increased on the day after both treatments (by 0.04 and 0.06 for truck and walk, respectively), which is coherent with the increase in SCC. Lipolysis and milk coagulation properties were not affected by walk or truck transport on the day following these treatments.

Table VI.3. Effect of the day of treatment on milk yield, milk composition, lipolysis, pH and coagulation properties.

		Day				
	Treatment	-1	0	1	2	SEM
Milk yield	Truck	4.29^{b}	4.16^{b}	4.49^{b}	4.51^{b}	0.382
(kg/milking)	Walk	5.19^{a}	4.19^{b}	4.41^{b}	4.46^{b}	
	Control	4.41^{b}	4.75^{ab}	4.47^{b}	4.52^{b}	
Milk composition						
Fat (g/kg)	Truck	43.2^{bc}	49.6^{a}	43.6^{bc}	41.0^{c}	2.00
	Walk	41.4^{bc}	46.5^{ab}	43.9^{bc}	43.1^{bc}	
	Control	42.3^{bc}	44.2^{bc}	46.0^{abc}	41.8^{bc}	
Protein (g/kg)	Truck	31.1	30.7	30.4	30.0	0.72
	Walk	31.2	30.8	30.6	30.8	
	Control	31.9	30.9	31.2	31.3	
Fat-to-protein ratio	Truck	1.38^{bc}	1.62^{a}	1.43^{bc}	1.37^{bc}	0.046
	Walk	1.32^{c}	1.52^{ab}	1.44^{abc}	1.41^{bc}	
	Control	1.33^{c}	1.43^{bc}	1.48^{abc}	1.33^{c}	
Casein (g/kg)	Truck	26.9	26.5	26.2	25.9	0.61
	Walk	26.9	26.5	26.3	26.5	
	Control	27.4	26.7	26.9	26.8	
Casein-to-protein ratio	Truck	0.863^{a}	0.862^{abc}	0.862^{abc}	0.858^{abc}	0.004
	Walk	0.861^{abc}	0.858^{bc}	0.860^{abc}	0.860^{abc}	
	Control	0.860^{abc}	0.862^{abc}	0.863^{ab}	0.857^{c}	
Lactose (g/kg)	Truck	47.7^{ab}	47.6^{abc}	48.1^{a}	46.6^{cd}	0.58
	Walk	47.0^{bc}	45.9^{d}	47.2^{abc}	47.5^{abc}	
	Control	47.1^{abc}	47.7^{ab}	47.6^{ab}	47.1^{abc}	
Urea (mg/L)	Truck	307^{abc}	310^{ab}	244^{d}	248^{d}	15.9
	Walk	279^{c}	306^{abc}	292^{bc}	287^{bc}	
	Control	303^{abc}	326^{a}	292^{bc}	294^{bc}	
SCC (× 10^3/mL)	Truck	325^{b}	589^{a}	477^{ab}	444^{ab}	233.7
	Walk	321^{b}	567^{a}	472^{a}	526^{ab}	
	Control	416^{ab}	399^{ab}	614^{a}	740^{ab}	
Lipolysis	Truck	1.25	1.26	1.01	0.98	0.191
(Meq oleic acid/100 g fat)	Walk	1.07	0.95	0.95	1.05	
	Control	1.15	0.98	1.02	1.27	
Milk pH	Truck	6.67^{d}	6.71^{abc}	6.68^{cd}	6.71^{abc}	0.016
	Walk	6.68^{cd}	6.74^{a}	6.69^{bcd}	6.72^{abc}	
	Control	6.68^{cd}	6.70^{abcd}	6.69^{bcd}	6.73^{ab}	
Coagulation properties						
RCT (min)	Truck	17.2	18.2	17.6	18.2	1.03
	Walk	18.0	18.8	19.7	19.4	
	Control	17.7	19.7	19.6	19.9	
a_{30} (mm)	Truck	23.3	23.1	20.8	22.4	2.39
	Walk	20.5	22.7	18.8	19.8	
	Control	18.4	19.4	18.7	20.0	

*P-values are reported in Table VI.1. $^{a-d}$Values without common superscript differs at P<0.05.

Table VI.4. Effect of walk or truck on blood metabolites of dairy cows.

	Treatment	Hour -12	Hour +0.25	Hour +6	SEM
NEFA	Truck	0.125^{c}	0.539^{a}	0.113^{c}	0.036
	Walk	0.094^{c}	0.321^{b}	0.131^{c}	
	Control	0.099^{c}	0.168^{c}	0.123^{c}	
BHB (mmol/L)	Truck	0.412^{bc}	0.381^{c}	0.441^{abc}	0.042
	Walk	0.497^{a}	0.297^{d}	0.464^{ab}	
	Control	0.484^{ab}	0.288^{d}	0.419^{bc}	
Glucose	Truck	0.478^{b}	0.614^{a}	0.498^{b}	0.027
	Walk	0.469^{b}	0.450^{b}	0.436^{b}	
	Control	0.484^{b}	0.454^{b}	0.447^{b}	
Urea	Truck	0.227^{abcd}	0.206^{cd}	0.218^{abcd}	0.017
	Walk	0.203^{d}	0.210^{bcd}	0.238^{ab}	
	Control	0.236^{abc}	0.204^{d}	0.243^{a}	

*P-values are reported in Table VI.1.

$^{a\text{-}d}$Values without common superscript differs at P<0.05.

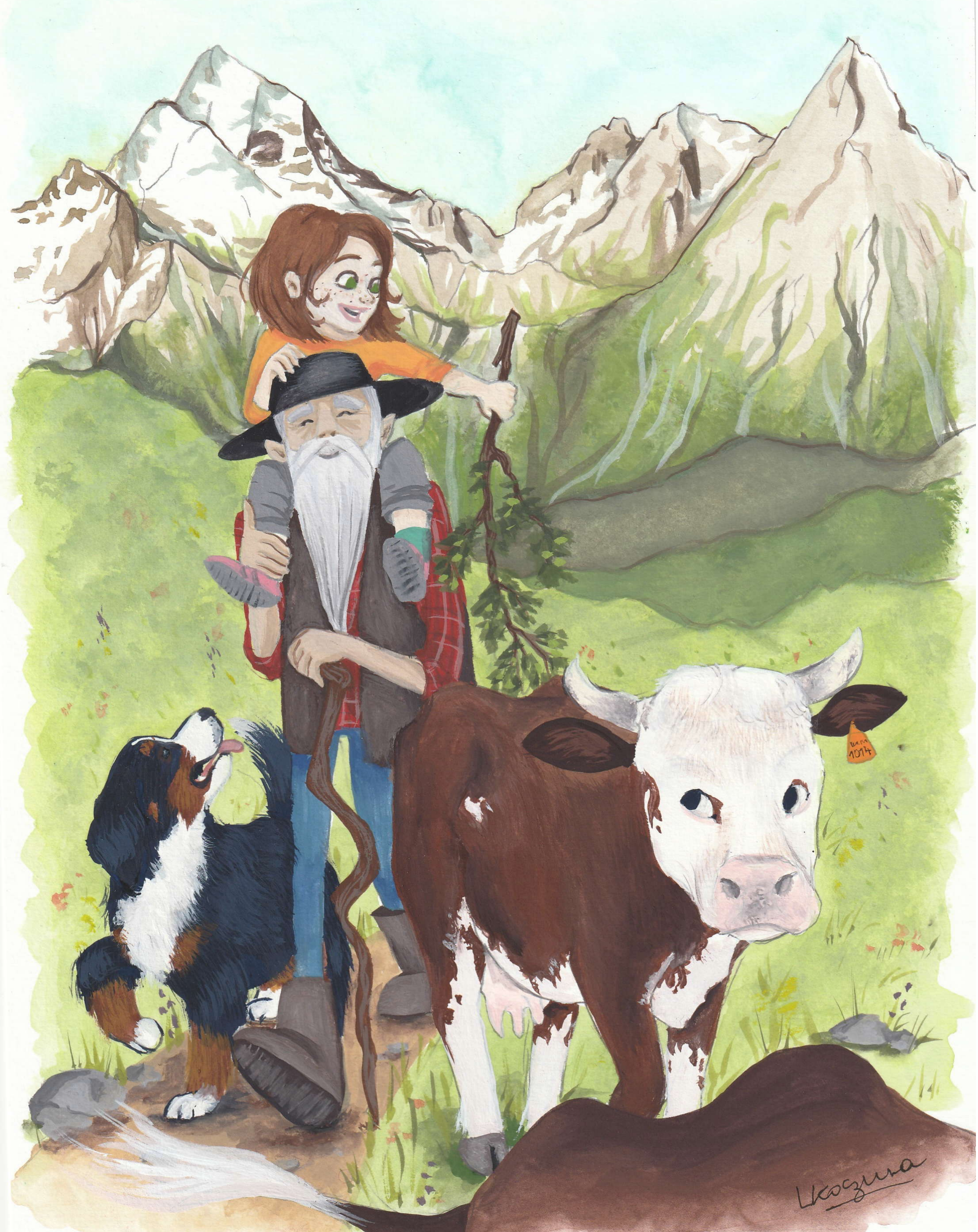
3.4. Lack of breed-type differences within moving treatments

In the end, unlike our hypothesis, the 3 breeds did not perform differently the day after the treatment (interactions breed × treatment × day not significant, Table VI.1.). It was expected of cows with a higher genetic merit for milk production to suffer more from walk and truck treatments, as demonstrated by Coulon and Pradel (1997) with Montbéliardes and Tarentaises cows. Indeed, the decrease in absolute MY was twice as high in the higher yielding Montbéliardes than in Tarentaises. In the present experiment, low genetic merit Va were in addition already adapted and used to transhumance involving walk or truck, unlike Mo and Ho. Maybe the experience of the latter breeds in walking between different pastures and the stable for each milking helped them coping with the different treatments. In the end, no better adaptation of Va in the short-term to walk or truck transportation was observed, compared to Mo and Ho. At their own scale, cows have to make trade-offs by mobilising body fat reserves either to give priority to lactation at the expense of reproduction, or, conversely, give priority to reproduction at the expense of the MY (Ollion et al. 2016). Enhanced differences might be observed in early lactation, when the need for trade-off is even higher. After energy demanding treatments such as these investigated in the present study, a further investigation taking into account the main life functions is necessary.

4. Conclusion

In conclusion, when conditions of transhumance applied in farm practice were simulated under experimental conditions, a 6–km long walk decreased MY and increased plasma NEFA and milk SCC. The present study allowed to quantify for the first time the effects of a 1–hour truck transport on dairy cows. Conversely to walk, truck did not decrease MY, but still increased plasma NEFA and impaired milk quality by increasing both milk fat content and SCC. In the end, neither walk nor truck effects were more pronounced in less experienced cows with a higher genetic merit for MY. No short-term advantage of certain dairy breeds after transhumance were obvious in late-lactating cows. In the future, it would be interesting to further investigate broader criteria on the long term to deepen the comparison of the three breeds. Moreover, further studies should include actual cheese manufacturing in order to assess the consequences of walk and truck transportation of cows on the final product.

CHAPTER VII
General conclusions and perspectives

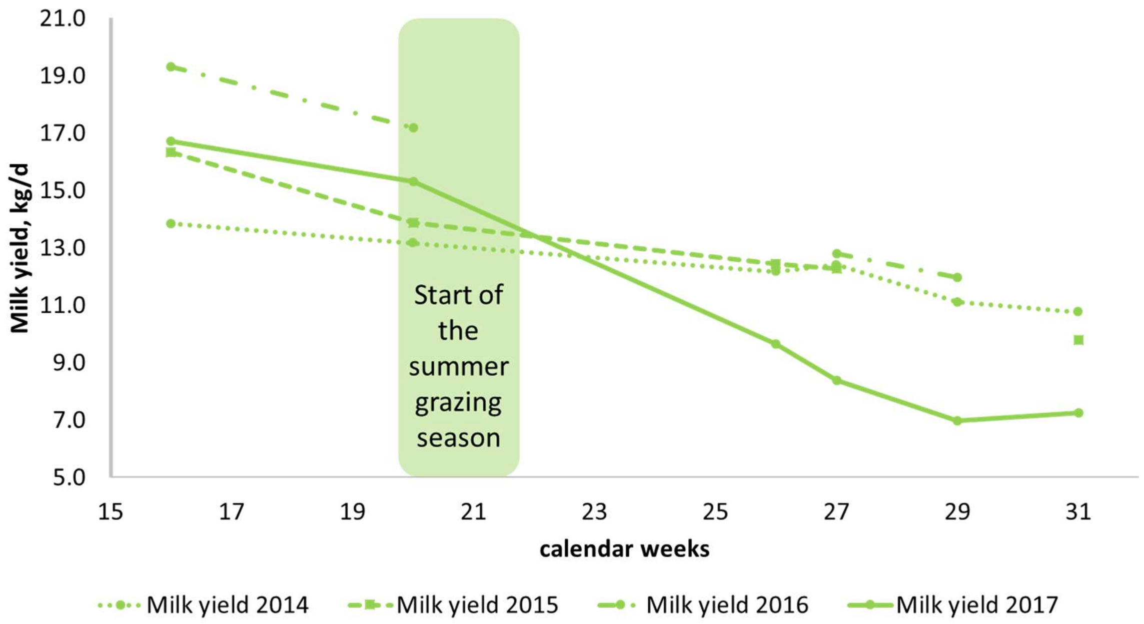


The aim of the present doctoral thesis was to experimentally investigate the potential better ability of local autochthonous breeds to valorise highland pastures in mountain dairy systems, through the case of the Valdostana (Va) breed. First, the objective was to qualify and quantify the behaviour, performance and milk quality of Va in their local environment. Then, their resilience was tested in an experimental comparison with more specialised dairy breeds (namely, Montbéliarde (Mo) and Holstein (Ho)), through an in depth investigation of diet selection on mountain biodiverse pastures. Initiated based on empiric observations from the local farmers and thanks to on-farm samples, our studies also aimed at identifying the short-term effects of moving from the lowland to the highland pastures on Va's milk quality, as well as give first insights on changes in cheese's texture and taste. Eventually, these on-farm effects on milk quality were confirmed and completed under experimental conditions, whilst comparing the three dairy breeds. Observations from Va in their local environment and directly on farm (described in chapters II, III and V) will help discussing the results obtained along the comparison of the three breeds (described in chapters IV and VI). First, the issue of the comparison between the autochthonous Va and more specialised dairy breeds will be debated. Then, considerations about the behaviour of dairy cows on mountain pastures obtained in the frame of the several studies of the present thesis will be discussed. Besides, new elements on the short-term effects of transhumance on milk and cheese quality will be pointed out and discussed. Ultimately, the results of the present doctoral thesis will allow the discussion on a larger scale of mountain dairy systems and their perspectives.

A) Which dairy breed is more effective in a mountain system?

1. Global performance of the three dairy breeds across the experiments

The observation of Va in their local environment (described in chapters II and III) showed that on the medium term (scale of the summer grazing season), their performance was affected to some extent by summer transhumance, even though they are local cows adapted to their environment. During the different summer seasons (from 2014 to 2017), Va repeatedly experienced a decrease in MY, especially at the start of the grazing season (Figure VII.1.). Indeed, between the end of the winter period (week 16) and the start of the summer grazing season (week 20), the MY of Va in Aosta Valley (2014 to 2016) decreased by 10 % on average. This proportion is coherent with the observations of Zendri et al. (2016a), when changing from winter permanent farms to highland pastures (Figure VII.2.). Estimated from the latter figure, local breeds decreased their MY by 10% between May and June, and the Ho by 8%. This can be related to the advancing stage of lactation (linked to seasonal calving in mountain systems), together with the extra energy required to cope with mountain conditions and transport during transhumance (Zendri et al. 2016a). In our experiments, the average decrease in MY for Va after 7 weeks of mountain grazing was 23 %. Zendri et al. (2016a) also observed that local cows decreased their MY by about 33 % between June and September, whereas Ho experienced a bigger drop in MY (-56% between June and September). Besides, Farruggia et al. (2014) observed a huge drop in MY with Ho and Mo managed on the same biodiverse mountain pasture than that used in our experiment (-50% between June and the end of August). Therefore, in our 2017 experiments, it was expected for Ho and Mo to decrease their MY by a higher proportion than Va.



season	Loss in MY between week 16 and 20	Loss in MY between week 16 and 27
2014	-5%	-10 %
2015	-15%	-25 %
2016	-11%	-34 %
2017 (France)	-8%	-50 %

Figure VII.1. Evolution of the milk yield (MY) of Valdostana (Va) cows during the 2014, 2015, 2016 and 2017 summer grazing seasons (only multiparous cows).

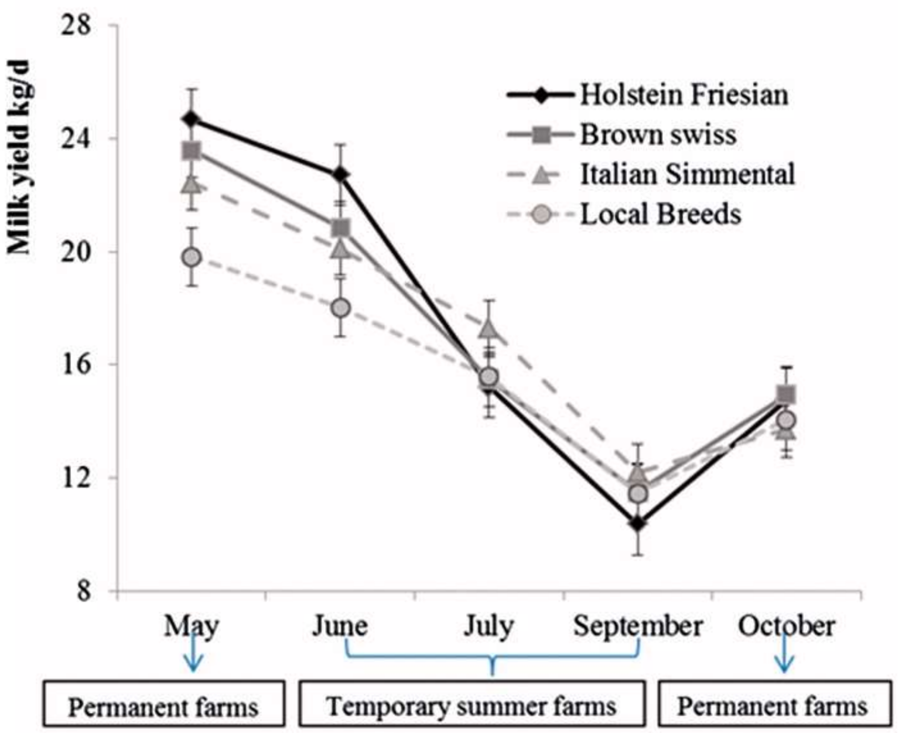


Figure VII.2. Evolution of the milk yield (MY) of cows of different breeds before, during, and after summer transhumance in Italy (Zendri et al. 2016a).

However, not so many distinct differences were observed in the short term between the three breeds during our experiments. Were the mountain conditions experienced by Ho, Mo and Va in 2017 similar to those of Va's native environment or not hard enough to observe differences between breeds? Did the transportation of Va to the new environment in France affect their performance before the experiments? To assess these two points, it is important to replace the performance in the summer season scale by also taking into account the arrival of Va in France.

Figure VII.3. Focus on the evolution of the milk yield (MY) of Valdostana (Va) cows during the pre-experimental period and the transport from Italy to France in 2017.

The decrease in MY observed in week 20 (*i.e.* right after starting the grazing season) in 2017 for Va was similar to that of previous grazing seasons in Aosta Valley, even though cows were transported to France during week 18 (Figure VII.3.). Actually, after their arrival, Va experienced a MY decrease for 3 days and then totally recovered their initial production, which shows that they were resilient enough to cope with the change in environment and long transportation without further consequences for the experiments aiming at comparing them with Ho and Mo. After 7 weeks of mountain grazing, their MY decrease was then 50 % (Figure VII.1.), which is higher than what could be observed by Zendri et al. (2016) with local Italian breeds. This confirms that the decrease in performance of Va (Figure VII.3.) is actually exclusively due to the harsh grazing conditions experienced in the Massif Central (France) and the absence of concentrate feed; which were conditions needed to test the hypothesis of enhanced different selective behaviour according to the MY potential of each breed.

When expressed in proportion of the initial production, the decrease in MY across the overall 2017 grazing season was similar for the three breeds. Indeed, between weeks 17 and 27, Mo lost 45 %, Ho decreased MY by 54 % and Va by 50 %. This refutes our hypothesis that Ho and Mo would decrease their MY by a higher proportion than Va. Actually, compared to the observations of previous seasons and the literature (Farruggia et al. 2014; Zendri et al. 2016), Va are the ones who decrease their MY even more in 2017. This questions their strategy to save energy and their choice of trade-off. Indeed, in harsh conditions and without concentrate feed, they do not seem to put priority on lactation. It is thus interesting to further investigate other criteria, such as milk quality, grazing behaviour, body condition and reproduction ability, in order to identify which main life function (between lactation, reproduction or ability to survive; Blanc et al. 2006) Va do prioritise and how it defines their robustness. It is noteworthy that the similar drop in MY proportion across the 2017 season actually resulted in a shrinkage of the gap between the effective production levels. Indeed, in week 17, Va were yielding on average 9.1 kg/day less than Mo and Ho (Figure VII.3.). In week 27, this difference was only 5.8 and 3.6 kg/day for Mo and Ho, respectively. It would mean that in optimal conditions and with concentrate supplementation, Ho and Mo are able to clearly differentiate from Va. Conversely, when confronted to harsh grazing conditions such as those they were exposed to in 2017, they struggle more and their production level is more comparable to that of Va. Roche et al. (2018) explain that genotypes that were selected within systems that have greater access to imported feed produce more when offered additional feed, or when compared with genotypes selected from systems that import less feed. This is coherent with the performance of the three dairy breeds across our experiments, and involve a genotype × environment interaction that will be further developed later in the discussion.

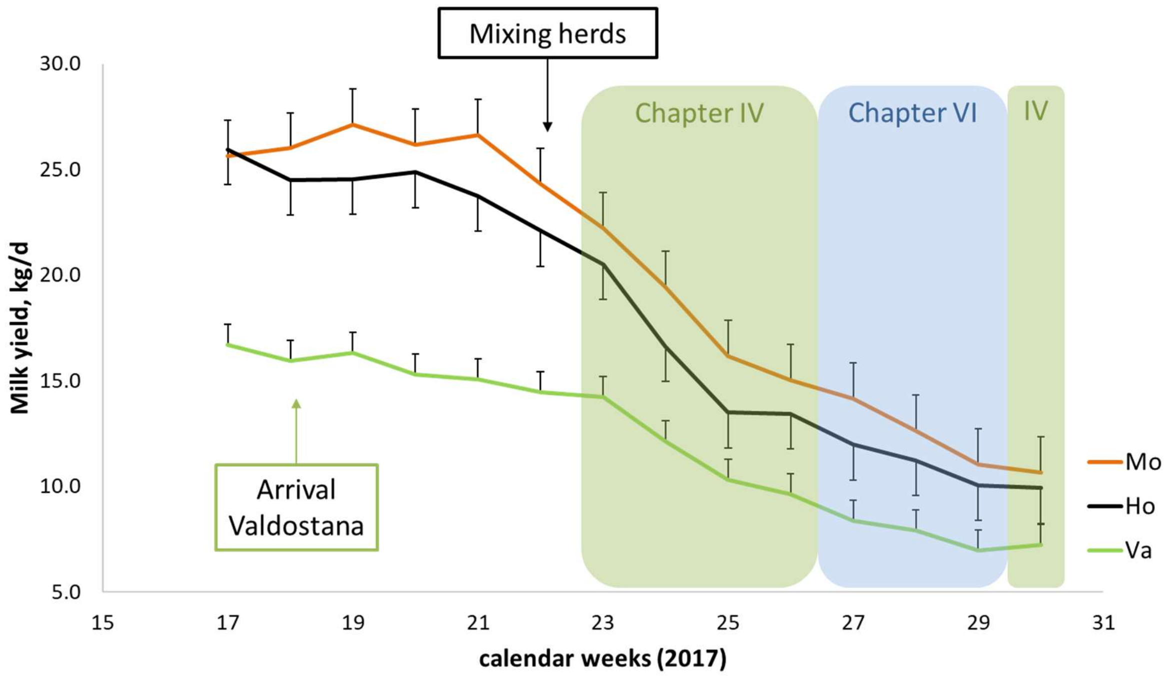


Figure VII.4. Evolution of the milk yield of the three dairy cow breeds during the 2017 grazing season. Mo: Montbéliarde, Ho: Holstein, Va: Valdostana Red Pied. Bars are standard deviations.

Finally, over the 2017 summer grazing season, Va were globally lighter and yielded less milk than Ho and Mo cows (Table VII.1.). However, their milk was richer in protein and casein than Ho's milk by 2.5 and 3.0 g/kg, respectively. The lower protein content of Ho's milk could be linked to their energetic status and highlight a negative energy balance during the grazing season (Coulon and Rémond, 1991), but is also genetically usual for this breed. Besides, a low protein and especially a low casein content might be a limitation to further cheese processing, as confirmed by the very low curd firmness and long rennet coagulation time observed in Ho's milk. The pH was also higher in Ho's milk, which impairs coagulation and may lead to difficulties in cheese processing. In conclusion, cows from the three breeds might have been affected by the harsh mountain grazing conditions of 2017 in a similar way concerning MY decrease, but milk's gross composition of Va and Mo was more interesting than that of Ho for further cheese processing.

Table VII.1. Global differences ($P < 0.05$) observed between the three dairy cow breeds (Ho: Holstein, Mo: Montbéliarde and Va: Valdostana) during summer 2017 in body weight, milk yield, milk composition, pH and coagulation properties.

	Ho	**Mo**	**Va**
Body weight (kg)	653	681	507
Milk yield (kg/day)	13.4	14.0	9.4
Milk composition			
Protein content (g/kg)	30.2	31.4	32.7
Casein content (g/kg)	25.1	26.7	28.1
Milk pH	6.75	6.69	6.66
Milk coagulation properties			
Rennet coagulation time (min)	22.7	16.5	16.9
Curd firmness (a_{30}, mm)	10.1	26.1	25.9

2. Consequences of breed-specific behaviour on milk quality

Our investigation of the diet selection showed that only a few differences occurred between breeds in the short term on mountain pastures. If any, they occurred between Ho and Va. Indeed, the latter had the smallest proportion of grasses in their bites, in favour of mature vegetation and forbs (chapter IV). It seems that Ho's strategy was to select vegetative grasses, which are more energetic, probably to sustain their lactation. On the other side, the indifference of Va to forbs might point out that they were not really selecting on the pasture but rather grazing *by stratum* (Morris, 2002), probably because they do not put priority on their milk production. The diet selection of Mo was intermediate. In the end, even though these little differences did not result in a clear better performance for one of the breeds, they might still affect the quality of milk. Indeed, more forbs in the diet means a higher intake in plant secondary compounds, thus an inhibition of the ruminal bio-hydrogenation (detailed in chapter I.II.). Consequently, there should be more poly-unsaturated fatty acids (PUFA), conjugated linoleic acids (CLA) and n-6 and n-3 FA in the milk fat from Va cows, compared to Ho. Preliminary results of the 2017 milk samples from the 3 breeds (Table VII.2.) suggest, in fact, a higher proportion of PUFA and n-3 FA in Va's milk fat compared to Ho's milk fat. It is thus necessary to deepen the investigation and comparison of the milk FA profiles of these three different breeds in different conditions, in order to confirm the systematically higher content in n-3 FA and PUFA of Va's milk. It is, however, still unclear to which extent the effect is due to the genotype, metabolism, behaviour or all combined, and need to be further investigated. Besides, the effects of the breed-specific behaviour on milk FA profile must be investigated on a longer term.

Table VII.2. Differences observed between the three breeds (Ho: Holstein, Mo: Montbéliarde and Va: Valdostana) in milk fatty acids (FA) profile. Results are presented as arithmetic means ± standard deviation. (Koczura et al. in preparation).

	Ho		Mo		Va	
Individual FA (g/100 g)						
C12:0	1.89	± 0.17	2.03	± 0.40	1.89	± 0.39
C14:0	8.36	± 0.58	8.79	± 1.36	8.45	± 1.45
C16:0	24.8	± 2.1	24.4	± 2.6	24.1	± 3.0
C18:0	12.9	± 1.2	11.3	± 1.7	12.5	± 1.9
C18:1 *trans* 11	2.13	± 0.31	2.02	± 0.83	2.54	± 0.58
C18:1 *cis* 9	24.8	± 1.8	26.1	± 2.6	24.5	± 7.0
C18:2 n-6	1.06	± 0.18	1.05	± 0.16	1.19	± 0.18
C18:3 n-3	0.76	± 0.11	0.81	± 0.11	0.91	± 0.12
Groups of FA (g/100 g)						
Total CLA	1.09	± 0.22	1.18	± 0.31	1.43	± 0.25
SFA	61.5	± 2.3	60.1	± 3.1	60.5	± 6.4
MUFA	33.3	± 2.0	34.6	± 2.8	33.6	± 6.4
PUFA	4.32	± 0.46	4.38	± 0.63	5.00	± 0.61
n-6 FA	1.37	± 0.22	1.29	± 0.16	1.45	± 0.21
n-3 FA	1.05	± 0.14	1.07	± 0.11	1.20	± 0.15
Ratio C14:1 *cis* 9 to C14:0	0.092	± 0.024	0.092	± 0.021	0.100	± 0.015

Table VII.3. Differences observed between the three breeds (Ho: Holstein, Mo: Montbéliarde and Va: Valdostana) in milk terpenes profile (logarithmic scale of arbitrary units: peak area). Results are presented as arithmetic means ± standard deviation. (Koczura et al. in preparation).

	Ho		Mo		Va	
Total terpenes	9.00	± 1.16	8.93	± 0.81	8.93	± 0.51
α-pinene	6.58	± 1.03	6.65	± 0.73	6.73	± 0.68
β-pinene	6.49	± 0.79	6.61	± 0.59	6.71	± 0.63
Limonene	6.78	± 2.70	7.25	± 2.54	6.71	± 2.28
α-caryophyllene	4.99	± 0.86	5.09	± 1.04	5.48	± 0.81
β-caryophyllene	6.77	± 1.26	6.86	± 1.29	7.23	± 0.71

The investigated mountain pastures in our experiment were rich in forbs, such as plants from the *Apiaceae* family and *Thymus serpyllum* in the upper zones. These plants are rich in pinenes and caryophyllenes especially (Rivas da Silva et al. 2012; Cutillas et al. 2018). Some of these molecules, when present in the diet of the cow, might be transferred to milk. As Va were found to feed on forbs more easily than Ho, it could be that the terpenes profile of their milk is richer in terms of terpenes quantity and/or diversity. Preliminary analyses on milk samples from 2017 suggest that the milk of cows from the three breeds contain the same total amount of terpenes, but with slight variations in their diversity (Table VII.3.). The pinenes and caryophyllenes amount seems to be higher in Va's milk than Ho's, with Mo being intermediate. The high standard deviations suggest a high individual variability, that could be due to the individual variations in grazing behaviour. Further investigations are strongly needed in order to qualify the diversity of terpenes found in milk of cows from the three breeds in contrasting conditions, and determine if the variations are rather due to the individual or the breed. Besides, Rivas da Silva et al. (2012) demonstrated that positive enantiomers of α- and β-pinene used in synergy (250 µg/mL) can have a bactericidal effect against methicillin-resistant *Staphylococcus aureus*. Even though concentrations of terpenes found in milk are low, it would be interesting to manufacture cheeses with the milk of the three breeds and investigate the link between terpenes profile, microbial development and potential further influence on cheese sensory properties.

3. Distinction between genotype and experience effects

Our experiments, together with the previous considerations on the three investigated breeds, suggest that the small differences in behaviour observed in our study are not resulting in short-term differences in performance. Nevertheless, they might be of high importance for further milk and cheese nutritional and sensorial quality. However, it is still unclear if these small differences are actually linked to the genotype of the breed or the experience of the animals with their environment. Even though Va cows adapted quite quickly to the new environment in France, their native management and breeding system was different than that of Ho and Mo. In Aosta Valley, cows were indeed grazing 24 hours a day, being allocated each day a new portion of the plot and milked directly on the pasture. Moreover, they were not selected based upon the same criteria as Ho and Mo and not complemented the same amount of concentrate. Ho and Mo were used to graze freely all day and night long and come back to the stable for milking. They were used to receive substantial concentrate complementation and selected on milk production. The previous grazing experience of the Va cows might have influenced them, by limiting their selection opportunities. They were used to graze *by stratum* (Morris, 2002). Ho and Mo, on the contrary, were given wide areas and could choose the patches they preferred.

Consequently, the question of the importance of genotype or experience effect is raised. It has been previously demonstrated and discussed that animals develop their selection of diverse diets when exposed to it in early life (chapter I.II.). However, in their native environment, Va with or without previous site-specific experience did not seem to behave or perform differently. The effect of experience might then come from a longer history. Indeed, the past of breeds and conditions for their selection and development could also have affected the grazing behaviour of the animals through the so-called genotype × environment interaction (Roche et al. 2018). Local breeds, that were reared and selected in their native environment, might have coevolved with the native vegetation and characteristics of their milieu, resulting in a strong combined effect of their genotype and experience on their grazing behaviour. Coevolution is defined as the “reciprocal genetic change in interacting species owing to natural selection imposed by each on the other” (Occhipinti, 2013). Many biologists consider that this process has generated the nowadays earth’s biological diversity. It is mostly used to qualify the fluctuation of allele frequency in plants, which allows them to develop defence strategies against herbivore to avoid extinction. It could also work on the other way, with animals adapting to the plants. It was once demonstrated by Laycock (1978) that some herbivores developed the ability to counteract adverse plant compounds because they coevolved with the native vegetation. These processes are extremely complex and need to be investigated on large temporal and geographical scales. It could however partly explain the contradictory results observed when comparing grazing behaviour of local cow breeds to more specialised dairy breeds.

In conclusion, our experiments allowed us to understand that the link between the dairy cow breed, grazing behaviour, performance and milk quality seems to be much more complex than expected. It is actually the coevolution interaction between the breed and its environment that needs to be further investigated and understood, for genetic selection or breed choice on dairy systems (also discussed by Roche et al. 2018). Further research must involve local rustic breeds and specialised dairy breeds which are raised together in the same system. Besides, as seasonal calving is performed in mountain dairy systems, our experiments always involved late-lactating cows. In early lactation, the requirements of the animals would be higher, which may lead to enhanced breed-specific differences and would be interesting to consider in the frame of sustainable lowland grazing systems. Moreover, there is a need to further investigate reproduction, longevity and broader criteria on the long term and system scale. Animals being born and raised on the zone for generations might actually be more important for the adequacy to the territory and further behaviour and performance. This aspect could also question the policies of PDO products and their specifications, which nowadays rely on local breeds to ensure the link of products to the *terroir*.

B) Grazing behaviour on mountain pastures

1. <u>Methods used to investigate grazing behaviour on mountain pastures</u>

During our studies at high altitude, the qualification of the "basic" grazing behaviour was possible thanks to sensors (described in details in chapter I.II.). The opportunity to use sensors on dairy cattle in the mountains is already a great improvement, as visual observations need a lot of time and workers and are limited in time. However, sensors are limited, as they provide information only on the time budget allocated and only few information about the bites. In order to go deeper in the characterisation and understanding of the grazing behaviour of dairy cattle in mountains, it is necessary to develop new methods and improve the current possibilities. Moreover, qualification of the grazing behaviour can give us more details about the observed diet selection, but it must be completed by information about the actual ingestion.

In order to quantify the daily intake, direct measurements based on the herbage mass before and after grazing are often used (Decruyenaere et al. 2009). However, this method gives reliable results only if the grazing period is short and the stocking rate high, which is usually not the case in all mountainous systems. Indirect measurements must be performed, through markers techniques for instance. Therefore, during the 2015 season, an experiment was led in order to test the efficiency of the n-alkane method (Mayes et al. 1986) to estimate the intake on mountain pastures. Unfortunately, this method is particularly challenging to implement in an mountain environment and did not allow us to correctly estimate the intake in the case of our experiment. The daily manipulation of the animals needed by this method is a problem while grazing on mountain pastures. Moreover, the high biodiversity of the mountain pastures must be a major source of error, as plant organs and species present very different alkanes profiles (Cortes et al. 2005). Slow release alkane capsules were successfully used in former intake studies with dairy cows under mountain grazing conditions (Leiber et al. 2004 and 2006), but it is difficult to take representative samples of what was actually eaten by the cows, as it must be also sampled with substantial visual observations.

Intake depends on three main parameters, which are grazing time, biting rate and bite mass (Decruyenaere et al. 2009). Therefore, new methods should be developed on field in order to be able to estimate it through the data already collected by the sensors. In addition, the analysis of faecal samples by NIRS (as performed in chapter IV), which has been proved accurate also on biodiverse pastures (Decruyenaere et al. 2009; Farruggia et al. 2014; Mesquita et al. 2016), may help understand and estimate better the digestibility on mountain pastures and might be useful for further experiments with intake estimations.

2. Time allocated to ingestion and rumination in mountain grazing

If the feeding behaviour of grazing dairy cows is well documented, it was not so often investigated on mountain pastures. The effects of high-altitude grazing were already investigated on the animal's metabolism and performance (chapter I.II.). However, to our knowledge, only few deeper analyses of the diet selection of dairy cows in high altitudes were performed before our studies (Hessle et al. 2014; Romanzin et al. 2018). In these mountainous conditions, cows have to walk more to look for their feed, are confronted to steep slopes and facing cold nights as well as warm hours in the middle of the day. Besides, because of the seasonal calving, they are already in an advanced stage of lactation and therefore need a lower feed intake than in early lactation. The grazing behaviour may thus slightly differ from what is known in the literature.

In our experiments, measurements of ingestion and rumination times were performed on individual cows during the 2014, 2015, 2016 and 2017 summer seasons. Between 2014 and 2016, Va cows were monitored using MSR sensors (described in chapter I.II.), whereas in 2017 all the cows were wearing Medria collars, equipped with an accelerometer. With these sensors, the feeding behaviour was registered 24 h/day, and the proportion of time spent ruminating or ingesting per day was calculated. These results are compared across years in Table VII.4.

Table VII.4. Proportion of time spent ruminating or ingesting by Valdostana (Va) cows during the different summer grazing seasons (arithmetic means).

Method	**MSR**			**Medria**
Year	**2014**	**2015**	**2016**	**2017**
Rumination (%)	22	27	23	39
Ingestion (%)	26	28	19	27

The average time allocated to ingestion at pasture by Va over the seasons seems to be lower than previous measurements with other breeds (Delagarde et al. 2001; Prendiville et al. 2010; Chilibroste et al. 2015; Romanzin et al. 2018). Indeed, according to Delagarde et al. (2001), an adult grazing dairy cow spends on average 450 to 650 min per day ingesting, and

350 to 550 min ruminating, which represents 31 to 45 % of the day for ingestion and 24 to 38 % of the day for rumination. These averages are most of the time calculated for specialised dairy breeds, which are characterised by a higher milk production and body weight than dual-purpose breeds and thus need a higher feed intake. The lower MY and BW of Va, and thus lower energy requirements, could partly explain the lower ingestion time compared to those observed in the literature (Table VII.3.). During the 2017 summer grazing season, Va spent 27 % of their day ingesting (*i.e.* 385 min/day), whereas the Mo and Ho were spending 31 % of their time ingesting (*i.e.* 453 and 454 min/day, respectively). Compared to the literature, this proportion is one of the lowest observed for this type of cows. Therefore, we could also hypothesise that the low ingestion time might be linked to the mountainous conditions previously enumerated. In 2017, Va's ingestion time was similar to that recorded during the previous mountain seasons. Their rumination time, however, seems increased compared to the previous seasons. This could confirm the harsh conditions encountered during summer 2017 after the heat wave in the Massif Central. Cows ingested more dry and mature vegetation, especially in late season (as described in the results of chapter IV), which is a fibrous diet. Rumination time is positively correlated to the intake of neutral detergent fibre (Santana et al. 2013). It would be interesting go deeper in this characterisation of the grazing behaviour of dairy cows on mountain pastures, and be able to assess the effect of high altitude on different behavioural parameters.

3. Looking for the best trade-off between grazing and physical effort

The specificity of mountain pastures is not only their biodiversity or vegetation structure, but also their topography, with sometimes very steep slopes. On regular pastures, cows usually prefer short and tall vegetative grasses, and therefore re-graze the same preferred spots. This "patch grazing" behaviour (Adler et al. 2001) was reported by several authors (Dumont et al. 2007a; Coppa et al. 2011a; Farruggia et al. 2014). Consequently, in our experiment involving steep slopes, we expected cows (especially specialised dairy breeds) to first graze the lower and flat zones, in order to avoid the extra physical effort needed to climb up. We observed that, regardless of breed, cows indeed re-grazed the lower and flat zones first. Only when the abundance of grasses and nutritional value of the herbage was decreasing, cows started to explore the further parts of the plots, including climbing the slopes. However, a different behaviour was observed between a low slope and a steep slope. Indeed, at a similar nutritional value between the lower and upper part of the slope, cows on a low slope went up, whereas cows on a steep slope stayed at the lower end (chapter IV). Cows had to make a decision for a trade-off between their access to the most palatable feed and the physical strain, which they apparently chose to avoid. In order to assess the resilience of and the adequacy of cows or breeds to a certain environment, the trade-off issue is of high importance. At their own scale, cows have to choose where to put priority between lactation, reproduction or ability to survive, and then adapt their behaviour and physiology (Blanc et al. 2006). Typically, this trade-off notion can be illustrated by cows in negative energy balance at the beginning of lactation. They are mobilising body fat reserves either to give priority to lactation at the expense of reproduction, or, conversely, give priority to reproduction at the expense of the MY (Ollion et al. 2016). In our case, in late lactation and on mountain pastures, cows might have avoided extra physical effort (*i.e.* slope climbing) in order to maintain their body condition and therefore reproduction. In conclusion, the grazing behaviour is determined by the choices of the animal in the trade-off between milk, reproduction or coping with the environmental conditions. According to Friggens et al. (2017), repeated measurements over time have a high potential for quantification of the animal's ability to cope with environmental challenges. Therefore, on mountain pastures, a deeper investigation on the long term, which takes into account the main life functions, is necessary.

C) Milk and cheese quality during transhumance

1. Differences between individual milk and bulk milk

In our studies, the composition of individual milk was systematically analysed. Individual milk can give information about the animal's status, and increases the number of repetitions for measurements of milk composition. However, when it comes to cheese production, the bulk milk composition comes into play. As described in chapter V, not all effects observed on individual milk might always be recovered in bulk milk, or not as strong as what is observed individually. Individual variations in coagulation are due to several parameters, from milk composition to genetics (detailed in chapter I, paragraph B.1.3.). When mixed together, the average composition of the bulk milk can strongly differ from some individual samples, and especially the proportion of genetic variants of proteins (such as κ-caseins) might be diluted.

As bulk milk is the sum of all individual milk, this can be due to a dilution effect, and effects observed on individual milk might not act additively. On one hand, as described in chapter II, V and VI, the proportion of individual milk samples that did not coagulate was of 13.2, 18.6 and 20 %, respectively. On the other hand, bulk milk samples from the different farms did all coagulate (chapter V). Observations described in chapter V mean that the individual milk of 80 % of the cows coagulated with an average RCT of 19.5 min, and 20 % of the samples coagulated with an RCT > 30 min. If we assume that these 20 % would have coagulated in 31 min, the average RCT of individual milk would be 21.8 min. However, the average RCT of bulk milk (composed of the mixed milk of all same individual cows) was 19.7 min. It shows that the 20 % of non-coagulating milk did not impair the coagulation time of bulk milk, and that measurements of coagulation properties in the highlands should always include both individual and bulk milk. Moreover, from the udder of the cow to the final vat, storage and transport of milk in mountain pastures can lead to contamination with microorganisms. It is therefore important to complement data on the effects of transhumance and transport of cows with bulk milk analyses. Besides, our first studies are not representative enough for the high diversity of farms in the mountains (around 200 alps in Aosta Valley) and composition of herds, which must be investigated more in depth.

2. Milk and cheese quality after moving the cows

Results presented in chapters V and VI confirmed *in situ* the effect of the walk on the quality of milk observed in extreme experimental conditions (D'Hour et al. 1994; Coulon and Pradel, 1997; Coulon et al. 1998; detailed in chapter I.II.). Individual and bulk milk from the day right after walking were richer in fat and somatic cells, which was linked to a decreased MY and to body fat mobilisation. Magrin et al. (2015) suggested that high yielding cows should be excluded from the extreme and intensive walked transhumance at the end of the summer season, since their high MY and energy expenditure for locomotion could not be counterbalanced. They are thus implying that such cows should be transported by truck back to the lowland farms. Our study brings new elements to assess this issue: truck transhumance appeared indeed to not decrease the MY of late lactating cows. However, in our experiment, the production levels were already lower than those observed by Magrin et al. (2015). We complemented the qualification of truck transhumance by showing that it affected milk quality in the same way than walked transhumance (results described in chapter VI). Besides, we demonstrated that coagulation properties are not directly impaired by the walking transhumance or truck transportation. As described in chapter V, all short-term transhumance effects on milk observed on-farm seemed to disappear after 5 days. However, the study under strict experimental conditions described in chapter VI stopped 2 days after transport. The resilience of the milk from the different breeds must be further investigated, with repetitions earlier in the season and higher MY. Moreover, as the results from the on-farm study (described in chapter V) showed, amongst all combined effects of transhumance, the changes in milk composition seem to barely affect the cheeses. Therefore, it would be interesting to use milk from cows transported in controlled conditions in order to test these effects on cheese chemical and sensory characteristics.

3. Milk and cheese quality in changing environments during transhumance

During the sensory analyses of Fontina cheeses, the panel has detected several defects in taste. It was, however, not possible to link them with the day of transhumance. Abnormal tastes such as “propionic”, “butyric”, “pungent” or “stable” were the most frequent. In our study, such tastes could not be linked to a higher proteolysis, and may rather be related to the development of microorganisms. As Fontina is produced directly on farm, each alp is equipped with specific buildings for cheese processing. Therefore, the issue of the change in buildings for cheese manufacturing between the lowlands and highlands, and then the successive alps, can be raised. When arriving on the alp, most of the time cheese-factory buildings there were not used since the previous year. The temperature and microbial environment in the first days of use of these successive buildings might strongly affect the cheese manufacture and later ripening. Indeed, our on-farm study (chapter V) underlined an increased pH in the 24–h-old Fontina in the day right after transhumance. A low temperature in the building during processing could have slowed down the acidification of the cheese in the first 24 h and impair its drainage, causing a high lactose content in the fresh cheese (Martin, oral communication). This could induce consequences on the final ripened cheese. Indeed, chemical reactions due to the microflora of the milk, the added starter cultures and possibly contaminating bacteria take place. Lactose is transformed into lactic acid by *Lactobacilli*, leading to the acidification of the cheese. A higher content of lactose in the fresh cheese would thus cause an increased acidification during ripening, leading to more acid final cheeses. This hypothesis was partly confirmed by the sensory panel (chapter V), that found the cheeses from the day after transhumance more “sour”. This mechanism and the possible influence of the temperature and conditions in the cheese manufacturing buildings the day after transhumance should be further investigated, in order to help farmers producing high quality cheese by adapting their process in the days around transhumance.

Contaminating bacteria such as *Enterobacteria*, *Enterococci* and *Staphylococci* may be enhancing proteolysis and lipolysis in cheeses, leading to unfavourable tastes. On thawed bulk milk and on a limited number of cheese samples from our on-farm study, the amount of colony forming units (CFU) of the previous types of bacteria were counted (data not shown). As already recorded by Dolci et al. (2014) and Giannino et al. (2009), the *Enterococci*, *Enterobacteria* and *Staphylococci* counts resulted to be highly farm dependent. No difference was observed within the transition itself. Still, farmers and cheesemakers in Aosta Valley report taste defects and abnormal swelling of the cheese paste in the days after the transhumance. It would be necessary to investigate more in depth the microbial contamination and evolution in Fontina from the different days of transhumance.

In conclusion, our studies showed that it was difficult to directly link changes in milk composition to cheese defects. The modifications in milk composition caused by the walk or truck are indeed happening already with short durations of transport, but they do not seem to affect milk coagulation properties, nor damage cheese manufacturing. It seems that transhumance is enhancing during a few milkings the already existing morning-evening difference in cheeses, rather than directly impairing their chemical and physical properties. It might be due to the environment during manufacturing, which means that improvements and adaptations are possible (controlled temperature, use of a different dosage of starter cultures, etc). To confirm this effect, it would be interesting to widen the study, already in the sampled zone, but also to other systems. Indeed, a very high diversity of mountain dairy systems and cheese processes can be encountered in the mountains. Our results suggest that bacteria species should be investigated, and that the microbial environment during transhumance might be of higher interest than milk composition itself. It would be interesting to develop on farm investigations followed by controlled production of cheeses in relation to the changing microbial environments and their possible origins: cow teats, litter, milking machines, water, grass or cheese vats.

4. Importance of the global morning-evening difference for Fontina cheese

Our experiments (described in chapters II and V) assessed for the first time the issue of morning or evening cheese. Some cheeses in the mountains are produced on farm after each milking, as it is the case for Fontina (detailed in chapter I.II.). The composition of morning and evening milk has repeatedly been studied under various conditions (Mc Dermott et al. 2017; Bergamaschi and Bittante, 2018). Usually, morning milk has lower contents of fat and SCC than evening milk, which is due to the longer milking interval during the night. However, the effects on coagulation properties and cheese are not well known yet. In our studies, the different composition between morning and evening milk did not result in differences in milk coagulation. However, a few differences in Fontina cheeses were observed (Table VI.5.).

Table VII.5. Differences ($P < 0.05$) found between cheeses produced from evening and morning milk.

	Evening	Morning
Fat in dry matter (%)	53	50
Brightness	76.4	74.1
Force at failure (N)	213	270
Hardness (note from 1=low to 7=high)	2.4	3.0
Melting (note from 1=low to 7=high)	4.7	4.2

Globally, cheeses from the evening were fatter, brighter and easier melting, which was directly linked to the milk composition. Conversely, cheeses from the morning were harder. These differences are not big enough to question the certification of these cheeses. Indeed, cheeses with notes of hardness and melting ranging from 3 to 5 are accepted as Fontina PDO (Roberto Ronc, head of the Fontina sensory panel, oral communication). Our results gives first scientific insights on the qualification of Fontina cheese according to the time of manufacturing, which confirms some empiric observations of the farmers. This qualification has to be further investigated on a larger number of cheeses and farms. Besides, differences between morning and evening may also occur for other types of cheeses and different processes should also be taken into account in further studies.

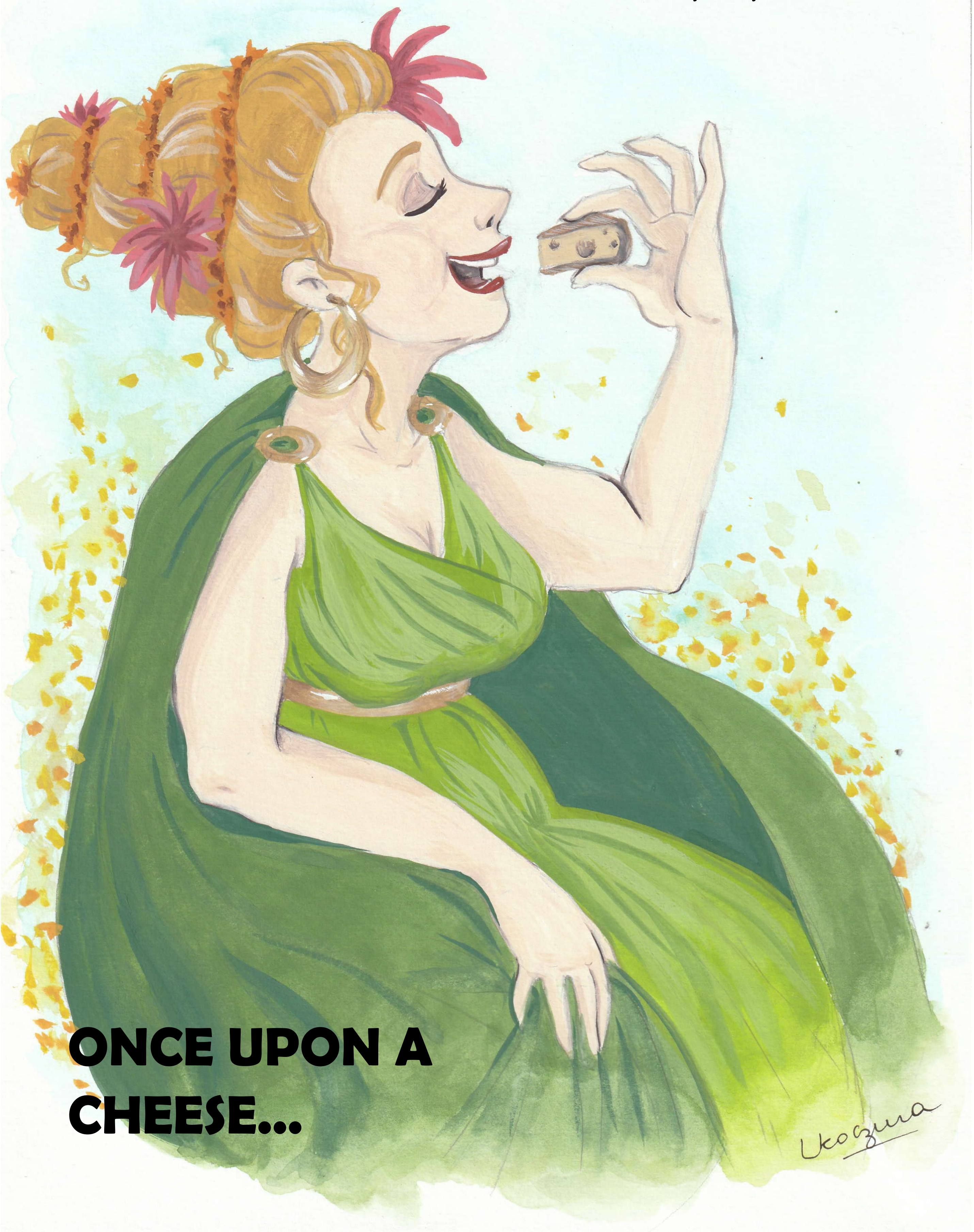
D) Mountain dairy systems modelling the future low-input dairy systems

Since decades, dairy cow breeds were mostly selected according to their production potential, and their ability to put priority on milk production (Roche et al. 2018). To sustain such production levels and therefore high energy requirements, feeding of dairy cows had to be adapted. More cereals and proteins were needed, and farmers slowly stopped their cows from grazing in order to stay inside and eat silage and concentrate (Delaby et al. 2014). Even though it helped increase food self-sufficiency, the question arises if this kind of systems can truly be considered economically and environmentally sustainable. Indeed, the dairy sector in Europe is currently undergoing an economic crisis, farmers and breeders are trying to produce more each year but product prices are decreasing. Feeds are often produced on another continent, production costs keep increasing, and competition for milk production is high. Moreover, as productive cows are usually bred inside and with a specific high energetic diet, pastures are no longer used or, if used, intensified. However, maintaining and grazing pastures is a key element to taking care of landscapes and biodiversity (Farruggia et al. 2008). They also can trap carbon emissions, contributing to the decrease of greenhouse effect (Salvador et al. 2017). The sustainable dairy system from the future must be low-input and more autonomous, and rely on the grass resources that do not compete with human food (Delaby et al. 2014). In addition, and to face changing climatic constraints, the future dairy cow must be resilient to external disturbance and show robustness (Friggens et al. 2017). As described by Roche et al. (2018), it means that cows should be able to achieve their nutritional requirements almost entirely from grazed pastures, with limited inputs of concentrates and feed supplements. They estimated that the future resilient dairy cow must produce reasonable quantities of milk (from 5500 to 7500 kg/lactation), walk long distances, become pregnant within 80 days of calving and be able to maintain adequate body condition. The same authors pointed out that in the nowadays context of sustainable intensification, grazing cows must also produce less CH_4 and excrete lower amount of nitrogen in urine.

As they were not selected and bred in grazing systems, our nowadays high genetic merit cows might not be able to valorise grass in their diet in such an efficient way. The ability of current dairy cows to maintain reasonable levels of production and high quality milk by exclusively grazing biodiverse pastures is thus questioned. Mountainous farming systems are a good example of such grazing low-input systems. The use of autochthonous local dairy cattle is preferred, most of the time for production of specific PDO products. Their adaptation to the territory and native breeding system was already highlighted (Couix et al, 2016). However, the present doctoral thesis did not demonstrate that autochthonous and lower yielding dairy breeds are better adapted to low-input systems in general. Actually, as underlined in the previous chapters, genetic background is not the exclusive explanation for cows' behaviour and performance. In fact, the interaction between genetics, environment and experience is the key factor for the future sustainable and resilient dairy cow (Dumont et al. 2014; Roche et al. 2018). This interaction has most probably been developed and valorised in an interesting way in transhumant mountain systems, with the local adapted breeds. Indeed, the greatest genotype × environment interaction leading to nowadays cows is actually due to different breeding objectives in different production systems, and, as a result, the suitability of different cows for different systems (Roche et al. 2018). Mountain dairy farming and transhumance might model the future agro-ecological dairy system. In the latter, the priority should not be put on milk quantity, but rather on the quality of dairy products, their certification (Dumont et al, 2014) and the ecosystem services provided (Dumont et al. 2013). Each country now has to develop an appropriate breeding objective to make sure that the cow of the future support a system robust to climatic and financial constraints (Roche et al. 2018). In conclusion, the objective of the adequacy of the animal with its native environment would lead to the optimisation of the transformation of local forage resources into high quality dairy products, a higher autonomy and therefore better social, economic and environmental sustainability.

CHAPTER VIII

Mountain dairy farming as a model for tomorrow's sustainable dairy system



ONCE UPON A CHEESE...

Once upon a time, in a kingdom far, far away, lived a young knight called Elinor. She had spent her entire life in a little village near the castle, and was raised to protect her beloved land and its Queen. However, over the past decade, she had seen the sky of the kingdom getting darker and the land less fertile. As the farms got bigger, the flowers and honeybees slowly disappeared, giving way to hectares of corn and soy...

Soon, not a single animal was to be seen grazing the fields. The Queen's favourite food, a rare cheese called Her Majesty's, originally made from raw cow's milk, was ultimately produced by the ton in enormous factories. It lost its particular and delicious taste. The Queen, who used to have a slice of it every evening, slowly became sick and depressed. The entire kingdom was eventually plunged into

DARKNESS

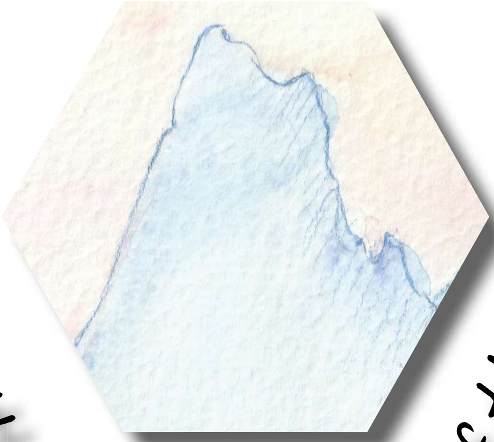
One gloomy afternoon, as she was passing a courtyard, Elinor overheard woeful former farmers talking about the Queen's critical state. Troubled, she stopped and listened.

"If this goes on, our whole kingdom will crumble to dust, as will the Queen... Only the old Mabeobsa could help us, but who knows where he is now?"

"The old Mabeobsa?" thought Elinor,

"I will find him and bring back harmony and serenity in our kingdom."

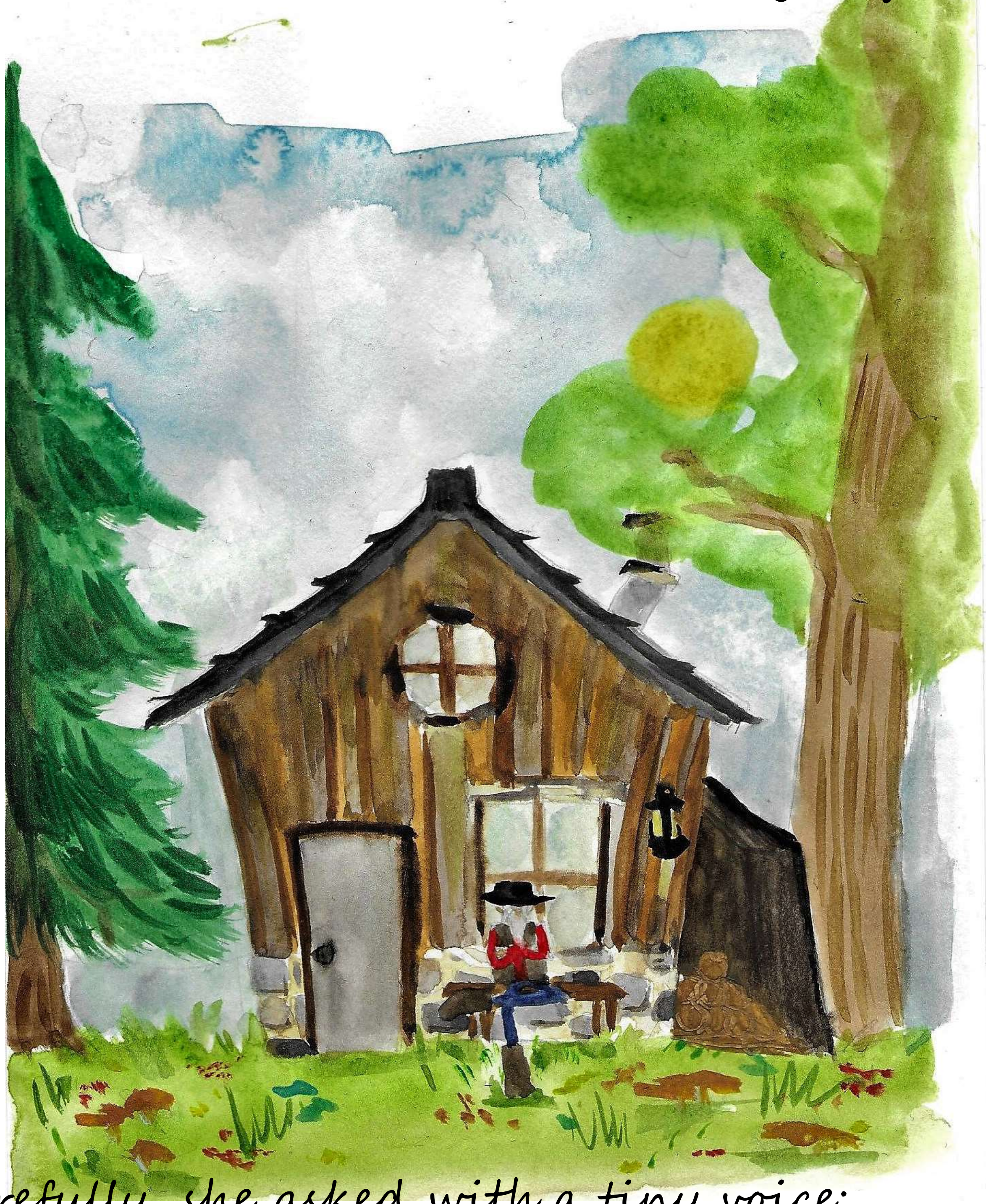
The following day, she took off on her steed,
aiming for



the high icy peaks of the NORTHERN MOUNTAINS

where the old Mabeobsa was last seen.

After weeks of hard and constant exploration, exhausted and hopeless, she finally arrived to a hut. The little house was warm and welcoming, and made her instantly feel relaxed and relieved. An old man was sitting in front.

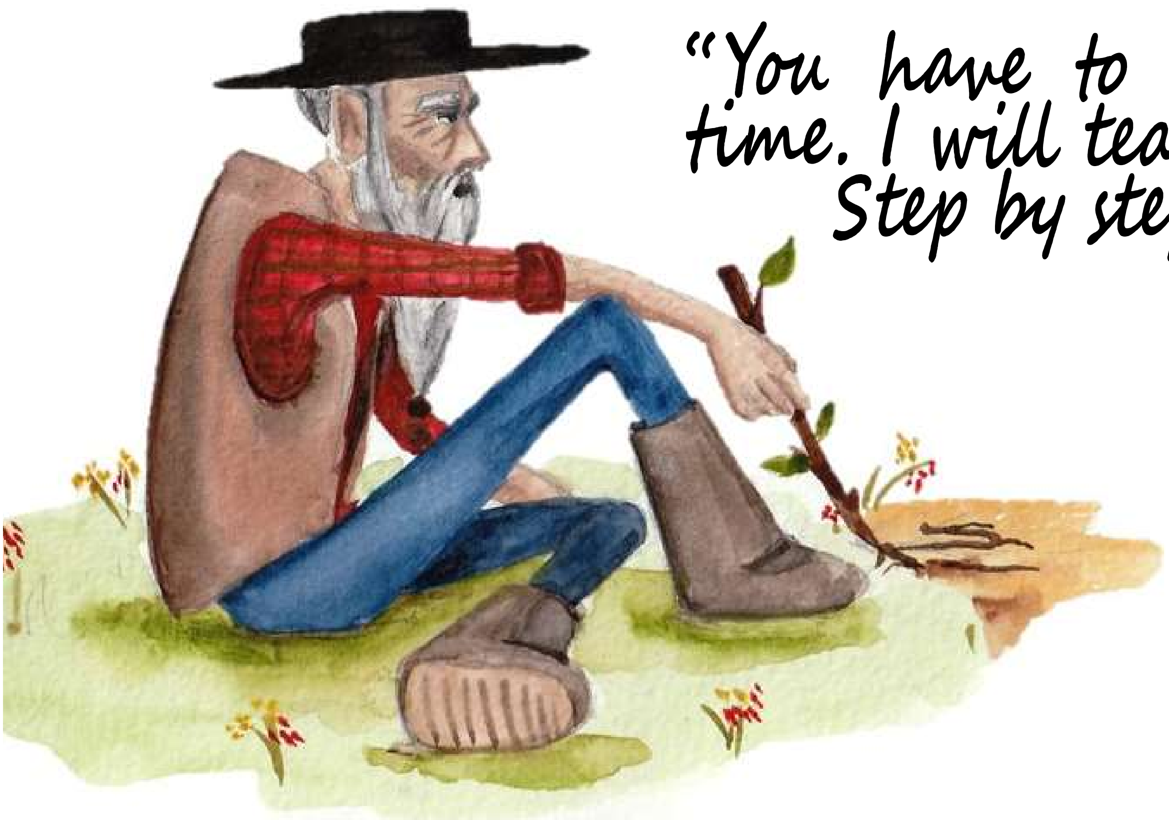


Carefully, she asked with a tiny voice:

"ARE YOU THE OLD MABEOBSA?"

"Young lady, it's been a long time since anyone has called me by that name... something must be wrong... is it the Queen?"

Elinor started to describe the poverty and sadness into which her kingdom had fallen. The old man listened carefully to her story, sometimes nodding and sometimes frowning. When she had finished, he remained silent for a long time. Elinor started to fear he could not save the kingdom. But suddenly, the old Mabeobsa looked up at her and said:

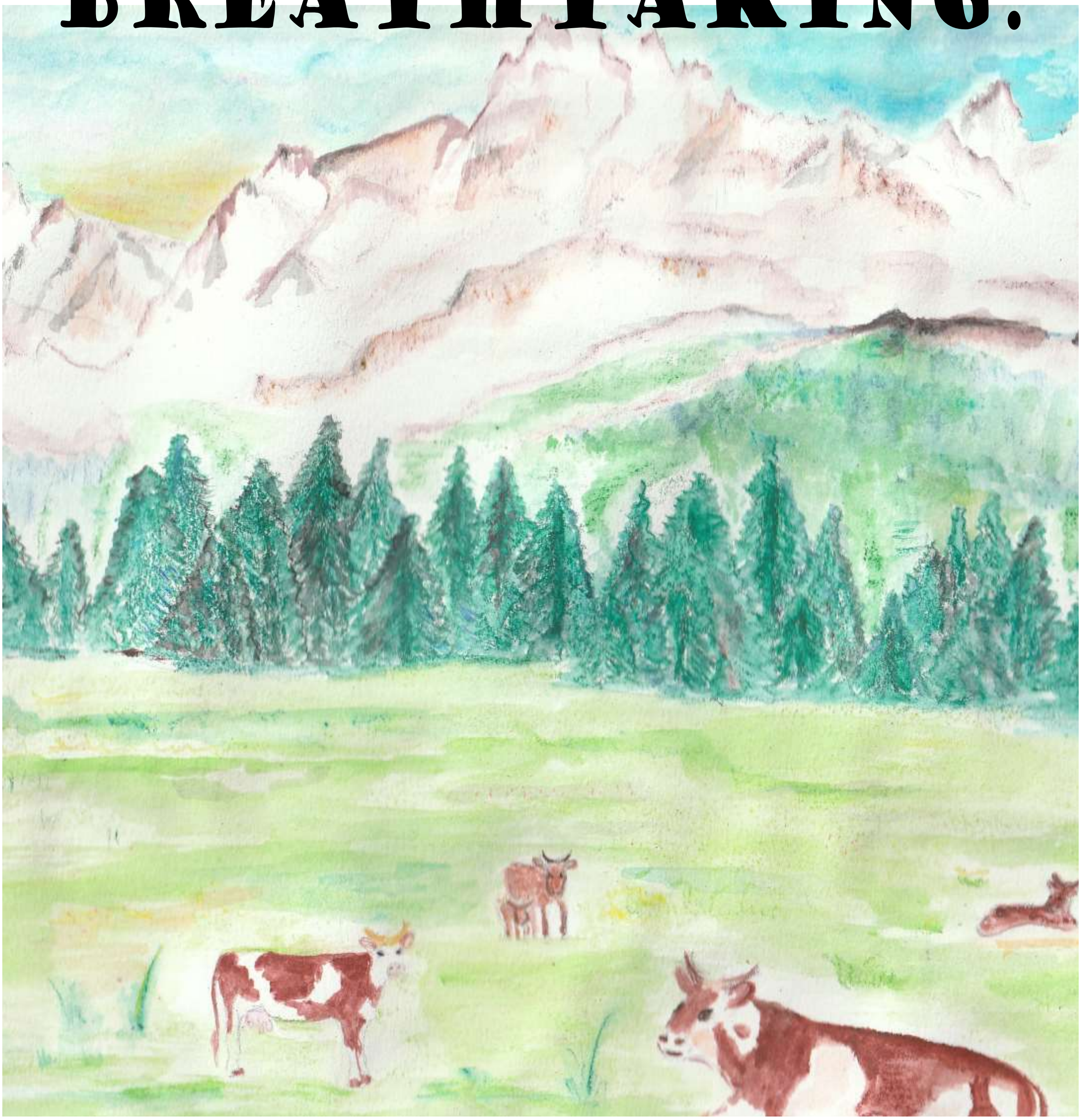


"You have to give it time. I will teach you. Step by step."

At first Elinor was irritated; she had hoped for an immediate answer. However, she agreed to stay and learn from the old Mabeobsa.

After a deserved restful night, she woke up ear-
ly to follow the old man in his everyday work.
What she discovered outside at dawn was

BREATHTAKING.



Mountains were still and quiet in the crisp morning air. Below the icy peaks, a herd of healthy and beautiful cows was peacefully grazing on a field of colourful flowers.

They milked the cows, and afterwards came back to the house to make cheese. Elinor carefully observed the old Mabeobsa's every move, who was absorbed in his work.

While the curd was slowly heating up in the cauldron, he invited her to take a lunch break and made her taste the cheese from the previous months.

As she was eating, it struck her: this cheese was perfect. The texture, the colour, the taste! Everything. Amazed, she asked the old man:

"OLD MABEOBSA...

WHAT'S YOUR SECRET?"

"My dear, there is no secret.
Please remember that

THERE IS MORE TO MAKING CHEESE THAN JUST MILK...

The whole system from the very beginning is crucial.

The **grass**,
the **environment**,
the **animals**,
how they are
taken care of...

... everything plays a huge role."

Elinor was challenged by these words. From this day on, she longed to understand. She followed the old man everywhere. After her observations, she started to make hypotheses...

Maybe the **diversity of the grassland** had an **impact on the animals**?

Maybe they did not **behave in the same way** depending on what we **offered them for feed**?

And what we were feeding them must have played a key role on the composition of their milk, she was already persuaded.

Perhaps the **taste of the cheese** was also linked to the **cows' health condition**, through **milk quality**?

She had so many questions and so few answers. When she asked the old Mabeobsa, he would always answer that she needed to give it time and to observe.

So she started collecting grass, milk and cheese samples. Eager for knowledge, she followed the animals for the entire summer grazing season on her loyal steed, observing what they would eat.

Every fifth bite, she would write down what the cow was choosing for her diet and try to link it to the taste and composition of the milk.

She measured the heart rates and stress levels of the cows, followed them during their seasonal migration.

The animals quickly got used to seeing her every day in the middle of the herd, and also observed her in a placid way.

Soon, winter came and she still had not found the answers to all of her questions. She nervously went to talk with the old Mabeobsa.

"The more answers I find, the more questions I raise... This is

NEVER ENDING

I will never make it in time to save the Queen!"

"Dear child", answered the amused old man. "**Who are we to seek absolute knowledge?** How can we claim to give an exact answer to all the questions we raise?"

Seeing Elinor's defeated expression, he added:

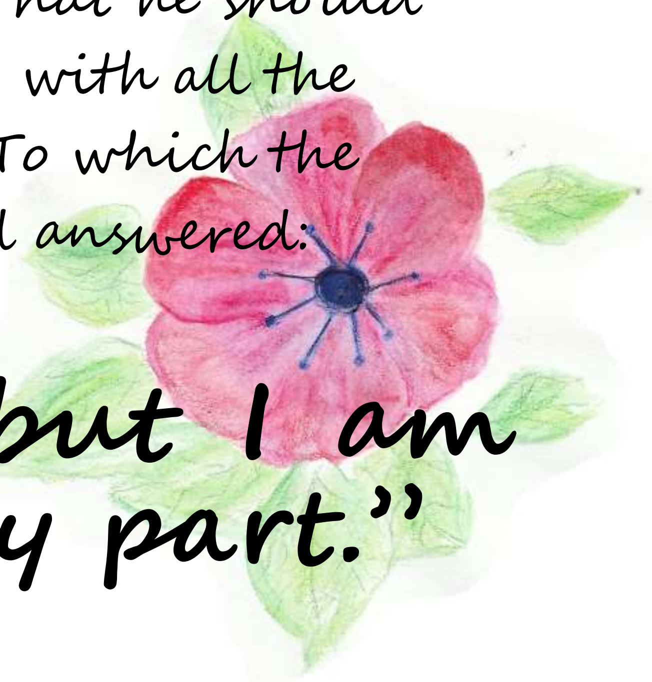
"I think you are now ready to go home and save the Queen. **You will never have all the answers** and must move forward with what you have found and understood so far."

"But... I am not sure this is enough to save the Queen!" complained the young girl again.

"I WILL TELL YOU A STORY" said Mabeobsa quietly.

Once, there was a huge forest fire. All animals were running away in fear, crying and screaming. However, in the middle of this mess, was a tiny hummingbird. He was flying endlessly from the river to the fire, each time throwing a few drops of water on the flames.

After a while, an old armadillo came to him, saying he was a fool and he would never manage to put out the fire. That he should rather fly away with all the other animals. To which the hummingbird answered:



"I know, but I am doing my part."

Elinor took a few minutes to meditate on the old man's words. He was right. She obviously could not save the whole kingdom on her own, even if she spent her whole life up there in the mountains, figuring out some of the answers.

"I understand", she said smiling.
"I will head back to the city tomorrow at dawn. Thank you so much for everything."

As planned, she packed all her samples, analyses and notes and left the next morning.

When she arrived to her hometown, her heart sank. The contrast with the mountains was heartbreaking: everything was **grey, depressing and monotonous.**
She immediately asked to meet the Queen, whose health was still in a **critical state**.

"My Queen", Elinor said kneeling in front of her. "I went to the old Mabeobsa. I can help you and the kingdom get better. The whole system is to be rethought, and we must give it time. But I think this can work."

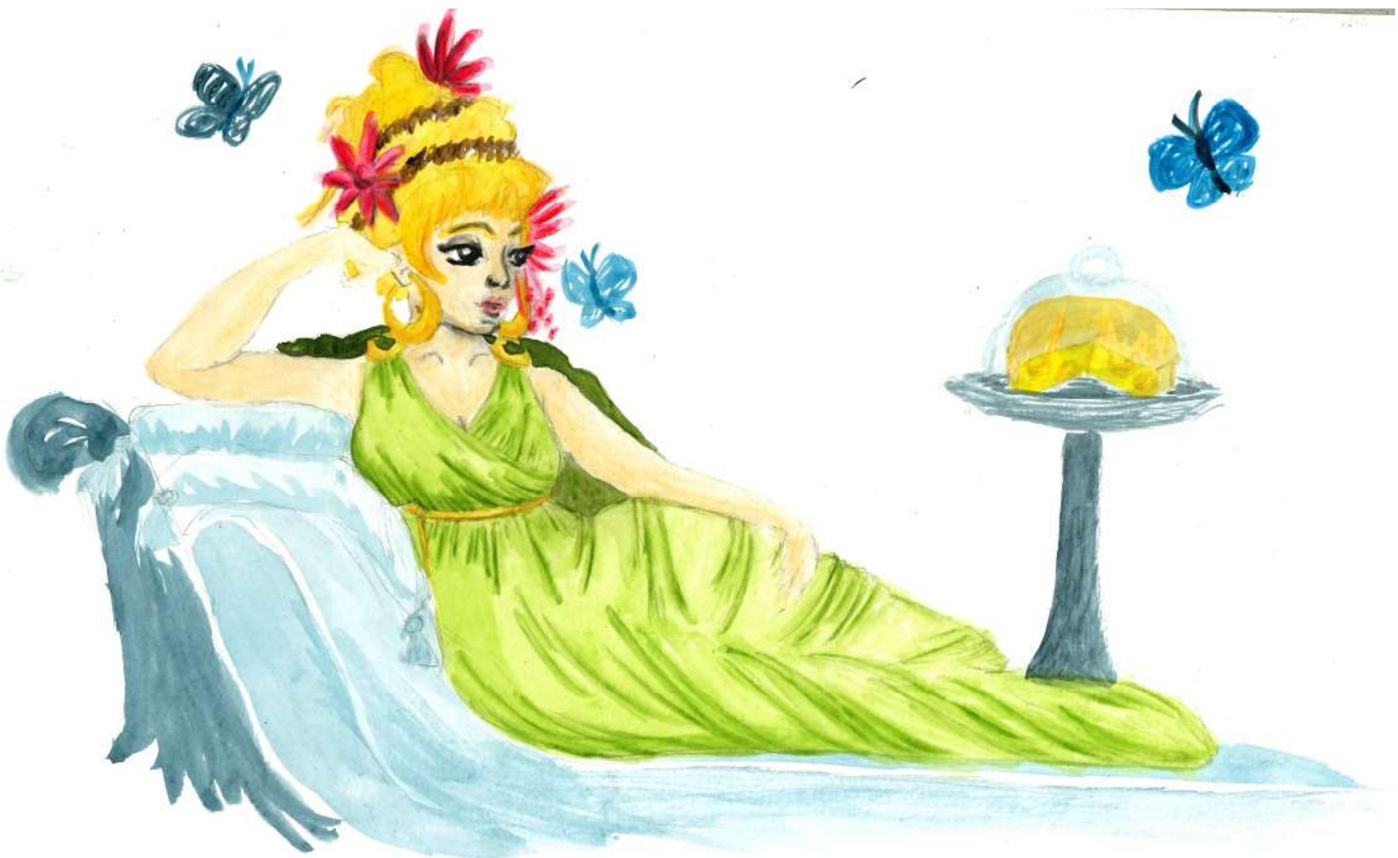
The Queen summoned her council and everyone listened attentively as Elinor presented her experiences and findings.

She told them the sustainability of the whole agricultural system should be developed.

She explained what she already understood in the field, raised new questions and suggested a few applied solutions to start changing the situation.

The word spread quickly, and soon the whole kingdom was reorganising its system.

Day after day, month after month, fields turned green again and Her Majesty's cheese began to be locally produced in the farms again. The Queen's health got better and people started to be hopeful and happy again.



Of course, not **all** solutions were found in such little time, and there were **no immediate and miraculous answers.** However, people were curious and eager for knowledge and improvement. Elinor opened a farm dedicated to research and training. From then on , they all lived

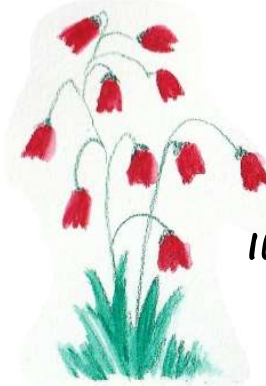
HAPPILY EVER AFTER

constantly questioning their system and its impact on their environment.

the
END

"Il nous faudra répondre à notre véritable vocation, qui n'est pas de produire et de consommer jusqu'à la fin de nos vies, mais d'aimer, d'admirer et de prendre soin de la vie sous toutes ses formes."

Pierre Rabhi



Inspired by a true story

Text: Madeline Koczura

Illustrations: Laurette and Madeline Koczura

17 April 2018

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Contribution to the presented papers

	Chapter II	**Chapter III**	**Chapter IV**	**Chapter V**	**Chapter VI**
Conceptualisation			×		×
Sampling/analyses	×		×	×	×
Data treatment	×	×	×	×	×
Statistics/software	×	×	×	×	×
Writing	50 %	×	×	×	×

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(Sir Edmund Hillary)