DAFNAE

Department of Agronomy Food
Natural resources Animals Environment

12TH INTERNATIONAL MEETING ON MOUNTAIN CHEESE

-Proceedings-

20-22 JUNE 2017 PADOVA, ITALY

PADOVA UP

PADOVA UNIVERSITY PRESS

Original title *12th International Meeting on Mountain cheese. 20-22 June 2017 Padova, Italy*

Università degli Studi di Padova
via 8 Febbraio 2, Padova
www.padovauniversitypress.it

ISBN 978-88-6938-059-4

12TH INTERNATIONAL MEETING ON MOUNTAIN CHEESE

20-22 JUNE 2017 PADOVA, ITALY

PADOVA UP

Organized by:

UNIVERSITÀ DEGLI STUDI DI PADOVA

DAFNAE
Department of Agronomy Food Natural resources Animals Environment

With the support of:

réseau Fromages de Terroirs

INRA
SCIENCE & IMPACT

Foreword

In constrained environments such as mountainous areas, the sustainability of animal production systems relies on their ability to combine economic performance and to provide a range of other ecosystems services allowing among other the maintenance of open landscapes and high biodiversity, particularly important for the attractiveness of these territories. The economic performance of the herbivore production systems is based on the added value of the high quality products they propose. Currently, the mountain cheeses production chains are facing major challenges due to increasing uncertainty on markets, regulations, policies, societal trends and environment. One of the hot topics for the mountain cheese production, often produced with raw milk, is to reach the very high safety standards required for market entry while preserving at the same time the link to terroir, at the origin of the unique diversity and richness of the mountain cheeses qualities. In this aim, an optimal management of the complex and interconnected ecosystems of the chain from field to animal, farm, milk and cheese, is required because each link greatly determines the final qualities of cheeses. The objectives of the Mountain Cheese Network are to exchange information and knowledge on these multidisciplinary issues and to foster research connections and create a dynamic between scientists and members of the cheese production chains.

This publication is the outcome of the 12th International Meeting on Mountain Cheese held in Padova (Italy), 20-22 June 2017: "**The terroir of mountain cheeses: a focus on microbes, animals and production syste**ms". It compiles the 25 oral communications and 8 posters presented in full session by 60 scientists and technicians from 8 countries who attended the meeting. All participants share the same idea that the extraordinary diversity and originality of mountain cheeses is a fantastic tool to convert the natural handicaps of mountain farming into strengths for the local development. The contributions were split into three scientific sessions with one invited conference and oral and poster communications:

1. Phenotyping of milk properties interesting for cheese-making
2. New insights on microbiota, from the environment of the farm to the cheese
3. The farming system and cheese qualities

The 12th International Meeting on Mountain Cheese was organised by the "Università Degli Studi di Padova – Department of Agronomy, Food, Natural resources, Animals, Environment - DAFNAE" with the contribution of the "Federazione Provinciale Allevatori Trento", the "Reseau Fromages de Terroirs" (France) and "INRA" (France). We warmly thank all authors for submitting papers, the members of the organising and scientific committees, the editorial team who carried out the revisions of the contributions and the chairpersons of the scientific sessions.

We wish you a pleasant and fruitful meeting on the fascinating topics related to mountain cheeses.

Bruno Martin and Enrico Sturaro

On behalf of the organizing and scientific committees

Perspectives of dairy cattle breeding in mountain dairy systems: new insight from Cowability/Cowplus projects

G. Bittante[1]*, A. Cecchinato[1]

Dep. DAFNAE, University of Padova, Viale dell'Università, 16 I-35020, Legnaro (PD)
*bittante@unipd.it

Abstract

The abolition of milk quotas in EU is causing an increase of milk production and a decrease of the prices of dairy products which can lead to dramatic effects on many dairy systems in the mountains areas. Mountains dairy chain cannot compete for productivity, efficiency and cost of production and, for its economic sustainability should rely on the production of very high quality products in order to achieve high prices. Genetics of cattle (breeds, crossbreeding, and selection) should contribute to this target through optimization of use of genetic resources and the improvement of the cattle populations. Cowability/Cowplus projects offered new insights on the efficiency of dairy productions of cattle populations reared in the mountain areas and especially of Jersey and dual purpose breeds originated in the Alps. The herd productivity level, and the dairy system, if not properly considered can lead to biased estimates of breeding values of bulls used in traditional low-input farms. New phenotypes important for improvement of quality of dairy products have shown to be heritable in a mountain territory. Among these: the new modeling traits of milk coagulation, curd firming and syneresis; the % cheese yields, milk nutrients retained in cheese and cheese-making efficiency traits; the chemical, physical and sensory characteristics of cheeses obtained; the detailed fatty acid profile of milk and of cheese and the transfer of individual fatty acids from milk to cheese; the volatile organic compound fingerprint of cheeses produced.

Keywords: cow's breed efficiency, genetic-environment interactions, new cheese phenotypes

Introduction

Dairy cattle rearing in the mountains represents a small proportion of milk production in the EU, but it has a great value because of the externalities it offers to the community. First of all, cattle breeding in the mountain areas is important for the ecological services linked to environment protection from hydrogeological threats and landscape conservation, moreover it contribute a lot to maintaining culture and traditions linked to the area and thus enhancing the tourism and the quality of life.

The abolition of milk quota system in EU is causing an increase of milk production, especially in the intensive farms of the plains, and a decrease of the prices of dairy products. This can lead to dramatic effects on many dairy systems in the European mountains. Indeed, the dairy chains in the mountains cannot compete for productivity, efficiency and cost of production with more favoured areas and, for its economic sustainability, the mountainous dairy chains should rely on the production of very high quality products that should be able to obtain high market prices. Objective of the present study is to analyse the role of genetics of dairy cattle in enhancing the sustainability of dairy farms in the mountain areas through the optimization of the genetic resources. In particular, the results obtained from the Cowability and Cowplus projects "Cattle farming in mountain areas: sustainability, animal functionality and product quality" (funded by the Autonomous Province of Trento, Italian Alps) will be summarized.

Efficiency of cattle breeds for dairy farming in the mountains

Many dairy farmers in the mountainous areas are trying to face the global competition in the dairy sector by copying the evolution characterizing the dairy farms in the favoured areas of

the plains, in terms of facilities, feeding, management and also genetics. Indeed, the Holstein-Friesian is replacing the traditional breeds originated in the Alpine environment, and particularly the dual purpose ones.

Comparing cattle breeds in the mountains for milk yield

In table 1 data obtained from milk recording of cows belonging to 3 dairy specialized breeds (Holstein Friesian, Brown Swiss and Jersey) and 3 dual purpose breeds of Alpine origin (Simmental, Rendena and Alpine Grey) are summarized. The Italian national data show clearly the much greater average milk production of Holsteins respect to all the other breeds. Obviously different breeds are reared in different areas of the Country characterized by different environmental conditions and dairy systems. Considering only the data obtained from all the herds controlled in one mountainous province in the north-eastern Italian Alps (Trento province), it could be noted that the superiority of Holsteins is still very evident but the differences among breeds is slightly lower than at national level. In any case, also within the same province, the farms and farmers rearing only Holsteins are different from those rearing local breeds. For this reason, to try to disentangle the effect of breed from that of farms and breeders, in the Cowplus project, 41 multi-breed farms have been selected to represent all the 6 breeds considered (2 to 5 different breeds reared per farm).

Table 1. Summary of effect of breed and of herd productivity level on traditional and new phenotypes and on cow efficiency traits (from Cowplus project).

	Breed of cows:							Herd level:	
	HF	BS	JE	-	SI	RE	AG	High	Low
Contrasts	-	*BS vs HF*	*JE vs HF+BS*	*HF+BS+JE vs SI+RE+AG*	*SI vs RE+AG*	*RE vs AG*	-	-	*L vs H*
Milk yield, kg/d:									
Italy, all farms	30.9	23.6	20.4	-	21.9	17.2	16.3	-	-
Trento province, all farms	29.7	24.1	16.6	-	20.9	16.7	14.5	-	-
Mixed herds, ave	28.4	25.0	21.1	-	20.2	16.9	13.1	28.0	18.5
Mixed herds, LSM	25.9	22.7***	17.0***	ns	23.0***	21.8**	18.7	26.1	17.0***
Milk, cheese corr.	22.8	23.5	20.3*	*	22.5***	20.5	19.4	26.3	16.7***
Cheese yield:									
RCT, min	19.8	19.0	13.0***	ns	18.3	15.4**	19.1	18.6	16.3**
Max curd firmn., mm	48.3	57.2***	63.0***		53.4	55.5	55.6	57.3	53.7*
$\%CY_{CURD}$	12.4	14.4***	17.1***	***	14.0	13.0**	14.1	14.5	13.8**
Ef-$\%CY_{CURD}$,%	96.1	102.4***	100.9	*	101.0	103.5	102.2		
Cheese, kg/d	3.2	3.3	2.8*	*	3.1**	2.8	2.7	3.6	2.3***
Body size:									
BW	645	643	384***	***	669***	592***	552	604	557***
BCS	2.7	2.9***	2.7	***	3.2	3.2	3.3	3.1	2.9***
Body protein, kg	94	92***	56***	***	94***	83	76	85	80***
Net energy req. MJ/d	126	120***	94***	ns	121***	110	102	130	95***
Productivity:									
Milk/BW	40	35***	45***	***	34	36*	33	43	32***
Cheese/BP	33	35	54***	***	33	33	33	43	31***
Efficiency:									
Energy, %	62.2	60.8*	63.2	***	59.7*	57.9	56.7	63.9	56.3***
Monetary, %	175	190***	200**	***	180	177	176	196	170***
IOFC, €/d	3.1	3.5	3.3	*	3.1	2.9	2.7	4.0	2.2***
Cows, N	471	663	40	-	158	103	73	920	588

Considering the raw averages of daily milk production of cows of the different breeds reared in the multi-breed farms the results were not much different from the averages obtained on all the farms of the province. But, obviously, also multi-breed farms represent different environments and dairy systems and the various breeds are not homogeneously distributed among them.

Effect of dairy system on milk yield of different breeds

The multi-breed farms were divided in high- and low-producing ones on the base of the average daily milk energy production after correcting data for breed, parity and DIM of cows reared (28.0 vs 18.5 k/d of milk, respectively). The majority of the farms of the first group were characterized by "modern" rearing systems (loose rearing, total mixed rations, high concentrate:forage ratio, milk parlour, etc.) whereas those belonging to the second group pertained mainly to "traditional" dairy systems (tied cows, hay, few compound feed, milked at the stall, etc.).

The large-framed breeds, Holstein, Brown Swiss and Simmental, were present in both groups of farms (the first breed more in the high-producing and the last more in the low-producing farms). Jerseys were present only in high-producing and the Rendena and Alpine Grey only on the low-producing farms. After having included the herd productivity level and the individual herds in the model, the least squares means (LSM) of daily milk yield were much closer respect to raw averages (the difference between the two extremes, Holstein and Alpine Grey, was 7.2 vs 15.3 kg/d for LSM and raw averages, respectively). These LSM values represent the different productivity of cows of different breeds when reared in the same farm and having the same age (parity and DIM) distribution. The interaction between breeds and herd productivity level could be tested only considering the breeds (Holstein, Brown Swiss and Simmental) present in both groups, but it was not significant for almost all the traits considered. It could be concluded that the genetic differences in daily milk production among different breeds in a mountain area roughly represent half of the overall variability observed among them, being the remaining variability due to farms and farmers.

From milk to cheese daily production

The differences among breeds are very evident also for milk quality, but in this case with a much different order among breeds. Holsteins produce the more diluted milk and Jerseys the more concentrated, with the Alpine breeds intermediate. Taking into account the different ability to produce cheese, the differences among breeds diminish further (only 4.1 kg/d between the extreme breeds). The best breed is no more Holstein, but Brown Swiss, even if not significantly, with similar production showed also by Simmentals, while the smaller framed-breeds were similar among them, producing only about 10% less than the larger-framed ones (Table 1).

The breeds studied are different not only in terms of milk yield and composition but also for the milk technological properties, that are particularly important for the production of traditional PDO high priced local cheeses. The modelling of the pattern of curd firming over time since the addiction of rennet (Stocco et al., 2017) showed that the milk from Holsteins was the worst and that from Jerseys the best in terms of coagulation properties (Table 1). The efficiency of cheese-making was studied for 508 individual cows of the Cowplus project producing of each of them a model cheese. For the fresh cheese-yield (cheese produced for every 100kg of milk processed) again the Holstein and the Jersey were the extreme breeds, with the Alpine ones being intermediate (Table 1). But cheese-yield depends not only on milk composition (protein and fat), but also on the efficiency with which the nutrients are retained in cheese instead of being lost in the whey. The efficiency of cheese-making was much greater for Alpine breeds, and especially Rendena, than for Holstein-Friesian, with Jersey intermediate. The final result of milk yield, composition and cheese-making efficiency was that the 3 large-framed breeds produced slightly more than 3 kg of fresh cheese per day and

per cow, whereas the small-framed cows produced slightly less than 3 kg/d (Table 1).

Productivity and efficiency of cheese production

But differences in body size and composition means differences in nutrient requirements for maintenance, growth and pregnancy and then differences in feeding cost of cows. Beyond the expected differences among breeds in terms of body weight (respect to Simmentals, Alpine Grey were more than 100 kg lighter, and Jerseys almost 300 kg lighter), the dual purpose breeds were characterized by a greater BCS respect to the specialized dairy cows and these means a fatter composition of body mass and lower maintenance requirements per unit weight (Table 1). It worth to note that metabolic weight could introduce bias in the estimation of nutrient requirements when different breeds are to be compared, whereas better results are yielded using body protein mass.

The concept of productivity is often based on scaling production by a dimension parameter. The simplest and raw productivity index is obtained expressing the daily milk production per unit body weight (g/kg). A more correct index is obtained expressing cheese production per unit body protein mass (more related to maintenance nutrient requirement of cows). Both productivity indices are listed in Table 1 and it can be seen that excluding the greater value of Jersey, all the other 5 breeds presented a very similar production of cheese per kg body protein mass.

The concept of efficiency relates output and input of a production process. From an energy point of view, it could be seen that milk energy produced by a lactating cow represents about 60% of total energy consumed to meet overall energy requirements of the cow. The specialized dairy breeds are slightly more efficient than dual purpose breeds for milk production (Table 1). In monetary terms, the efficiency is represented as the ratio between the value of the cheese produced and the cost of the feed needed for meeting energy requirements. The Jerseys allowed to obtain a value of cheese double than the cost of feeding, followed by Brown Swiss and by the dual purpose and the Holstein breeds. When the monetary efficiency was represented as a difference (IOFC: income over feed costs, in €/d) instead of a ratio, the ranking of breeds were slightly different (not being scaled by body size) with the extreme breeds represented by Brown Swiss and Alpine Grey and the other breeds being intermediate.

Breeding goals of dairy cattle in the mountains and other traits

These results lead to the conclusion that comparing different breeds reared in different areas and farms only considering average daily milk production can give a profoundly biased result. Especially in mountainous areas, comparisons should be done within the same rearing environment (farm) and need to consider differences not only in milk composition but also in cheese-making efficiency.

Moreover, an evaluation of the efficiency of cheese production should consider differences in body size and composition (and nutrient requirements) relating the value of product obtained to the production costs.

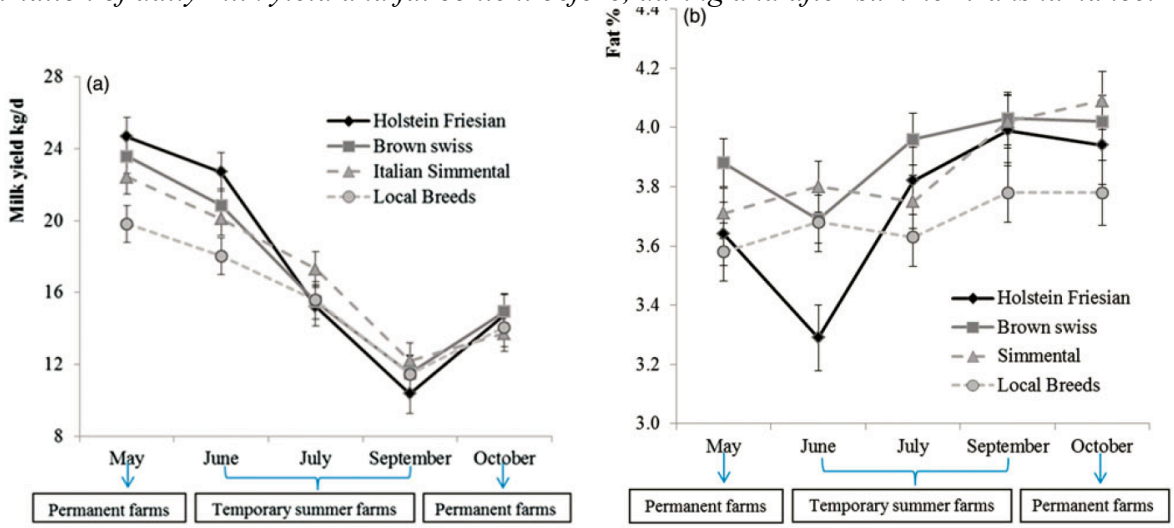
It is then evident that many other new phenotypes reward for consideration in future selection goals, especially in the mountains. Moreover, efficiency of cheese production should be combined with other income sources, and especially meat production through calves and culled cows, and reduction of rearing costs through improved fertility, longevity and overall fitness traits and adaptability of cows reared in the mountains.

Adaptability to summer pastures

A survey on 15 summer temporary farms in the Alpine pastures of Trento province showed clearly the different adaptability of various breeds reared in the permanent farms of the lowland (Zendri et al., 2016). Holsteins were the higher milk producers before the transhumance and the lower milk producers at the end of summer pasture. On the contrary,

cows belonging to Alpine dual purpose breeds exhibited a much smaller decrease of production during summer pastures (Figure 1).

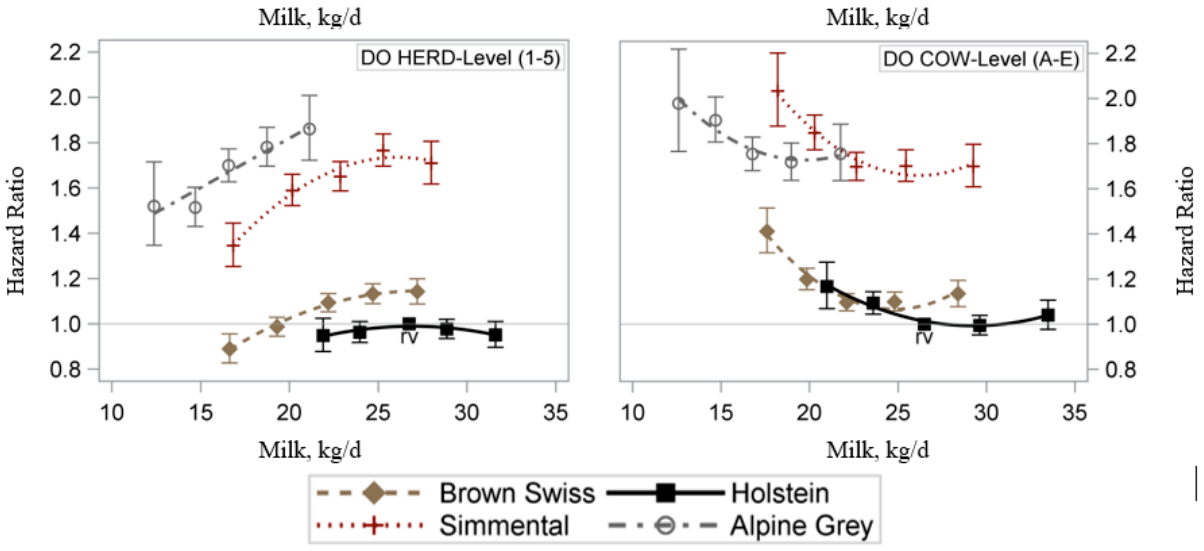
Figure 1. Different adaptability of various cattle breeds to Alpine pastures as shown by variation of daily milk yield and fat content before, during and after summer transhumance.



Breed × production level interactions on cows' fertility

From a large survey on fertility of cows reared in an Alpine province (Toledo-Alvarado et al., 2017) it was found that breeds are not only different among them, but also that they interact in a different way with milk production level. As shown in Figure 2, it is possible to see that, on average, the dual purpose breeds of Alpine origin (Simmental and Alpine Grey) are characterized by a greater fertility respect to dairy breeds and, among these, the Alpine (Brown Swiss) breed is slightly more fertile than Holstein. The effect of increasing herd productivity was favourable in all the Alpine breeds but not in Holsteins, whereas the increase of milk productivity of individual cows within herds leads to a decrease of fertility, at least till a daily production of about 25 kg of milk.

Figure 2. Hazard ratio estimates of days open of different cattle breeds reared in the mountains (Alto Adige / Süd Tirol province, Northeast Italian Alps) as affected by the level of productivity of the herd and by the level of productivity of the cow within herd.



Meat contribution to farm income

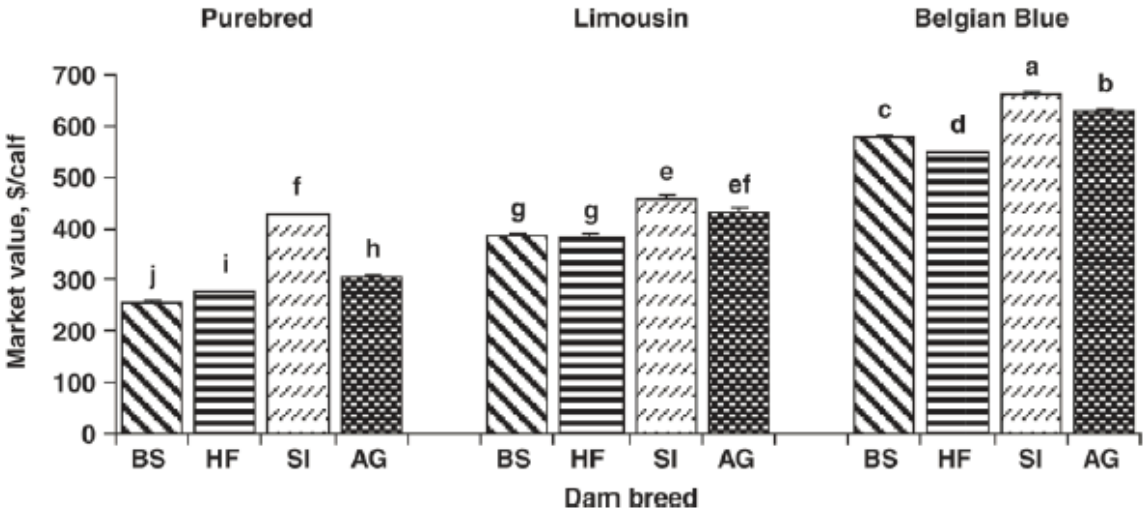
The number of cows culled per year in the mountains is lower for Alpine breeds because of their greater longevity and lower replacement rate, but their value is greater than Holsteins. As shown by a survey on culled cows in the mountainous province of Trento (Gallo et al., 2017). The culled cows belonging to the dual purpose Alpine breeds, and especially Simmentals, are more muscular and conditioned, show greater dressing percentage and carcass price (Table 2).

Table 2. Characteristics of culled cows from the mountainous province of Trento according their breed.

	HF	BS	SI	RE
Cows	233	238	44	40
Culling age, mo.	66	74	74	81
BW	618	607	667	546
BCS	2.7	2.9	3.2	3.0
Fleshiness	0.9	1.0	1.8	1.2
Carcass wt, kg	251	252	296	234
Carcass price, €/kg	2.0	2.1	2.3	2.1
Carcass value, €	519	536	713	506

If the number of culled cows is lower, the number of calves exceeding replacement needs that are sold for veal and beef production is much greater in the case of Alpine breeds. A survey on calves sold at public auctions in the province of Alto Adige / Süd Tirol (Dal Zotto et al., 2009) showed that purebred Simmental calves are sold at a price about twice that of purebred dairy calves and that the price of crossbred calves obtained from Belgian Blue bulls is about twice the price of those of their corresponding maternal purebred calves (Figure 3).

Figure 3. Average value of calves from mountainous farms sold at auction at an average age of 23 days according to their sire and dam breeds.



It is evident that crossbreeding with double muscled bulls is a viable option for Alpine cows, provided that their replacement rate is lower than in the case of Holsteins.

Genetic × environment interaction in estimating breeding values

Dairy farming in mountainous areas is different from plains and high-input areas, but, within mountainous areas, different dairy systems exist. This variability can affect the estimation of breeding values of animals reared in the different environment.

After having divided the herds of Holsteins and Brown Swiss cows reared in Trento province in two groups (High and Low) according to their average milk yield, a genetic analysis was

carried out separately for breed and productivity level. The unpublished results are summarized in table 3. As it can be seen the average milk yield was about 30% greater in High- vs Low-producing herds, whereas milk fat and protein content was not affected. The semen of the large majority of Holstein and Brown Swiss bulls was used in both groups of farms, and their daughters represented the large majority of cows in both groups.

Table 3. Effect of herd production level on estimates of genetic parameters of milk yield of Brown Swiss cows reared in mountains (Trento province, North-east Italy).

Breed	Brown Swiss:			Holstein Friesian:		
Herd level	High	Low	High/Low	High	Low	High/Low
Herd milk yield, kg/d	>25	<25	-	>30	<30	-
Data, N:						
Herds	59	132	-	40	77	-
Sires	842	993	-	1202	1948	-
Common sires	566		-	694		-
Cows	4,431	6,036	-	5,738	6,668	-
Cows from common sires	4,077	5,294	-	4,875	4,383	-
Production						
Milk, kg/d	27.7	21.0	1.32	32.9	25.2	1.31
Fat, %	4.10	4.07	1.01	3.96	3.86	1.02
Protein, %	3.73	3.63	1.03	3.41	3.42	1.00
Milk variances:						
Genetic	4.6	2.3	1.43	5.7	3.6	1.26
Permanent environmental	9.3	5.7	1.28	11.9	9.9	1.09
Residual	17.9	11.5	1.24	25.5	20.1	1.13
Phenotypic	31.8	19.5	1.28	43.1	33.6	1.13
Heritability, %	14.6	11.7	1.25	13.3	10.7	1.25
Repeatability, %	43.8	40.8	1.07	40.8	40.2	1.01
Genetic correlation, %	99.2		-	90.5		-

While genetic parameters of quality traits were not affected by herd productivity level, the milk yield yielded much greater variances in High- vs Low-producing farms and the root squared ratio was greater for genetic than for permanent environmental, residual and phenotypic variances. As a result, also heritability was greater in High- vs Low-producing farms (Table 3). Even though, the genetic correlation between the milk yield in the two environment was very high (0.91 for Holsteins and 0.99 for Brown Swiss), it is evident that, if these differences are not taken into account the estimation of breeding value of animals can be biased. In particular, bulls tested in Low-producing farms will obtain a breeding value more close to the average of the population, while those tested in High-producing farms will receive more extreme (both positive and negative) breeding values. Before the genomic era, the standardization according to the individual farm phenotypic variance was practiced, but only for breeds reared in large herds (excluding much of the farms in the mountains). Now new attention should be placed on this topic with genomic selection.

Heritability of new phenotypes

The heritability estimates (h^2) for the standard traits, normally recorded during milk recording schemes, varied from 10% (SCS) to 28% (protein) depending on the trait analysed and were all within the mean ± s.d. of the estimates reviewed by Bittante et al. (2012). Moving to novel phenotypes, like protein fractions assessed using the High Performance Liquid Chromatography (HPLC) method, the h^2 exhibited higher values especially for the β-CN content which showed an heritability estimate greater than 60% (Table 4). It is worth noting that such estimates were obtained by fitting an animal model without considering the large

effect of the well-known milk protein loci mainly located on chromosome 6 (Dadousis et al., 2017). With respect to phenotypes related to milk coagulation properties (MCP: standard and the new modeling parameters based on a bi-exponential four parameter model) the situation is slightly different because the estimates were lower respect to those of protein fractions, varying from 19% to 27%, for the standard MCP and 6% to 28% for the curd firmness (CF) parameters. It is worth noting that the incidence of herd variance on the total variation, for each of the MCP traits, was always lower (except for k_{SR} and CF_P) respect to the h^2, highlighting the importance of the selective breeding for enhancing MCP. Moving from milk to the cheese-related phenotypes, the situation of h^2 is intermediate between the protein fractions and the MCP traits. In fact, the heritability estimates varied from 21% (REC_{FAT}: recovery of fat into the curd) to 49% ($REC_{PROTEIN}$: recovery of protein into the curd) highlighting, even in this case, the relevance of the genetics for improving the cheese-making ability in dairy cattle. The cheese traits exhibited moderate genetic variation, in fact the h^2 varied from 4% (shear force) to 16% (protein content). The lower genetic variation is attributable to the potential environmental variability occurred during the two months of ripening of the individual cheeses.

It worth noting that, for the first time, it was demonstrated that also sensory profile of the cheese depends, even if for a small proportion (4%, for elasticity and smell, to 7% for acidity taste), from genetics of cow. Higher were the h^2 estimates obtained for many volatile organic compounds of ripened cheeses (Bergamaschi et al., 2016).

Table 4. Summary of heritability and herd effect of traditional and new phenotypes recorded in Brown Swiss cows reared in mountain (from Cowability project).

	h^2,%	herd,%		h^2,%	herd,%
Common traits:			Cheese-making:		
Milk yield	18		%CY_{CURD},	27	30
Fat, %	12		%CY_{SOLIDS}	26	21
Protein, %	28		REC_{FAT};%	21	32
SCS,	10		$REC_{PROTEIN}$, %	49	27
Protein fractions:			REC_{SOLIDS},%	27	21
Casein HPLC,%	10		REC_{ENERGY},%	23	30
α_{s1}-CN,%	33		Cheese traits:		
α_{s2}-CN,%	24		Protein,%	16	
β-CN,%	63		Fat,%	11	
κ-CN,%	51		Solids, %	6	
β-LG,%	33		pH	11	
α-LA,%	11		Salt,%	12	
Traditional MCP:			Color (average)	10	
RCT, min	27	14	Shear force	4	
k_{20}, min	23	3	Cheese sensory traits:		
a_{30}, mm	19	5	Flavor	5	
CF modelling:			Smell	4	
RCT_{eq}, min	26	14	Salty	5	
CF_P, mm	6	12	Acidity	7	
k_{CF}, %/min	23	14	Elasticity	4	
k_{SR}, %/min	8	11	Thoughness	5	
CF_{max}, mm	19	18	Moisture	5	
T_{max}, min	28	15			

In conclusion, results obtained suggest that genetic variation exists among the aforementioned novel phenotypes suggesting possible exploitation of such variation in breeding programs for dairy cattle aiming for genetic improvement of the cheese-making ability and quality.

Moreover, it should be considered that for many of these new phenotypes the predictions based on Fourier-transform Infrared Spectrometry have shown to be reliable and often more correlated to golden standard analyses from the genetic than phenotypic point of view (Ferragina et al., 2015; Bittante et al., 2014)

Acknowledgements
The authors would like to thank the Autonomous Province of Trento fur financial support, the Breeders associations of Trento and of Bolzano Provinces, the Consorzio Superbrown and ANARB for providing data and collaboration.

References
Bittante G., Ferragina A., Cipolat-Gotet C., Cecchinato A. 2014. J. Dairy Sci. 97:6560-6572.
Bittante G., Penasa M., Cecchinato A 2012. J. Dairy. Sci, 95: 6843-6870.
Bergamaschi M., Cecchinato A., Biasioli F., Gasperi F., Martin B., Bittante G.. 2016. Genet. Sel. Evol., 48:89.
Dadousis C., Pegolo S., Rosa G.J.M., Gianola D., Bittante G., Cecchinato A. 2017. J. Dairy Sci., 100: 1223-1231.
Dal Zotto R., Penasa M., De Marchi M., Cassandro M., López-Villalobos N., Bittante G. 2009. J. Anim. Sci., 87: 3053-3059.
Ferragina A., de los Campos G., Vazquez A., Cecchinato A., Bittante G. 2015. J. Dairy. Sci., 98: 8133-8151.
Gallo L., Sturaro E., Bittante G.. 2017. Animal., 11: 696-704
Toledo-Alvarado H., A. Cecchinato, G. Bittante. 2017. J. Dairy. Sci., In press.
Zendri F., Ramanzin M., Bittante G., Sturaro E. 2016. Ital. J. Anim. Sci., 15: 481-491.

Prediction of cheesemaking properties of Montbeliarde milks used for PDO/PGI cheeses production in Franche-Comté by mid-infrared spectrometry

C. Laithier[1]*, V. Wolf [2], M. El Jabri[1], P. Trossat[3], S. Gavoye[3], D. Pourchet[2], P. Grosperrin[2], E. Beuvier[4], O. Rolet-Répécaud[4], Y. Gaüzère[5], O. Belysheva[5], E. Notz[6], A. Delacroix-Buchet[7]

[1]Institut de l'Elevage, F-75595 Paris, France, [2]Conseil Elevage 25-90, F-25640 Roulans, France, [3]ACTALIA, F-39800 Poligny, France, [4]INRA, UR342 Technologie et Analyses Laitières, F-39801, Poligny, France, [5]ENILBIO, F-39801 Poligny, France, [6]CTFC, F-39800, Poligny, France, [7]INRA UMR1313 GABI, INRA, AgroParisTech, Université Paris-Saclay, F-78350 Jouy-en-Josas, France.
*cecile.laithier@idele.fr

Abstract
It is important for cheesemakers to obtain quality parameters for the cheesemaking properties (CMAP) of milk in order to secure quality and regularity in the cheese production. This is especially true for most Protected Designation Origin (PDO) cheeses where milks treatments are limited or forbidden. This study aimed to examine the feasibility of predicting, by mid-infrared spectroscopy (MIRS), CMAP of French Montbéliarde milks from the Franche-Comté cheese production area (5 PDO + 1 PGI cheeses). The investigation was conducted at three production levels - individual milk (n = 250), herd milk (n = 100) and cheese dairy milk (n = 70) - considering the requests of all actors in the dairy sector. CMAP reference analyses included measures of the laboratory curd yield and according to two models of cheese technology (soft and pressed cooked), the rennet curd firmness. We observed that the CMAP variability depended largely on the studied scale. CMAP were more variable for individual cow milks compared with herd bulk milks and even more dairy vat milks. The model predictions of the laboratory curd yield developed using the individual milks dataset were considered as excellent. Further investigations remain in progress.

Keywords: cheesemaking properties, mid-infrared prediction, cow milk, Montbéliarde breed, PDO cheese

Introduction
Managing cheesemaking properties (CMAP) of milk is important for cheesemakers to secure quality and regularity in the cheese production, especially for Protected Designation Origin (PDO) and Protected Geographical Indication cheeses (PGI) where milks treatments are limited or forbidden. Franche-Comté is the first producing region of PDO cheeses in France. The PDO cheese sector in Franche-Comté bands together producers, cheesemakers and cheese ripeners based on the sharing of the added-value between the different actors. Managing CMAP has become a concern, shared by the regional PDO inter-branch organization and, more generally, with the entire French cheese sector and national dairy inter-branch.
Many studies have been carried out in the past ten years to assess the possibility of using mid-infrared spectrometry (MIRS) to predict CMAP of individual milks of various cow breeds in order to manage it routinely, time effectively and at low cost (Dal Zotto *et al.*, 2008; De Marchi *et al.*, 2009 and 2013; Visentin *et al.*, 2015). The Montbéliarde breed, the second French cow breed, represents 95% of the milking cows of Franche-Comté. To our knowledge, the FROM'MIR program is the first to evaluate by MIRS the CMAP of Montbéliarde milks not only at the scale of the individual milk but also at the scales of the herd tank milk and the dairy vat milk to fulfill the expectations of the cheesemakers.

Material and methods

250 Montbéliarde cows were sampled in commercial herds across 3 French departments (Doubs, Jura and Haute-Saône) located in the area of PDO and PGI cheeses. The sampling periods were during January-March 2016 and April-June 2016. The objective of the sampling was to maximize genetic and milk composition diversity. Two criteria were used for this selection: the lactoproteins phenotypes based on the results of a previous study (Fang *et al*., 2016) and the lactation stage. Cows with rare alleles of lactoproteins were systematically collected. Samples were obtained either from the morning milking (n = 98), the evening milking (n = 99) or the mixture of two successive milkings, in proportion by volume (n = 53).
100 commercial herds were selected from an initial dataset of 2100 PDO and PGI herds of Franche-Comté and their herd tank milks were sampled at two periods (February-March 2015 and May-June 2015). Selection was carried out in order to hedge the variability of the dairy farming practices (geographic location, calving season and average annual herd protein content of the previous milking campaign). Samples were collected in the tank containing two successive milkings excepted in farms where the milk is delivered to the dairy after each milking *(lait de coulée*). In this particular case, the herd tank was sampled twice, namely two samples of two consecutive milkings were collected then mixed in proportion of each milking volume. Seventy dairy vats were sampled - before addition of starters- at the same periods as herd tank milks in 55 dairies located in the PDO cheeses area (15 dairies were collected at both periods). Dairies were selected to maximize the expected milk composition variability (collection area, dairy size). Animals, herds and dairies were selected independently.
Every sample of whole fresh raw milk was collected under strict hygienic conditions immediately cooled at 4°C and analyzed within 24 h. Individual milk with SCC>10^6/ml and UFC >10^5/ml were discarded. Aliquots were preserved by bronopol and analyzed by MIR spectroscopy using MilkoScan FT6000 (Foss, Hillerod, Denmark) over spectral range from wavenumber 5011 to 925 cm^{-1}. All MIR spectra were standardized by piecewise direct standardization, which matches slave-instrument spectra on master-instrument spectra (Grelet *et al.*, 2015). Following Foss recommendations (Foss, 1998), only informative wavelength bands not tainted by water molecule were kept as data (446 wavelengths). Other whole raw fresh milk aliquots were analyzed by CMAP reference methods. Milk coagulation properties during rennet coagulation were measured, after heating 30 min at 32°C, using a Formoptic adapted from the mechanical Formagraph, by Chr. Hansen and ENILbio, to facilitate data computerization. In short, the position of the pendulum is transmitted via optic transceivers to a computer system which converts this position into volts in relation to time. For easy viewing, these volts are expressed into firmness index (FI in volt*10). Milk samples were standardized with lactic acid and coagulated according to two models of cheese technology (soft and pressed cooked) i.e. respectively, either at pH 6.45 with an equivalent of 25 ml of rennet extract (520 mg of chymosin/l) for 100 l of milk or at pH 6.60 with an equivalent of 14 ml of rennet extract (810 mg of chymosin/l) for 100 l of milk, each measured as duplicates. The rennet curd firmness (aR) was expressed in FI. Laboratory curd yields were measured in duplicate by centrifugation (2700 *g*, 15 min) of coagulated milk, 50 ml at pH 6.6 and 32°C (method adapted from Hurtaud *et al.*, 1995). Milk and whey dry matter (DM) were measured for calculation of curd yields in DM.
On individual milk dataset (n= 250), different calibration equations were developed using different regression models (Bayesian, RF-PLS, UVE-PLS). Partial Least Square (PLS) applied after variable selection methods outperform other models for predicting CMAP parameters in terms of predictive ability (El Jabri *et al*., 2017). Calibration equations were developed on individual milk dataset (n = 250) and entire dataset (all samples: individual + herd + vat milks, n = 420) using PLS regression analysis with external validation, by dividing the data set in 2 subgroups: 2/3 calibration set and 1/3 validation set. To assess and compare the equations, statistical parameters were computed using R: software (version 3.2.3., https://r-project.org/): mean, standard deviation (sd), Standard Error of prediction (SEP),

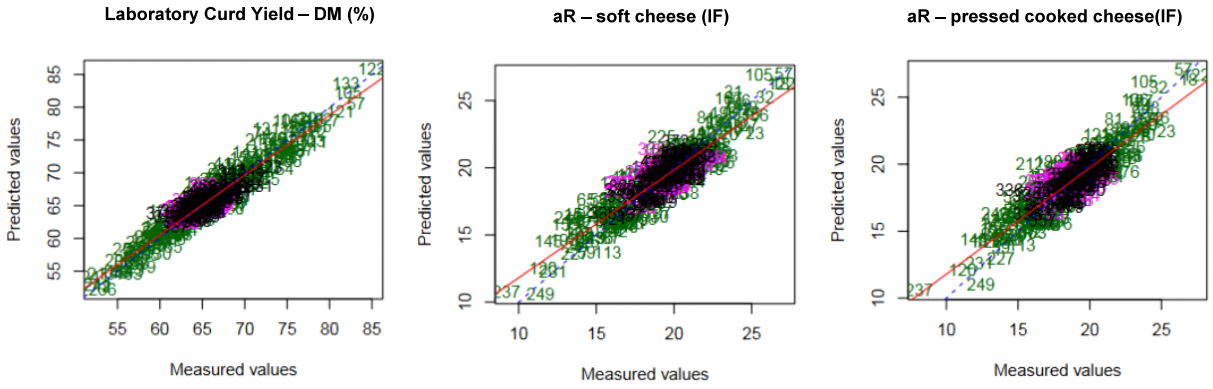
coefficient of determination (R^2) and Ratio to Performance Deviation of external validation (RPD). We consider the model/predictions fair with RPD between 1.4 and 1.8, good with RPD between 1.8 and 2, very good with RPD between 2 and 2.5 and excellent with RPD > 2.5 (Viscarra Rossel *et al*., 2006). The accuracy was also calculated, because of its potential importance in case of further applications, either to access the CMAP of dairy vat milk in routine or to simulate milk price if a milk pricing system would be based on milk technological traits.

Results and discussion

Descriptive statistics showed that CV for all measured parameters fell rapidly from individual to bulk milks and ranged respectively for individual milks and herd milks from 9.9 and 3.5 for the laboratory curd yield, from 17.5 to 7.0 % for the aR soft or pressed cooked cheeses. CV were even lower for dairy vat milk: 2.4 % (laboratory curd yield-DM), 5.0 % (aR pressed cooked cheese) and 6.2 % (aR soft cheese). The entire dataset variation ranged from 8.0 % (laboratory curd yield) to 14.0 % and 14.2 % (aR pressed cooked and soft cheese respectively).

Scatter plots of laboratory curd yield and aR of all samples confirmed (Figure 1) that even if the number of herd and dairy vat milks were limited compared to the number of individual milks, the variability of herd and dairy vat milks was included in the variability of individual milks.

Figure 1. Scatter plots of predicted (y-axis) versus measured values (x-axis) of milk samples analyzed for laboratory curd yield-DM (%), aR soft cheese (FI) and aR pressed cooked cheese (FI). Individual samples are represented in green, herd milk in pink and dairy vat milk in black.



The prediction equation (see Table 1) for laboratory curd yield-DM was considered as excellent for equations developed on both the entire dataset (RPD = 3.05) and individual milks dataset (RPD = 3.39). The prediction of aR in soft cheeses and pressed cooked cheeses were considered as good for both datasets with RPD around 1.8-1.9. Finally, the equations of a2R in soft cheeses are considered as fair for both datasets (RPD around 1.7 for both datasets).

Table 1: Fitting statistics of prediction models in external validation for cheese-making properties

Trait	N	SEP	Accuracy	R^2	RPD	Level
Laboratory curd yield-DM	123	1,67	3,34	0,89	3,05	All samples
Laboratory curd yield-DM	74	1,84	3,68	0,91	3,39	Individual
aR- soft cheese	120	1,50	3,00	0,73	1,92	All samples
aR- soft cheese	73	1,55	3.09	0,72	1,88	Individual
a2R- soft cheese	119	1,34	2.68	0,64	1,67	All samples
a2R- soft cheese	72	1,31	2.62	0,66	1,71	Individual
aR- pressed cooked cheese	121	1,41	2.81	0,72	1,88	All samples
aR- pressed cooked cheese	72	1,46	2.91	0,70	1,81	Individual

n = number of samples for external validation; SEP = standard error of prediction of external validation; R^2 = coefficient of determination of external validation; RPD = Ratio to Performance Deviation of external validation; Laboratory curd yield-DM = Laboratory curd yield in Dry Matter; aR = curd firmness at once the rennet coagulation time; a2R = curd firmness at twice the rennet coagulation time

The MIRS equations developed on individual milks and on all milks (individual + herd + dairy vat milks) to predict laboratory curd yield-DM and curd firmness were equivalent in terms of quality of prediction and ranged from fair to excellent quality models. On the contrary, equations developed on herd milks and dairy vat milks datasets exclusively (data not shown) were less robust confirming that variability in the calibration dataset is an essential condition to develop robust predicting models (De Marchi *et al.,* 2013). Prediction accuracy of the models could be improved by increasing the number of milk samples and reference analyses (Visentin *et al*., 2015).
For genetics purpose, predicting models must reflect the variability obtained with reference methods (Visentin *et al*., 2015). In our study, for a given parameter, the predicting models developed either on the entire dataset or on the individual dataset have an equal R^2, meaning that they can be used equally and indifferently for genetic purposes.

Conclusions

In our study, CMAP were more variable for individual milks compared to herd milks and even more dairy vat milks. Models developed with individual datasets to predict laboratory curd yield in dry matter were considered excellent. Cheeses will be manufactured and ripened at a pilot scale to confirm these results. It will allow to check that CMAP evaluated under laboratory conditions are in accordance with results measured in real conditions. This validation will open new perspectives for evaluating curd yield in dry matter routinely and at low cost at the individual scale. The models developed within this project will be used in the FROM'MIR project itself to assess genetic variability of CMAP within the population of Montbéliarde cows. Another objective is to identify factors contributing to the CMAP variation at different scales of the PDO and PGI milk production systems (cow, herd, dairy) by using MIRS predicting methods if their accuracy is acceptable or, failing that, reference analytical methods.

Acknowledgements

This study was funded by the French Ministry of Agriculture, Agro-food and Forest, the French Dairy Interbranch Organization (CNIEL), the Regional Union of Protected Designation cheeses of Franche-Comté (URFAC) and the Regional Council of Bourgogne Franche-Comté, under the project FROM'MIR

References

Dal Zotto R., De Marchi M., Cecchinato A., Penasa M., Cassandro M., Carnier P., Gallo L., Bittante G. 2008. J. Dairy Sci., 91: 4103-4112.
De Marchi M., Fagan C.C., O'Donnell C.P., Cecchinato A., Dal Zotto R., Cassandro M., Penasa M. 2009. J. Dairy Sci., 92: 423-432.

De Marchi M., Toffanin V., Cassandro M., Penasa M., 2013. J. Dairy Sci., 96: 1-9.
El Jabri M., Sanchez M-P., Laithier C., Doutart E., Wolf V., Pourchet D., Grosperrin P., Beuvier E., Rolet-Répécaud O., Gaüzère Y., Belysheva O., Delacroix-Buchet A., Boichard D. 2017 Chimiométrie XVIII, 30 January-1st February Paris, France.
Fang Z.H., Visker M.H.P.W., Miranda G., Delacroix-Buchet A., Bovenhuis H., Martin P. 2016 J. Dairy Sci., 99: 8168-8177.
Foss. Reference Manual of Milkoscan FT120, Type 71200, Denmark, 1998.
Grelet C., Fernandez P. J., Dardenne P., Baeten V., Dehareng F., 2015. J. Dairy Sci., 98: 2150-2160.
Hurtaud C., Rulquin H., Delaite M., Vérité R., 1995. Ann. Zootech., 44: 385-398.
Viscarra Rossel R.A., McGlynn R.N., McBratney, A. B. 2006. Geoderma, 137: 70-82.
Visentin G., McDermott A., McParland D., Berry D. P., Kenny O.A., Brodkorb A., Fenelon M. A., De Marchi M. 2015. J. Dairy Sci., 98: 6620-6629.

Volatile Organic Compounds of milk, cream, fresh cheese, whey, ricotta, scotta, and ripened cheese obtained during summer Alpine pasture

M. Bergamaschi[1]*, B. Martin[2-3], G. Bittante[1]
[1]Dep. DAFNAE, University of Padova, Viale dell'Università, 16 I-35020, Legnaro (PD)
[2]INRA, UMR 1213 Herbivores, F-63122 Saint-Genès-Champanelle, France
[3]Clermont Université, VetAgro Sup, BP 10448, F-63000 Clermont-Ferrand, France
*matteo.bergamaschi@unipd.it

Abstract
The aim of this experiment was the characterization of volatile organic compounds (**VOCs**) in dairy products (**DPs**) derived from cheese- and ricotta-making performed in a temporary farm during summer transhumance in Alpine pastures. A total of 148 cows were grazed on pastures from June to September. Samples were collected from cheese-making performed every 2 weeks in the small dairy of the farm according to traditional artisanal procedures for obtaining Malga cheese. Forty-nine VOCs in milk, cream, fresh cheese (curd), whey, ricotta, scotta, and cheeses ripened for 6 and 12 months were determined by a SPME/GC-MS. The comparison between VOCs of whole evening milk, skimmed milk, whole morning milk, and milk in vat showed that the creaming process affect the major part of VOCs followed by the effects of milking and mixing. Cream, in contrast to fresh cheese and ricotta, shown higher content of fatty acids and other compounds (sulphurs, terpenes), while the ricotta shown higher proportions of aldehydes than the other DPs. Whey and scotta, respect to milk, shown a higher concentration of VOCs with the exceptions of esters, sulphurs, and lactones. The release of VOCs (mainly fatty acids) increased dramatically during the first 6 months of ripening, and again during the following 6. In conclusion, the evolution of VOCs characterized clearly the aroma profile of 11 dairy products sampled during cheese- and ricotta-making and the ripened Malga cheeses obtained were characterized by a great content of many specific VOCs.

Keywords: cow, dairy product, volatile organic compound, SPME/GC-MS

Introduction
The sensory aspects of food are useful in the market for discriminating different products. Volatile Organic Compounds (**VOCs**) are descriptors of the sensory quality traits of food related to the flavour (Liaw et al., 2011). Several works have been published on the effect of animal diets, type of grass, and botanical composition of pastures on VOCs and sensory proprieties of dairy products (**DPs**) (Carpino et al., 2004; Bovolenta et al., 2014). In addition, differences in the sensory traits of cheeses obtained using milk either from animals grazing on highland or lowland pastures have been reported (Martin et al., 2005; Coppa et al., 2011). In particular, grazing of cows on pastures provides milk with odour-active compounds that may be responsible for the specific aroma of milk and cheese (Carpino et al., 2004). Recent studies have been focused in quantifying the effect of different dairy systems, individual animal characteristics, farm altitude and quantity of concentrate in the diet, on VOCs of the cheese (Bovolenta et al., 2014; Bergamaschi et al., 2015). Concerning the volatile profile of cream and ricotta, as well as of by-products of the cheese-making process (like whey and scotta), the literature is scarce. The aim of this work was the characterization of VOCs in different DPs collected from an artisanal cheese- and ricotta-making obtained in a temporary farm during summer transhumance in Alpine pastures.

Material and methods
The experiment was carried out from June to September at Malga Juribello (Trento, Italy

1,860 m above sea level). Details about pasture, cow' productive traits, cheese-, and ricotta-making were reported in Bergamaschi et al. (2016). Briefly, 148 cows belonging to different breeds were grazed on pasture day and night. The feeding strategy was pasture-based supplemented with a compound feed given twice daily during the milking. A total of 7 cheese-making were performed in the small dairy of the temporary farm processing the bulk milk collected every 2 weeks according to the traditional artisanal procedure used for Malga cheese production. Raw whole milk (250 L) from the evening milking was collected in an open flat tank to permit the natural creaming. The following morning the cream was separated from milk and the skimmed milk was transferred into a vat and mixed with 250 L of freshly collected morning whole milk. The milk in vat (500 L) was heated to 27°C, inoculated with 250 g of full fat yogurt composed of pasteurized milk, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* (Latte Trento, Trento, Italy), and rennetted adding 25 g of commercial rennet (Naturen extra 1,030 NB, 1,030 IMCU/g; Chr. Hansen A\S, Denmark). The curd was cut, cooked at 45°C, put in moulds, pressed, salted, and ripened for 6 and 12 months. After that, the whey was transferred into a smaller vat and heated to 90°C, then 0.750 L of vinegar was added to catalyse the coagulation. The resulting ricotta was separated, weighted, and placed into mould. A total of 11 DPs were collected from each of the 7 cheese-making session: 4 types of bulk milk (whole evening milk, naturally creamed evening milk, whole morning milk, and milk mixed in the vat); 3 fresh products (cream, curd, ricotta); 2 by-products (whey, scotta); and 2 ripened cheeses (6 and 12 months). Analyses of the chemical traits of the 11 DPs were discussed in details in the previous paper (Bergamaschi et al., 2016). Volatile Organic Compounds were measured by SPME/GC-MS at Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy). For the extraction of VOCs the method used was a modified version of procedure described by Bovolenta et al. (2014). Briefly, 3 g of each of the 11 DPs were placed in glass vials (20 mL, Supelco, Bellefonte, PA) adding 2 g of sodium chloride (Aldrich, Milan, Italy), 4 mL of bi-distilled water, 50 µl of a solution of one internal standard (4-methyl-pentan-2-one = 0.0049 mg/mL, Aldrich, Milan, Italy). Each sample was measured in triplicate. The oven temperature of the GC was programmed at: 40°C for 3 min, 40 to 180°C at 4°C/min, 180°C for 6 min, and 180 to 220°C at 5°C/min. Results are expressed as µg/kg equivalent to the internal standard (4-methyl-pentan-2-one). The relative concentration in µg/kg of each VOC plus 1 was expressed as a ln to obtain a Gaussian-like and processed using a mixed model: $y_{ijk} = \mu + DP_i + date_j + e_{ijk}$. Where y_{ijk} is the VOCs content; μ is the overall mean; DP_i is the fixed effect of the i^{th} dairy product (i = 1 to 11); $date_j$ is the random effect of the j^{th} cheese-making session (j = 1 to 7); e_{ijk} is the residual random error term $\sim N(0, \sigma^2)$. In addition, orthogonal contrasts were used to evaluate the effect of milk treatments, the differences among the fresh products, the nutrient depletion of milk, and ripening period of cheese.

Results and discussion

A total of 49 VOCs were detected by SPME/GC-MS and belong to the following chemical classes: alcohols (13), aldehydes (9), esters (8), free fatty acids (6), ketones (5), lactones (2), sulphurs (2), terpenes (2), phenol (1) and benzene (1). As shown in Table 1 the evening milk after natural overnight creaming presented a relative larger amount of VOCs than evening whole milk. However, this pattern was not observed for all the chemical classes of VOCs. In fact, the decrease regarded "other" compounds (sulphurs, terpenes), while alcohols and aldehydes increased, and fatty acids and ketones were not affected as a whole. These differences may be related to the time (overnight) spent for natural creaming and related metabolic and microbial activities in milk (Gatti et al., 2014). The milking (evening *vs* morning) yielded milk with a similar relative content of VOCs and also a similar quantity of different chemical classes of compounds, with the only exception of the smaller relative content of ketones in the morning milk than in the evening one (Table 1). The different environmental light and temperature can modify the circadian rhythms of the cows,

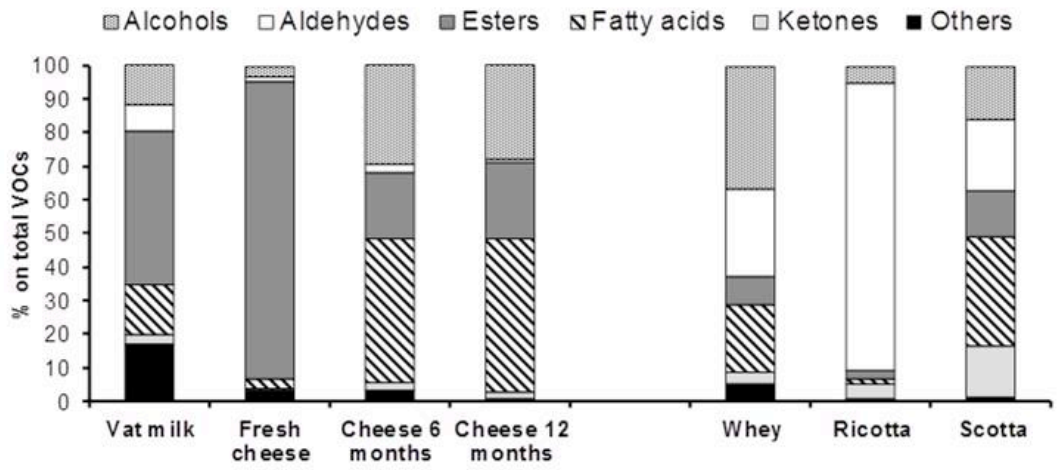
physiological state, foraging behaviour, and their productive performances (Das et al., 2016). In our study, overall the relative VOC content of milk mixture obtained in the vat from evening skimmed milk and whole morning milk was not different from intermediate expected value. The relative abundance of VOCs were much greater in fresh products (cream, fresh cheese, ricotta) than in milk, and also very different among them (*data not shown*). Esters and fatty acids were the 2 chemical classes more abundant in terms of relative quantity in fresh DPs. Cream, in contrast to the other 2 fresh DPs, shown higher content of fatty acids, sulphurs, and terpenes, while the ricotta shown higher proportions of aldehydes than the other DPs. The progressive depletion of milk nutrients in the dairy fluids, especially fat and protein, during cheese- and ricotta-making affects the VOCs belonging to different chemical families. The VOC profile of milk was composed by similar VOCs of whey, while scotta had some esters not present in milk and whey (*data not shown*). The proportions of VOCs across the cheese- and ricotta-making chain are summarized in Figure 1. The percentage of the quantity of VOCs was estimated multiplying the weight of the DPs sampled during the 7 cheese-making session and the VOCs concentrations in µg/kg equivalent to the internal standard. The VOC profile of vat milk before starter and rennet additions was mainly composed by esters (45.6%), while the fresh cheese produced was characterized by a ester proportion almost twice than those in the milk vat. At the end of cheese-making the proportion of alcohols and aldehydes of the whey was 24.9% and 18.3% more than the proportion in vat milk. Moving to the ricotta-making steps, it is possible to see from Figure 1 that the proportions of esters, fatty acids and ketones in the residual scotta were 5.5%, 12.2%, and 11.7% more than the proportions in whey before its heating and acidification, and that the VOC profile of ricotta was mainly composed by aldehydes (85.6%). The third step, cheese ripening for 6 and 12 months, caused a modification of the proportions of VOCs that increased during the first 6 months of ripening mainly in terms of alcohols (+26.5%) and fatty acids (+39.6%), while the increase during the following 6 months was slightly lower. Bovolenta et al. (2014) demonstrated that a nutrient-rich pasture lead to an increase of the proportion of several alcohols and fatty acids characterizing the VOC profile of Montasio cheese. The results in the Figure 1 shown the complex evolution of VOCs in DPs allowing to see the partition of each chemical family in different DPs, the disappearance, and especially the appearance of the different VOCs as a result of milk native enzymatic actions, microbiological activity, physical treatments, and interactions among these factors. In Mediterranean area of production, Martin et al. (2005) in a study regarding the dairy products, suggested also an effect of nature of forages and technological factors on sensory properties of cheese.

Table 1: Effect of natural creaming, milking, and mixing on volatile organic compounds (VOC) and their chemical families expressed as natural logarithm of concentration (µg/kg).

Chemical family	*Milk LSM:*				Date (%)[2]	RMSE[3]	*Effect (P-value)*		
	Evening Whole *(A)*	Evening skimmed *(B)*	Morning whole *(C)*	Mixture (Vat)[1] *(D)*			Creaming[4] *AvsB*	Milking[5] *AvsC*	Mixing[6] *Dvs(B+C)*
∑VOC	4.61	4.23	4.65	4.36	2.2	0.37	**	ns	ns
Alcohols	1.85	2.04	1.89	2.44	31.3	0.24	**	ns	***
Aldehydes	1.65	2.20	1.56	1.94	27.3	0.33	***	ns	ns
Esters	3.78	3.14	3.97	3.43	0.1	1.04	ns	ns	ns
Fatty acids	2.40	2.34	2.56	2.49	0.2	0.47	ns	ns	ns
Ketones	1.33	1.33	1.07	1.21	38.8	0.17	ns	***	ns
Others VOC	2.87	2.44	2.87	2.66	35.4	0.25	***	ns	ns

[1]It is obtained mixing the evening skimmed milk with the morning whole milk in equal part; [2]Incidence of cheese-making date calculated dividing the variance of cheese-making on total variance; [3]RMSE= root means square error; [4]Contrast between whole milk from the evening milking *vs* evening skimmed milk; [5]Contrast between whole milk from the evening milking *vs* whole milk from the morning milking; [6]Contrast between milk collected from the vat *vs* the mean of the milk from morning milking and evening skimmed milk.

Figure 1: Proportions of Volatile Organic Compounds (VOCs) in the headspace of different dairy products obtained from artisanal cheese- and ricotta-making.



Conclusions

The volatile organic compounds (VOCs) of whole evening milk, skimmed milk, whole morning milk, milk in vat, cream, fresh cheese, whey, ricotta, scotta, and ripened cheeses were analysed by SPME/GC-MS and characterized the flavour of dairy products obtained from artisanal cheese- and ricotta-making carried out on Alpine pastures. The evolution of VOCs depends by specific technological aspects of cheese- and ricotta-making processing such as natural creaming, temperature, and ripening period. We conclude that distinctive VOCs can be used as markers of product and process and that the summer Alpine transhumance influences the quality of dairy products within the same cheese-making process.

Acknowledgements. The authors would like to thank Flavia Gasperi, Franco Biasioli, Emanuela Betta, and Eugenio Aprea (Centre and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy) for SPME/GC-MS analysis.

References

Bergamaschi M., Aprea E., Betta E., Biasioli F., Cipolat-Gotet C., Cecchinato A., Bittante G., Gasperi F. 2015. J. Dairy Sci., 98: 2183-2196.
Bergamaschi M., Cipolat-Gotet C., Stocco G., Valorz C., Bazzoli I., Sturaro E., Ramanzin M., Bittante G. 2016. J. Dairy Sci., 99: 9631-9646.
Bovolenta S., Romanzin A., Corazzin M., Spanghero M., Aprea E., Gasperi F., Piasentier E. 2014. J. Dairy Sci., 97: 7373-7385.
Carpino S., Mallia S., La Terra S., Melilli C., Licitra G., Acree T. E., Barbano D. M., Van Soest P. J. 2004. J. Dairy Sci., 87: 816-830.
Coppa M., Verdier-Metz I., Ferlay A., Pradel P., Didienne R., Farruggia A., Montel M. C., Martin B. 2011. Int. Dairy J., 21: 815-822.
Das R., Sailo L., Verma N., Bharti P., Saikia J. 2016. Vet. World., 9:260.
Gatti M., Bottari B., Lazzi C., Neviani E., Mucchetti G. 2014. J. Dairy Sci., 97: 573-591.
Liaw I., Miracle R. E., Jervis S. M., Listiyani M. A. D., Drake M. A. 2011. J. Food Sci., 76: 1188-1194.
Martin B., Verdier-Metz I., Buchin S., Hurtaud C., Coulon J.B. 2005. J. Anim. Sci., 81: 205-212.

Characterization of the non-genetic causes of variation of bovine milk calcium concentrations in French farms

P. Gaignon [1-2]*, C. Hurtaud[1], M. Gelé [3], A. Boudon[1]
[1] PEGASE, Agrocampus Ouest, INRA, 35590, Saint-Gilles, France
[2] CMI, 18 avenue F.Roosevelt, BP80139, 35400, Saint-Malo, France
[3]Institut de l'Elevage, 49105, Angers, France
*pierre.gaignon@inra.fr

Abstract
Our objective was to identify and quantify non-genetic factors of variation in CaC (Calcium Concentration) in milk samples collected from a significant pool of French dairy farms. This study was based on program PhénoFinLait that consisted in a survey performed between 2009 and 2010 in about a thousand dairy farms located in the major French milk production areas, with three breeds: Holstein, Normande and Montbeliarde. Information about cows' diet was gathered and individual milk samples were collected in order to extract their mid-infrared (MIR) spectra. More than 200,000 MIR spectra were measured. We estimated CaC in milk samples from their MIR spectra. From the composition of the cow diets collected, we characterized 7 feeding strategies. The feeding strategy affected milk Ca with the constant fact that the diets based on fresh or conserved grass induced lower milk CaC regardless of the month of the year ($p<0.05$). The difference in CaC can be up to 100 mg/kg between two extreme diets at a given month, which was as important as the drop in CaC observed at the beginning of lactation. This study reinforced the idea that the diet of cows has an influence on milk CaC.

Keywords: calcium, milk, feeding strategies

Introduction
The main factors of variation of calcium concentration of cow milk reported in the literature are the genetics of the cow and their stage of lactation (Alais, 1984; van Hulzen *et al*., 2009). However, milk calcium concentration (CaC) is considered to be little affected by cow's diet, diets low in calcium driving to decreased milk production rather than milk CaC reduction(Alais, 1984; Suttle, 2010). However, experiments run at the UMR PEGASE showed that milk CaC can vary significantly according to the nature of the diets (Hurtaud *et al*., 2013). The aim of this study was to evaluate the effect of the cow feeding strategy on the annual dynamics of milk CaC content in several areas of France with the 3 main breeds i.e. Holstein, Montbeliarde and Normande. We assumed that cow's diet affects CaC in bovine milk but may not be the only explanation of the seasonal variation of milk CaC content.

Material and methods
Field prospection
The data were issued from the program PhénoFinLait, which consisted in a survey performed through the major areas of milk production in France. Between October 2009 and October 2010, 945 farms were enquired. During this period, several visits (5 on average) were performed in each farm, to follow evolution of herd diets during a complete year. During each visit, interviewers collected data about dairy cows (parity, stage of lactation, stage of gestation, etc…) and their diet (description of the composition of the diets based on 54 variables). They also collected individual milk samples and MIR spectra were measured for a part of the herd (Foss FTS).Within each enquired farms, interviewers selected as much as possible the same cows, visit after visit. The survey resulted into 252,519 milk spectra from 63,818 dairy cows, divided between the 3 main breeds in France: Holstein, Montbeliarde and

Normande, spread over 13 departments. Program PhénoFinLait has been fully described by Gelé *et al.* (2014).

Prediction of milk calcium using MIR spectrum

A prediction equation, specific to our study, was estimated to predict CaC in milk from MIR spectra. To achieve this, milk calcium contents of 495 frozen milk samples taken from the bank of samples of program PhénoFinlait were analyzed by atomic absorption spectrometry after diluting the samples with nitric acid (Brûlé *et al*., 1974). Those samples were chosen to represent the diversity of the whole bank of samples of program PhénoFinLait (parity, lactation stage, breed, department, cow diet, etc.…). The calibration was performed with a partial least square regression using the PLS procedure of SAS.

Characterization of feeding strategy

For each visit in each farm, a mean diet was estimated by averaging the proportions of each feed in the diet. Feeding strategies of farms were characterized over 3 periods: winter season (from 15th November to end of March), early summer season (from 1st April to 15th June), and late summer season (from 16th June to 15th October). Only farms that were investigated every season were used to characterize the feeding strategies. As a feeding strategy consisted in a characterization of the evolution of the diet over the year, each season had to bring the same amount of information. A multiple factor analyses (MFA) was therefore performed to characterize feeding strategies (Escofier and Pagès, 1994) with R (R Core Team, 2013) and package *FactoMineR*. A MFA is a generalization of principal component analysis for comparison of multiple data tables (Abdi *et al*., 2013). An ascending hierarchical classification was then performed on the factor scores using package *FactoMineR*.

Factors of variations of milk calcium concentration

An ANOVA with a mixed model was performed using PROC MIXED with SAS to characterize factors of variations of CaC. The selected model was:

Milk Calcium Content= Month of Lactation + parity + Calendar Month*Feeding Strategy + Individual +ε,

where all explanatory variables were considered as qualitative factors. All explanatory variables were included as fixed factors, excepting "Individual" factor that was a random factor. The model was run independently for each breed. Within breed, some strategies were removed when they had not enough data or when they were unbalanced throughout the year.

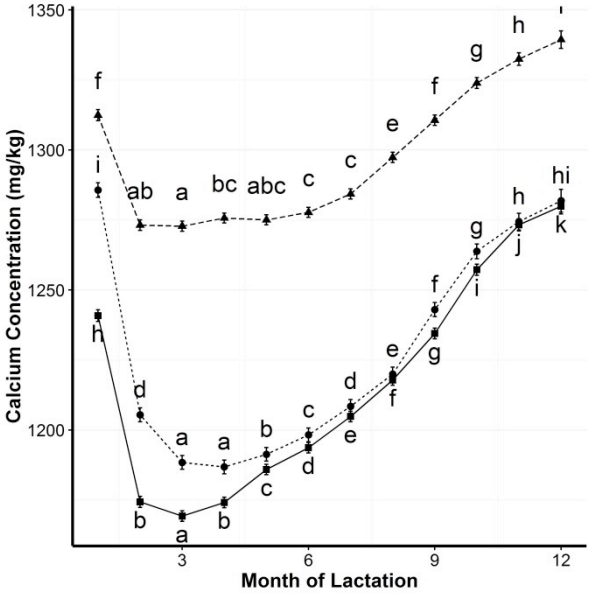


Figure 2 : Effect of stage of lactation on calcium Concentration

●: Montbeliarde, ▲: Normande, ■: Holstein. Letters are results of post-Hoc and group difference analyses for each breed. (Adjusted means corrected from all effects included in the model except the stage of lactation)

Result and discussion

Milk CaC was clearly affected by the stage of lactation for the 3 considered breeds ($p < 0.05$, Figure 1). CaC decreased sharply after the first month and lowest values were observed between 2nd and 5th month of lactation. From the 5th month, CaC increased gradually until the end of lactation. Milk CaC was higher for Normande compared with Montbeliarde or Holstein, with lower range of variation during lactation. Milk CaC was remained higher for Montbeliarde compared with Holstein, with similar dynamics during the lactation.

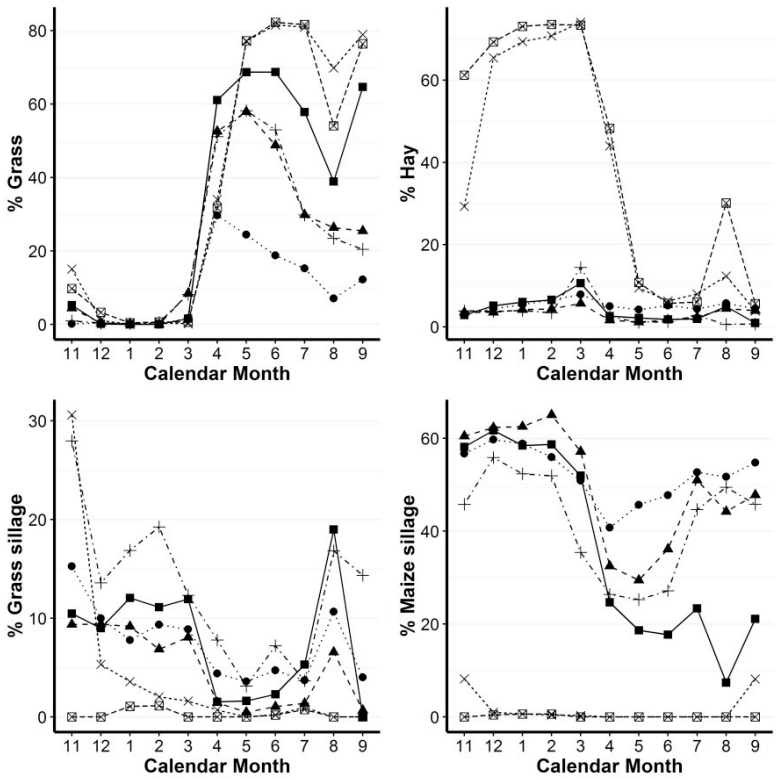


Figure 2: Evolution of main forages for the 7 feeding strategies during the investigated period; Grazing& FC Hay: ×, Grazing & BD Hay:☒, Max Grazing: ■, Grazing & Maize Silag : ▲, Maize Silage: ●, Grazed temp pasture:+

The MFA and classification resulted into 7 feeding strategies: The nature of the forage used was the major factor of strategy characterization. Strategies were named according to the relative importance of the main forages and the distribution of their contribution to the diet during the year (Figure 2). The strategies '*Grazing & FC Hay*' and '*Grazing & BD Hay*' represented feed systems based on grazed pasture during spring and summer and on field cured hay (FC) or barn-dried (BD) hay in winter. The strategy '*Max Grazing*' consisted in a maximal use of grazing in spring and summer, and in diets based on maize silage in winter. The strategy '*Grazing and Maize Silage*' was based on maize silage but with a part of grass when possible. The strategy '*Maize Silage*' was based on this forage for all seasons. The strategy '*Grazed temp pasture*' was based on non-permanent pasture for spring and summer and on maize silage in winter.

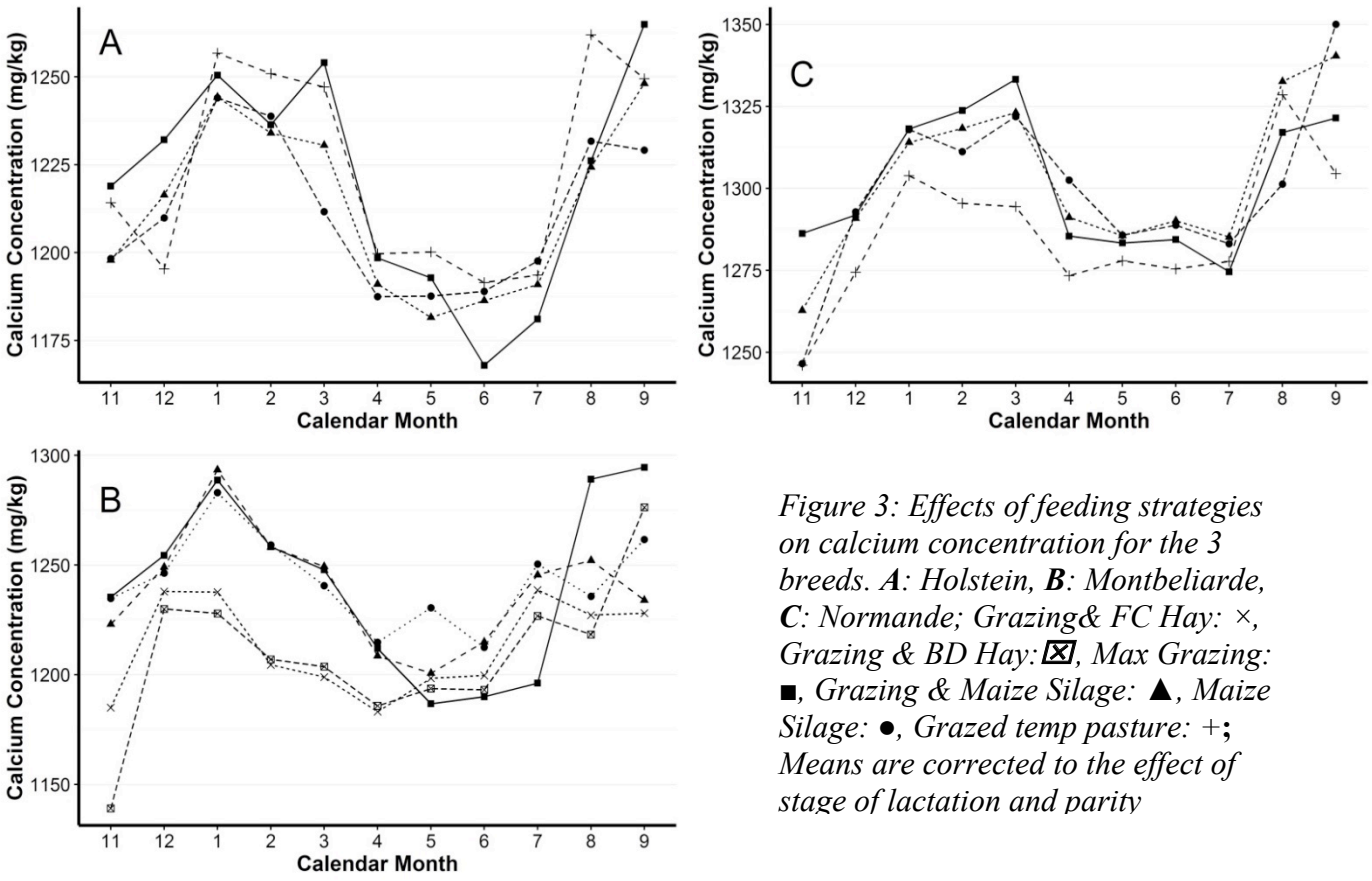


*Figure 3: Effects of feeding strategies on calcium concentration for the 3 breeds. **A**: Holstein, **B**: Montbeliarde, **C**: Normande; Grazing& FC Hay: ×, Grazing & BD Hay:☒, Max Grazing: ■, Grazing & Maize Silage: ▲, Maize Silage: ●, Grazed temp pasture: +; Means are corrected to the effect of stage of lactation and parity*

When performing the analyses of the effect the feeding strategies on the milk CaC, the data of strategies under-represented, i.e. less than 2,000 data within strategy and breed, or unevenly distributed throughout the year had to be removed for some breed. The strategies '*Grazing & FC hay*' and '*Grazing & BD Hay*' were only kept for Montbeliarde because they were underrepresented within Hostein and Normande. Indeed, those 2 feeding strategies were very specific to the areas of production where Montbeliarde is the predominant breed. Similarly, the strategy '*Grazed temp pasture*' was under represented within Montbeliard breed because it was very specific to the west of France where Montbeliarde is less represented.
The feeding strategy clearly affected the annual dynamics of milk CaC (Figure 3; effect of the interaction calendar month x feeding strategy: $p < 0.05$). CaC in milk has a huge drop in spring for each breed, i.e. between March and May. This could be related to the fact that for most of strategies, cows started grazing at this period. For Holstein, considering the summer months, CaC was higher for strategies based on higher proportion of maize silage. For Montbeliarde, the difference between strategies was more marked in winter, with higher CaC with strategies based on maize silage, and lower CaC with those based on hay. The difference in CaC between both types of strategies was high up to 100 mg/kg more calcium in milk obtained with strategies based on maize silage rather than hay (Graph B, Figure 3). For Normande, the strategy effect was less clear that for the 2 others breeds.
When considering the 3 breeds, the variability linked to the diet was important, with about 150 mg/kg of difference over the year between strategies. This difference was comparable to the maximal difference in CaC between the 3 breeds within a same feeding strategy and a similar stage of lactation.

Conclusions

This study clearly showed that the feeding strategy of the dairy system affected the annual variation of milk CaC. Variations in CaC seemed to be, at least partially, explained by the nature of the forages fed to the cows. Maize silage led to higher CaC in milk, in comparison with grass or hay. This was observable when comparing the strategies within breed and when considering the drop of CaC observed when cows started grazing in spring. The nature of the forage fed to the cows seems to be a factor explaining an important part of the variability of CaC, comparable to the variability explained by breed.

Acknowledgements

The *PhénoFinLait* project has received financial support from ANR (ANR-08-GANI-034 Lactoscan), APIS-GENE, CASDAR, CNIEL, FranceAgriMer, France Génétique Elevage and the French Ministry of Agriculture.

References

Abdi H., Williams L.J., Valentin D. 2013. Wiley Interdiscip. Rev. Comput. Stat. 5: 149-179.
Alais C. *Science du lait : principes des techniques laitières*.1984. Edition SEPAIC.
Brûlé G., Maubois J.L., Fauquant J. 1974. 54: 600-615. Escofier, B., and J. Pagès. 1994. Comput. Stat. Data Anal. 18: 121-140.
Gelé M., Minery S., Astruc J.-M., Brunschwig P., Ferrand-Calmels M., Lagriffoul G., Larroque H., Legarto J., Leray O., Martin P., Miranda G., Palhiere I., Trossat P., Brochard M. 2014. INRA Prod. Anim., 27: 255-268.
Van Hulzen K.J.E., R.C. Sprong, R. van der Meer, J. a. M. van Arendonk. 2009. J. Dairy Sci., 92:5754-5759.
Hurtaud C., M. Dutreuil, M. Coppa, C. Agabriel, B. Martin. 2013. Dairy Sci. Technol., 94: 103-123.
Suttle N.F. *Mineral Nutrition of Livestock*. 2010. CABI.

New insights on microbiota, from the environment of the farm to the cheese

M. Frétin[1,2], F. Irlinger[3], I. Verdier-Metz[1], A. Ferlay[2], B. Martin[2], M.C. Montel[1], C. Delbès[1]*
[1]UMR 545 Fromage, UCA, INRA, F-15000 Aurillac, France
[2]UMR 1213 Herbivores, INRA, F-63122 Saint-Genès-Champanelle, France
[3]UMR 782 GMPA, INRA-AgroParisTech, F-78850 Thiverval Grignon, France
*celine.delbes@inra.fr

Abstract
"Metagenomic" approaches are revolutionizing food microbial ecology and open new perspectives to investigate the microbial diversity of dairy products. Potential applications of these tools to gain knowledge on environmental reservoirs of microbial diversity for milk and cheese, and on biochemical drivers of cheese microbiota development are presented. Recent studies disclose the role of cow teat skin as a potential source of microbial diversity for milk and cheese, and show that the bacterial assembly from teat skin until ripened cheeses may be modified by the grazing systems. In addition, fat composition was identified as a potential driver of cheese microbial community dynamics. In order to identify drivers of the different dimensions of cheese quality, metagenomic data must be analysed in an integrated approach including production practices, physico- and bio-chemistry data sets, which remains a statistical challenge.

Keywords: microbiota, metagenomics, diversity reservoirs, farming system, biochemical drivers

Introduction
One of the characteristics of traditional cheeses, whether produced from raw or heat-treated milk, is to harbour a complex microbial community, which is also specific of the cheese type considered. The microbiota of raw milk plays a primordial role in the development of cheese flavour, its shelf-life and safety (Montel et al., 2014). It is obviously strongly influenced by the overall management system of the farm and tightly connected with animal rearing and health. The strict hygiene procedures adopted by farmers over the last decades resulted in a drastic loss of diversity of cheese organoleptic qualities. The current main concern of traditional cheese producers is to manage trade-off between safety, microbial diversity and sensory richness. They could rely on the high potential offered by the microbial biodiversity maintained primarily in small production systems, such as those found in mountainous areas. This requires to gain knowledge about the management of microbial resources throughout the cheese process chain. Knowledge of the microbial diversity of milk and cheese was at first derived from culture-dependent methods using different culture media. The recent advances in high-throughput sequencing (HTS) technologies, applied for "metagenomic" approaches, are revolutionizing food microbial ecology, deepening insight into complex fermentation systems. We will summarize potential applications of these new tools to investigate the microbial diversity of dairy products, in particularly to gain knowledge on environmental reservoirs of microbial diversity for milk and cheese, and on biochemical drivers of cheese microbiota development.

New tools for microbial ecology of dairy products
Thanks to the development of HTS and bioinformatics tools, the in-depth characterization of the composition of microbial communities in different matrices is now possible. The DNA-based approach named "metabarcoding" consists in the deep-sequencing of variable regions of the bacterial rRNA gene or of the fungal ITS (Internal Transcribed Spacers). It is the most common HTS application in food microbial ecology (Cocolin and Ercolini, 2015). The DNA sequences generated through HTS are clustered into OTUs (Operational Taxonomic Units)

which are further assigned to microbial species or genera. Proportions of the different OTUs within each ecosystem sample are determined, and can be compared across samples. These new approaches provide a powerful means to analyse dominant and subdominant populations and their dynamics in highly complex ecosystems. They offer the opportunity to monitor microbial populations across different successive habitats. However, they are susceptible to biases related to the fact that DNA can come from dormant or even dead cells. Therefore, some studies choose to sequence rRNA to characterize the active fraction of microbial communities of cheeses since RNA has been suggested as an indicator for metabolically active microbiota (Dolci et al., 2014).
These approaches allowed to reveal more extensively the richness of microbial communities colonizing milk (Oikonomou et al., 2014; Quigley et al., 2013), cheeses (Dugat-Bony et al., 2016), and cheesemaking environment (Bokulich et al., 2013). They showed how cheese microbiota structure can vary according to the origin of the milk and its treatment (pasteurization or not) (Quigley et al., 2012), the type of cheese technology (Wolfe et al., 2014; Dugat-Bony et al., 2016), the occurrence of sensory defect (Quigley et al., 2016), the cheese packaging (Duval et al., 2016), and the cheese-making plant environments (Bokulich and Mills, 2013; Calasso et al., 2015).
In order to identify biotic and abiotic drivers of cheese microbiota, metagenomic data must be associated with metadata that characterize ecosystems in terms of physico- and bio-chemical composition and production practices. However, analysing the relations within such massive and composite data requires appropriate statistical tools and remains challenging.

Farm environments as reservoirs of microbial diversity for milk and cheese
The composition of the milk microbiota depends first on the composition of microbial ecosystems directly in contact with the milk such as the animal's teat canal and surface, the dairy equipment such as milking machine, milk line and tank, and second on various environmental microbial sources which are not directly in contact with the milk such as bedding material, faeces, feed, drinking and washing water, stable and milking parlour air, milker (Montel et al., 2014).
The teat skin of cows is located at the crossroads of environmental microbial sources. It has long been considered as a main reservoir of microbial diversity of raw milk because many bacterial genera found in raw milk were detected on teat skin (Vacheyrou et al., 2011; Verdier-Metz et al., 2012). Recently, using HTS, Doyle et al. (2016) evaluated and ranked possible sources of microbiota for raw milk, with teat surface being the most significant, followed by the faeces. Verdier-Metz et al. (2012) showed that the microbial community of cow teat skin varied from one farm to another. The microorganisms can colonize the teat skin by contact with the bedding material that depends on animal feeding and housing conditions. The composition of animal feeding, particularly the balance between forage and concentrate. is known to affect the structure of the microbial community in the rumen as well as in the faeces (Zened et al., 2013; Kim et al., 2014). On teat skin, higher counts in *Lactobacillus* and *Enterococcus* were associated with a silage-based diet as compared with a hay-based diet (Monsallier et al., 2012). The hygienic practices of farmers during milking (washing of milking equipment, pre- and post-milking teat care practices) can modify the balance of teat skin microbiota (Doyle et al., 2016; Monsallier et al., 2012). The soil surface and phyllosphere microbiota of the grazed paddocks, especially in the areas used by cows for bedding, could also be a major factor influencing the microbial balance of teat skin. Individual dairy cow characteristics, such as lactation number, were found to interact with farming practices to affect the microbial levels on teat skin (Monsallier et al., 2012). However, inter-individual variations on teat skin microbial diversity profiles have not yet been investigated using HTS approaches. The dairy farm environment and the farming practices have become a focus of interest for researchers studying cheese microbial ecology since they may influence the milk microbiota. But to our knowledge, only one recent study

has explored the link between the microbiota of teat skin and the microbiota of raw milk cheeses. In this study by Frétin et al. (2017), we aimed to explore whether the bacterial assembly from teat skin of cows until ripened cheeses may be modified by the grazing systems. For this purpose, we implemented an experiment including two herds from the same farm reared separately for two years in two grazing systems, whose milk was collected and transformed under identical conditions. We investigated the bacterial populations of teat skin, raw milk, cheese rind and core using high-throughput 16S rRNA gene sequencing. We highlighted that 85% of the OTUs detected in milk and 27% of those detected in cheese were previously found on teat skin. For the first time, our results disclose the important role of cow teat skin as a potential secondary source for microbial diversity in cheese. The grazing system markedly affected the microbiota of cow teat skin but the differences were much lower in milks and cheeses. We hypothesize that first, the microbial biofilm on the milking equipment, as well as the starter and ripening cultures contributed to conceal the effect of grazing systems in milk and cheese. Second, the environment and equipment of cheese-making and ripening could further influence the microbiota of raw milk cheeses (Irlinger et al., 2015). Indeed, using metabarcoding, Bokulich and Mills (2013) brought new elements to understand the potential transfers of environmental microorganisms detected on cheese processing equipment to cheese surface. They identified facility-specific "house" microbiota, which may play a role in shaping site-specific product characteristics.

Milk biochemical composition as a driver of cheese microbiota development

Milk and cheese microbiota plays a key-role in the development of cheese sensory properties through enzymatic activities involved in proteolysis and lipolysis, which in return can affect cheese texture and flavour. The effect of animal feeding on the sensory characteristics of cheeses may be explained at least in part by a differential development of the microbiota in response to changes in the biochemical composition of milk.

Several authors showed that the flavour of raw milk cheeses was more intense and diversified when cows grazed fresh grass in comparison to preserved forages, especially maize silage (Bonanno et al., 2013; Martin et al., 2005). These differences have not been fully explained yet. They could be due to fatty acids since milk from cows fed on pasture are richer in unsaturated fatty acids (UFA) compared to milk of cows fed on maize silage. Differences in rind thickness depending on the animal feeding and related to differences in the composition in fatty acids were noticed with different uncooked pressed cheeses (Coppa et al., 2011; Frétin et al., 2017; Lerch et al., 2015). The development of cheese rind is highly connected with the development of the rind microbiota. However, only few studies investigated the interaction between milk fat component and cheese microorganisms. Several authors showed that reducing cheese fat content had rather adverse effects on bacterial growth, but inconsistent results were obtained regarding starter LAB (Broadbent et al., 2013; Porcellato et al., 2013). Using a culture-dependent approach, Buchin et al. (1998) analysed uncooked pressed cheeses produced from skimmed milk added with fat issued from either pasture or hay diets, and found no difference in the levels of the main microbial groups.

In a recent study by Frétin et al. (2017), we aimed to evaluate the role of milk fat on the dynamics of bacterial and fungal communities in cheese, using HTS metabarcoding approach. We set up an experimental design based on two groups of cows fed either grazed grass or maize silage that produced milks with very different fatty acid profiles. Cantal cheeses made from skimmed milk added with maize cream had a thicker rind than those made with pasture cream. We suggested that the higher concentration of polyunsaturated free fatty acids (such as c9t11-CLA and C18:3n-3) found on "pasture" cheese rind could have an antimicrobial effect and therefore explain their thinner rind. The relative abundance of fungal OTUs corresponding potentially to strains added as starters, such as *Sporendonema casei,* was lower on "pasture" cheese rind. When added to cheese, this mould is considered the main strain involved in Cantal cheese rind development. Weaker differences were observed in pasteurized

cheeses, which suggest an interaction between fat components, starter strains and the native microbiota. For the first time, our results showed that the milk fat composition is able to drive the microbial community dynamics in cheese. In this specific study, the interactions evidenced between chemical and microbial composition of milk and cheese are limited to raw cheese rind with high free fatty acid content (high lipolysis). Nevertheless, these results pave the way to further developments dedicated to the comprehension of the influence of animal diets on cheese sensory properties.

In particular, differences between sensory properties of cheeses derived from animal grazing grasslands with different botanical compositions are frequently reported (Martin et al., 2005). The possible influence of grassland botanical composition on cheese flavour is often attributed to the direct transfer from forages to milk and cheese of plant secondary metabolites such as terpenoids (Viallon et al., 2000). These compounds vary widely according to the botanical species composition of the grasslands (Mariaca et al., 1997) and it was suggested that they could influence the development or activity of cheese microbiota because of their antimicrobial properties (Martin et al., 2005). Indeed, relations between cheese terpenoids and specific sensory traits (Buchin et al., 1999; Bendall et al. 2001) have been suggested but never understood. In this field, the use of HTS technologies could help understanding the possible influence of milk terpenoids on cheese microbiota and therefore on the development of flavour of ripened cheeses.

Conclusions

Metagenomic approaches provide a powerful means to characterize microbial diversity. Combined with data on farming and production practices, metagenomic data offer the opportunity to identify biotic and abiotic drivers of cheese microbiota. However, a particular effort will have to be made to develop statistical approaches for the analysis of massive and composite data sets. Further researches are needed on environmental reservoirs of microbiota, such as phyllosphere and soil surface, to better understand how the microbiota of these reservoirs assemble from cow teat skin to cheeses. Questions are opened on the biotic drivers of microbiota development, in relation to the interactions of the microbial populations with each other but also with their animal host. Also, the effect of changing milk biochemical components, modulated by animal diet, on the development of the microbiota in cheese would deserve further investigation.

References

Bonanno A., Tornambè G., Bellina V., De Pasquale C., Mazza F., Maniaci G., Di Grigoli A., 2013. J. Dairy Sci., 96: 710-724.

Broadbent J.R., Brighton C., McMahon D.J., Farkye N.Y., Johnson M.E., Steele J.L. 2013. J. Dairy Sci., 96: 1-11.

Bokulich N.A., Mills D.A. 2013. Appl. Environ. Microbiol. 79, 5214-5223.

Buchin S., Delague V., Duboz G., Berdague J.L., Beuvier E., Pochet S., Grappin R. 1998. J. Dairy Sci., 81: 3097-3108.

Calasso M., Ercolini D., Mancini L., Stellato G., Minervini F., Di Cagno R., De Angelis M., Gobbetti M. 2015. Food Microbiol., 54: 115-126.

Cocolin L., Ercolini D. 2015. Curr.Opin. Food Sci., 2: 43-50.

Coppa M., Verdier-Metz I., Ferlay A., Pradel P., Didienne R., Farruggia A., Montel M.C., Martin B. 2011. Int. Dairy J., 21: 815-822.

Dolci P., De Filippis F., La Storia A., Ercolini D., Cocolin L. 2014. Int. J. Food Microbiol., 185: 127-135.

Dugat-Bony E., Garnier L., Denonfoux J., Ferreira S., Sarthou A.S., Bonnarme P., Irlinger F. 2016. Int. J. Food Microbiol., 238: 265-273.

Duval P., Chatelard-Chauvin C., Gayard C., Rifa E., Bouchard P., Hulin S., Picque D., Montel M.C. 2016. Int. Dairy J., 56: 198-207.

Doyle C.J., Gleeson D., O'Toole P.W., Cotter P.D. 2017. Appl. Environ. Microbiol., 83 (2).
Frétin M., Ferlay A., Verdier-Metz I., Fournier F., Montel M.-C., Farruggia A., Delbès C., Martin B. 2017. Inter. Dairy J., 64: 56-67.
Kim M., Kim J., Kuehn L.A., Bono J.L., Berry E.D., Kalchayanand N., Freetly H.C., Benson A.K., Wells J.E. 2014. J. Anim Sci., 92: 683-694.
Mariaca R.G., Berger T.F.H., Gauch R., Imhof M.I., Jeangros B., Bosset J.O. 1997. J. Agric. Food Chem., 45: 4423-4434.
Lerch S., Ferlay, A., Graulet B., Cirié C., Verdier-Metz I., Montel M.C., Chilliard Y., Martin B. 2015. Int. Dairy J., 51: 65-74.
Martin B., Verdier-Metz I., Buchin S., Hurtaud C., Coulon J.-B. 2005. Anim. Sci., 81: 205-212.
Monsallier F., Verdier-Metz I., Agabriel C., Martin B., Montel M.C. 2012. Dairy Sci. Tech., 92: 265-278.
Montel M.-C., Buchin S., Mallet A., Delbes-Paus C., Vuitton D.A., Desmasures N., Berthier F. 2014. Int. J. Food Microbiol., 177: 136-154.
Porcellato D., Østlie H.M., Brede M.E., Martinovic A., Skeie S.B. 2013. Int Dairy J., 33.2 104-111.
Quigley L., O'Sullivan O., Beresford T.P., Ross R.P., Fitzgerald G.F., Cotter P.D. 2012. Appl. Environ. Microbiol., 78: 5717-5723.
Quigley L., McCarthy R., O'Sullivan O., Beresford T.P., Fitzgerald G.F., Ross R.P., Stanton C., Cotter P.D. 2013. J. Dairy Sci., 96: 4928-4937.
Quigley L., O'Sullivan D.J., Daly D., O'Sullivan O., Burdikova Z., Vana R., Beresford T.P., Ross R.P., Fitzgerald G.F., McSweeney P.L.H., et al. 2016. mSystems 1, e00023-16.
Vacheyrou M., Normand A.-C., Guyot P., Cassagne C., Piarroux R., Bouton Y. 2011. Int. J. Food Microbiol., 146: 253-262.
Verdier-Metz I., Gagne G., Bornes S., Monsallier F., Veisseire P., Delbès-Paus C., Montel M.-C. 2012. Appl. Environ. Microbiol., 78: 326-333.
Viallon C., Martin B., Verdier-Metz I., Pradel P., Garel J.P., Coulon J.B., Berdagué J.L., 2000. Lait. 8: 12–16.
Wolfe B.E., Button J.E., Santarelli M., Dutton R.J. 2014. Cell., 158: 422-433.
Zened A., Combes S., Cauquil L., Mariette J., Klopp C., Bouchez O., Troegeler-Meynadier A., Enjalbert F. 2013. FEMS Microbiol. Ecol., 83. 504-514

Microbiological characteristics of Trachanas, a traditional fermented dairy product from Cyprus

D. Bozoudi, M. Agathokleous, I. Anastasiou, P. Papademas, D. Tsaltas*

Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3036 Limassol, Cyprus
*dimitris.tsaltas@cut.ac.cy

Abstract

The purpose of this study was to characterize the autochthonous microbiota of Cypriot Trachanas, a traditional fermented ewes' milk product. For this, 12 samples of raw and fermented milk, as well as natural starter culture were collected, in order to count, isolate and identify the main species present, during Trachanas fermentation. In total, 163 colonies were retrieved and identified by sequencing analysis at species level. Lactic acid bacteria (LAB) were the predominant group, followed by yeasts. *Lactococcus*, *Lactobacillus* and *Enterococcus* were frequently isolated from raw milk, and *Lactobacillus casei/paracasei* predominated in the starter culture. *Lactococcus lactis* was isolated in high frequency (27.9% of the isolates) at the beginning, while *Lactobacillus* spp. (20%) and *Saccharomyces unisporus* (17.9%) at the end of fermentation. After assessing their technological potential, selected strains could be used as starters to ferment milk for artisanal Trachanas production.

Keywords: Cypriot Trachanas, LAB, yeasts, 16S rRNA gene sequencing, ITS

Introduction

Trachanas is a dry mixture of fermented milk and crushed grain of wheat, produced in Cyprus and is the most known and characteristic traditional product of the country. Trachanas have high nutritive value, low pH and sour taste. Lactic acid bacteria (LAB) and yeasts are responsible for milk acidification, as well as for the formation of several volatile odour compounds (Carpino *et al*., 2010).The milk used for Trachanas production is primarily of sheep, although goat and mixtures of them are also common. The fermentation is initiated by autochthonous milk microbiota, although traditional yogurt is used in warmer areas as a starter, to avoid technological and safety problems.

It is well known that the typicality of traditional dairy products is linked mainly to the microbes originating from the milk (Berthier *et al.,* 2001). The biodiversity of these microorganisms could therefore be considered as a fundamental factor for the features and quality of these artisanal products (Morandi *et al.,* 2011). Raw milk's microbiota is known to be an expression of the local ecosystem and that it can have an impact on the characteristics of the final product (Scintu & Piredda, 2007). Since the typicity is linked to the "terroir", it realizes and expresses the effect of the "terroir" on a product, distinguishing the product linked with this territory from similar ones produced elsewhere. Traditional Cypriot Trachanas is produced with raw sheep locally produced milk, which means that the product is closely linked to the ecosystem of the production zone.

The objective of this study was to count, isolate and identify those microorganisms which are related to milk fermentation for Trachanas production, in order to be further studied and selected as starters. Changes in microbiological counts and composition of Trachanas during fermentation, were studied. Progress of fermentation for both the inoculum batch as well as of the final used fermented milk, will be presented.

Material and methods

The samples were taken from a small, family-owned facility, at Paphos district, Cyprus. Twenty liters of inoculum (starter culture), prepared from naturally fermented ewes' milk,

was used to initiate the fermentation of 380 L of raw milk. Samples of raw and fermented milk, as well as the inoculum, were collected at four consecutive weeks at midsummer. A subsample of 1 L was collected for a time period of 5 days, before the addition of broken wheat and the boiling of Trachanas. The samples were evaluated for total aerobic bacteria, LAB, yeasts and moulds counts. Totally, 163 isolates were retrieved randomly from raw milk, fermented milk during 5 days and the inoculum, from all media used. The identification of the isolates was performed by sequencing the 16S rRNA gene (Luo *et al.,* 2010) and the ITS region (Tristezza *et al*., 2014) for bacteria and yeasts, respectively, and comparing the data with those from the GenBank database using the BLAST algorithm. The pH of all samples was also determined. Statistical analysis was performed through the implementation of appropriate one-way ANOVA model (SPSS v.15) after a log transformation of bacterial counts.

Results and discussion

The results on microbial counts in raw milk and traditional starter samples are presented in Table 1. LAB constituted the predominant microbiota of the raw milk (2.99-3.79 $\log_{10}$cfu/ml), lower than those found by Gaya et al., (1999). The mean level of yeasts found in raw milk samples was 2.78 $\log_{10}$cfu/ml, as reported previously (Desmasures *et al.,* 1997; Fadda *et al.,* 2004). It is documented that yeasts occur in raw milk at significant levels (Fleet, 1990), probably due to competitive utilization for the growth substrates by psychrotrophic bacteria of milk, or owing to inhibition by metabolites excreted by bacteria (Viljoen, 2001). LAB counts of the artisanal culture studied were found at high levels (5.48-7.56 $\log_{10}$cfu/ml; Table 1). This artisanal starter culture is composed of fermented milk taken from the previously produced batch when the pH decreases to 3.8, in order to inoculate the new batch. Thus, this natural starter is continuously evolving as an undefined mixture of LAB. During the fermentation period, LAB reached their higher numbers at the day 4, while total aerobic counts and LAB on MRS agar pH 5.7 at day 3. A significant ($P<0.05$) decrease in their population was noticed at the end of the fermentation, probably due to the acidic conditions created by the drastic reduction of pH caused by NSLAB (3.8 pH units; Table 1). Sengun *et al*., (2009) reports that LAB counts and acidity of Turkish Tarhana depends on the availability of fermentative substrates and the fermentation time and temperature used in the production. On the other hand, yeasts kept their high levels ($P<0.05$) until the end of fermentation process (Lazos *et al*., 1993; Daglioglu *et al*., 2002). Despite the increase in yeast numbers, the population of LAB remains constant or continues to increase probably due to a symbiotic effect whereby both populations benefit from the interaction.

In total, 163 bacteria and yeasts colonies were isolated from raw and fermented milk samples, as well as the artisanal starter culture, and submitted to molecular identification (Figure 1). LAB was the predominant group of microorganisms (72.6% of the isolates), followed by yeasts (22.7%). Some other species were sporadically found. The strains under consideration shared 96-100% similarity with the respective species.

From raw milk samples, LAB species (*Lactococcus, Lactobacillus* and *Enterococcus*) were frequently isolated, *Streptococcus* spp. and *Macrococcus caseolyticus* were scarcely found, while no yeasts were isolated (Figure 1). These results are not unusual, since it is well known that LAB, adventitious bacteria and sometimes yeasts, naturally belong to the microbiota of raw milk (Quigley *et al*., 2013). Regarding the starter culture added to raw milk, it seems that the species *Lactobacillus casei/paracasei* predominated over the other microorganisms.

Lactococcus lactis and *Lactococcus* spp. were isolated at high frequency (35% of the isolates) from the fermented milk, which is in accordance with the log counts of LAB in M17 agar that favours the growth of Gram positive catalase negative cocci (Table 1). These bacteria originate from raw milk where naturally occurring (Berthier *et al.,* 2001) and they multiply when they find optimal conditions. Partial substitution of *Lactococcus* sp. microbiota by *Lactobacillus* spp. was undertaken after day 3 (Figure 1).

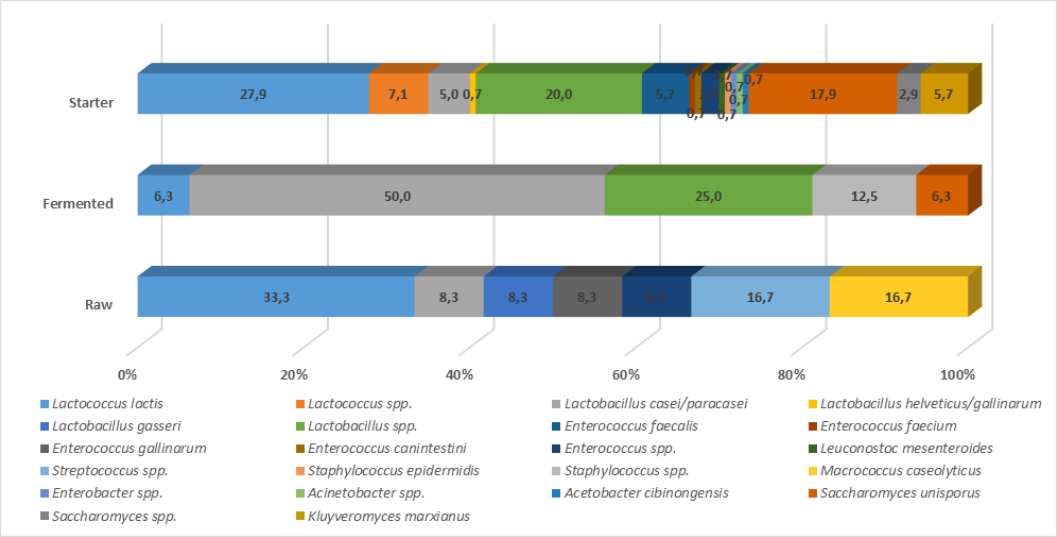
Table 1. Populations (log_{10}cfu/ml)[1] and pH values of raw milk and traditional starter used for Trachana production.

			Fermented milk				
	Raw milk	**Starter**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
LAB on MRS agar pH 6.2	3.79±0.5	7.56±0.4	5.20^{a}±0.2	7.30^{bc}±1.0	7.71^{c}±1.1	7.92^{c}±0.4	6.89^{b}±0.6
LAB on MRS agar pH 5.7	2.99±0.5	7.52±0.3	5.13^{a}±0.2	6.83^{b}±0.8	7.76^{c}±0.4	7.36^{bc}±0.5	6.97^{b}±0.4
LAB on M17 agar	3.84±0.4	5.48±0.7	3.82^{a}±0.5	5.18^{b}±0.4	5.48^{b}±0.2	5.62^{b}±0.7	5.49^{b}±0.3
Yeasts	2.78±0.6	5.23±0.7	3.23^{a}±0.7	3.93^{ab}±0.5	4.98^{bc}±0.9	5.53^{c}±0.8	5.54^{c}±0.7
Total Aerobic Counts	4.66±0.3	8.26±0.1	5.89^{a}±0.2	7.59^{bc}±1.1	8.41^{d}±0.1	8.14^{c}±0.2	7.23^{b}±1.1
pH	6.8±0.10	3.8±0.02	6.7^{a}±0.05	6.6^{a}±0.05	4.3^{b}±0.02	4.2^{b}±0.03	3.8^{c}±0.01

[1] Mean values of four samples counted in duplicate (x ± SD; n = 8)
[a, b, c, d]: Comparison using the LSD criterion between days of fermentation, P<0.05.

Yeasts were retrieved only from the fermented milk. Since no yeasts were isolated from raw milk samples and/or the starter culture, an environmental contamination is implied (Fleet, 1990). *Saccharomyces unisporus* predominated to the fermented milk, as reported for traditional koumiss (Montanari *et al.,* 1996). Additionally, *Saccharomyces unisporus* and *Lactobacillus* spp. seems to predominate at the end of milk fermentation. This is most likely the reason for high percentage in *Lactobacillus* species isolations of the added starter, since a part of the old batch is used to ferment the new one. *Macrococcus caseolyticus* and *Streptococcus* spp. found in raw milk were not isolated from any sample during the fermentation process, probably due to the presence of other bacteria, such as LAB and yeasts, and the nutritional competitiveness in the milk microenvironment (Angelidis *et al.,* 2015). On the other hand, there were microorganisms (i.e. *Lb. helveticus*, some *Enterococcus* species, etc.; Figure 1) isolated from the fermented milk, which were found neither in milk samples nor in the added starter culture. It is noteworthy to mention, that these strains were picked from petri dishes after cultivation, thus divergences in bacterial species detection could be due to different reasons, such as the high selectivity of some media towards specific microorganisms which do not find optimal conditions for their growth (Dolci *et al.,* 2008).

Figure 1. Numbers and kinds of microbial strains isolated from raw and fermented milk samples, as well as the inoculum used for the production of Trachanas.



Conclusions

At the present work the composition of autochthonous microbiota of traditional Cypriot Trachanas was studied. A great biodiversity was observed in respect of the predominant species present in milk during fermentation. Indications have been observed that *Lactococcus*, *Lactobacillus* and yeast species may drive the milk fermentation, but further studies are needed in order to establish these observations. The future determination of the technological properties of these isolates would allow the reproducibility of the product within its area, along with studying the microbiology of Trachanas coming from other areas of the country.

Acknowledgements

The authors gratefully acknowledge the material provided by C. Katsouras and the financial support from the Cyprus University of Technology Start Up Fund to Dr. Dimitris Tsaltas.

References

Angelidis A.S. 2015. The microbiology of raw milk. pp. 22-69. In: Dairy Microbiology, A practical approach. Papademas P. (ed.). CRC Press, USA.
Berthier F., Beuvier E., Dasen A., Grappin R. 2001. Int. Dairy J., 11: 293-305.
Carpino S., Rapisarda T., Belvedere S., Papademas P., Neocleous M., Scadt I., Pasta C., Licitra G. 2010. Dairy Sci. Technol. 90: 715-727.
Desmasures N., Bazin F., Gueguen M. 1997. J. of Appl. Microbiol. 83: 53-58.
Dolci P., Alessandria V., Zeppa G., Rantsiou K., Cocolin L. 2008. Food Microbiol., 25: 392-399.
Fadda M.E., Mossa V., Pisano M.B., Deplano M., Cosentino S. 2004. Int. J. of Food Microbiol., 95: 51-59.
Fleet, G.H. 1990. J. of Wine Res. 1: 211-223.
Gaya P., Babin M., Medina M., Nunez M. 1999. J. Appl. Microbiol., 87: 849-855.
Luo J., Lin W., Guo Y. 2010. Appl. Microbiol. Biotechn., 86: 377-384.
Montanari G., Zambonelli C., Grazia L., Kamesheva G.K., Shigaeva M.K. 1996. J. Dairy Res., 63: 327-331.
Morandi S., Brasca M., Lodi R. 2011. Technological, phenotypic and genotypic characterisation of wild lactic acid bacteria involved in the production of Bitto PDO Italian cheese. Dairy Sci. Technol., 91: 341-359.
Quigley L., O'Sullivan O., Stanton C., Beresford T.P., Ross R.P., Fitzgerald G.F., Cotter P.D. 2013. FEMS Microbiol. Rev., 37: 664-698.
Scintu M.F., Piredda G. 2007. Small Rumin. Res., 68: 221-231.
Sengun I.Y., Nielse D.S., Karapinar M., Jakobsen M. 2009. Int. J. of Food Microbiol., 135: 105-111.
Tristezza M., Fantastico L., Vetrano C., Bleve G., Corallo D., Grieco F., Mita G., Grieco F. 2014. Int. J. of Microbiol., 897428: 1-11.
Viljoen B.C. 2001. Int. J. Food Microbiol., 69: 37-44.

Influence of an autochthonous starter culture on the microbial dynamics of PDO Silter cheese

T. Silvetti[1]*, P. Cremonesi[2], S. Morandi[1], E. Capra[2], C. Albano[1], R. Giannico[3], I. De Noni[4], M. Brasca[1]

[1]Institute of Sciences of Food Production (CNR), 20133, Milan, Italy
[2]Institute of Agricultural Biology and Biotechnology (CNR), 26900, Lodi, Italy
[3]Fondazione Parco Tecnologico Padano, 26900, Lodi, Italy
[4]DeFENS, University of Milan (UNIMI), 20133, Milan, Italy
*tiziana.silvetti@ispa.cnr.it

Abstract
The aim of the study was to evaluate the influence of an autochthonous starter culture on the diversity and dynamics of PDO Silter cheese microbiota during ripening. To this end, a polyphasic approach comprising both culture-dependent and –independent methods was performed to compare cheeses produced with and without the addition of the autochthonous starter culture.
The starter determined higher lactic acid bacteria (LAB) level till 30 days of ripening, and the higher content of different LAB concurred to reduce detrimental microorganisms in curd. Discrepancies in the proportion of *Enterococcus* were observed between culture-dependent and –independent methods but the pivotal role of *Streptococcus* and *Lactococcus* genera was highlighted by both approaches.

Keywords**:** autochthonous starter, cheese, microbial dynamics, Next Generation Sequencing, raw milk

Introduction
Protected Designation of Origin (PDO) Silter cheese is a traditional, half fat, hard, raw milk cheese produced in the Valle Camonica and in the Prealpine zones on the eastern side of Lake Iseo, in the province of Brescia (Northern Italy). The bacterial diversity and richness of artisanal raw milk cheeses, deriving from the geographic area of origin and the cheese-making practices, deeply impact on the final product quality and sensorial properties. The adoption of autochthonous starters can be useful in optimizing the fermentation in order to limit sensory defects or health risk associated to some members of the raw milk consortium, preserving the typicality of each cheese (Renes *et al.*, 2014).
The aim of this study was to evaluate the influence of an autochthonous starter culture on the diversity and dynamics of PDO Silter cheese microbiota during ripening. To this end, a polyphasic approach comprising both culture-dependent and –independent methods was performed to compare cheeses produced with and without the addition of the autochthonous starter culture.

Materials and methods
Autochthonous starter culture
LAB strains isolated from the PDO Silter production process (milk, curd and cheese) and characterized in a previous study (Vanoni, 2007) were combined to formulate a starter culture that was scaled-up to industrial level. This lyophilized starter contained: *Lactococcus lactis* ST87 (10^6 cfu mL^{-1}), *Leuconostoc pseudomesenteroides* ST23 and *Ln. mesenteroides* ST32 (10^4 cfu mL^{-1}), *Streptococcus thermophilus* ST56 and *S. thermophilus* ST182 (10^6 cfu mL^{-1}).
Cheese production and sampling
Four batches of Silter cheese were manufactured in two different dairies (B and R): for each producer, a batch with the addition of the selected autochthonous starter culture in the vat (experimental, E) and a batch with no starter (control, C) were simultaneously prepared following the production specification (MIPAF, 2015). The curd was sampled immediately and cheese

samples were collected at different ripening periods (30, 60 and 200 days). Microbiological analyses were performed within 24 h after sample arrival.

Lactic acid bacteria (LAB) enumeration and RAPD-PCR typing

Microbiological analysis (total lactic acid bacteria, lactococci and streptococci, enterococci and *Leuconostoc* spp.) were performed as previously reported by Morandi *et al*. (2011).

In order to assess the survival and persistence of the LAB strains composing the autochthonous starter during the ripening, 103 strains were isolated and typed (RAPD-PCR with three primers M13, D11344 and D8635) as previously described (Morandi *et al*., 2011; Morandi *et al*., 2015).

Genomic DNA extraction and Illumina analysis

The bacterial DNA was extracted using an optimized protocol (Cremonesi *et al*., 2006) with some modifications in sample pre-treatment. The 16S rRNA gene amplicons on V3-V4 region sequenced on a paired 2x300 bp run on a Miseq platform (Illumina, San Diego, CA, USA).

Results and discussion

The addition of the autochthonous starter culture determined a higher LAB content in E cheeses than C cheese of both dairies up to 30 days. Lactococci and Streptococci dominated throughout ripening, whereas Lactobacilli achieved important level after 200 days. A greater number of *Leuconostoc* spp. was detected in E samples. Until 60 days of ripening, higher level of *Leuconostoc* spp. coincided to a lower number of *Enterococcus* spp. in both dairy productions. This confirms the results of a previous work that evidenced the inhibitory activity of *Leuconostoc* spp. against enterococcal growth (Morandi *et al.*, 2013).

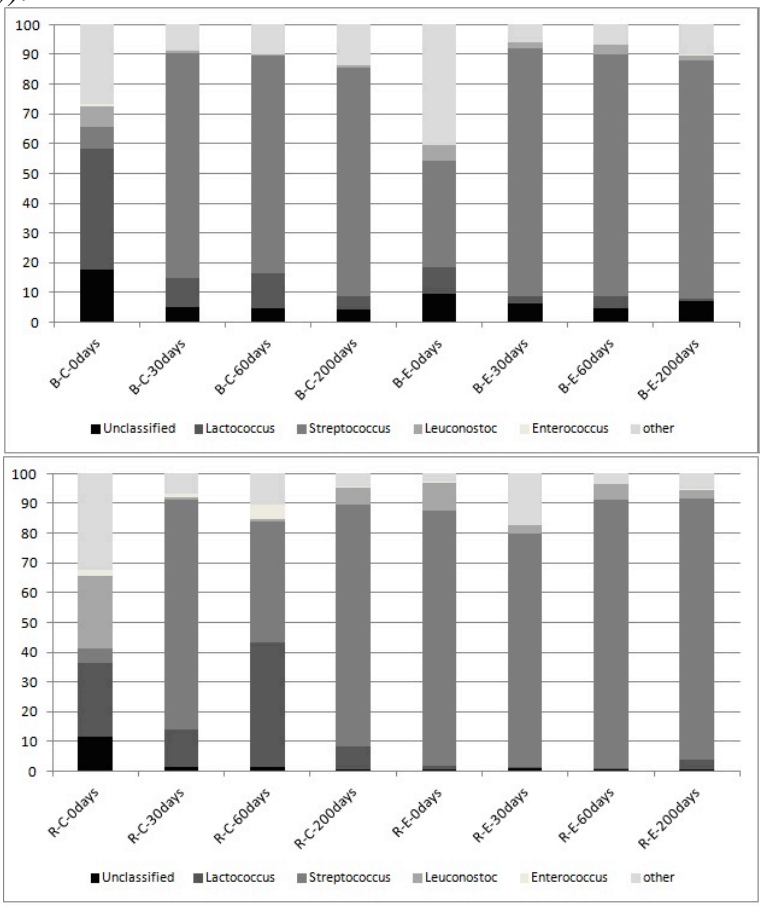
Table 1: Persistence of the autochtonous starter LAB strains in the two dairies (B and R) during ripening (0, 30, 60, 200 days). (SC: lyophilized starter culture).

Strains	Producers	Ripening (days)				
		SC	0	30	60	200
Lc. lactis ST87	B	■	■	■		
	R	■	■	■	■	■
Ln. pseudomesenteroides ST23	B	■				
	R	■				
Ln. mesenteroides ST32	B	■	■	■	■	
	R	■	■	■	■	
S. thermophilus ST56	B	■	■	■	■	■
	R	■	■	■	■	■
S. thermophilus ST182	B	■				
	R	■				

The persistence of the autochthonous starter strains from curd to ripened cheese differed (Table 1). In both dairies, *S. thermophilus* ST56 endured till the end of the ripening period and *Ln. mesenteroides* ST32 was isolated up to 60 days. In contrast, *Lc. lactis* ST87 showed a different behaviour during cheese-making B and R. *S. thermophilus* ST182 and *Ln. pseudomesenteroides* ST23 were not detected even in curd. These findings could be due to the strain-specific survival under different stress conditions occurring in cheese-making process.

Figure 1: Genus level abundance profiles for the microorganisms of interest using 16S rRNA sequence classification during ripening (BC and RC samples without autochthonous starter for

producers B and R, respectively; BE and RE samples with the autochthonous starter for producers B and R, respectively).



As reported in Figure 1, the metagenomic analysis supported the culture-dependent results. In fact, *Streptococcus* and *Lactococcus* (in particular, *S. thermophilus*, *S. vestibularis*, *Lc. lactis* and *Lc. raffinolactis*) were the most representative genera from curd to ripened cheese. Moreover, several undesirable bacteria, such as *Pseudomonas*, *Staphylococcus*, *Serratia*, *Chryseobacterium*, and *Enterobacter*, were observed in curd with higher abundance in C samples (data not shown). A larger number of unclassified species was also revealed in cheese produced without starter addition. Illumina sequencing detected *Enterococcus* spp. in low level in samples without the autochthonous starter, despite their presence at high cell numbers as demonstrated through cultivation. Actually, these discrepancies revealed by culture-dependent and -independent methods could be attributable to DNA extraction procedures and primer sets as described by Starke *et al.* (2014).

Conclusions

The addition of the autochthonous starter culture positively influenced the LAB cheese content and allowed to control cheese-making reducing undesirable bacteria in curd and unclassified bacteria during cheese ripening.

The combination of complementary culture-dependent and -independent techniques is still necessary for a deeper comprehension over time of the microbial community and its evolution, strictly involved in defining the cheese quality.

Acknowledgments

This study was partly performed within the research projects VALTEMAS supported by the Regione Lombardia.

References

Cremonesi P., Castiglioni B., Malferrari G., Biunno I., Vimercati C., Moroni P., Morandi S., Luzzana, M. 2006. J. Dairy Sci., 89: 163-169.
MIPAF 2015. https://www.politicheagricole.it/.
Morandi S., Brasca M., Lodi R. 2011. Dairy Sci. Technol., 91: 341-359.
Morandi S., Cremonesi P., Silvetti T., Brasca M. 2013 J Dairy Res., 80: 457-466.
Morandi S., Silvetti T, Miranda Lopez J.M., Brasca M. 2015. J. Food Safety., 35: 193-205.
Renes E., Diezhandino I., Fernández D., Ferrazza R.E., Tornadijo M.E., Fresno J.M. 2014. Food Microbiol., 44: 271-277.
Starke I.C., Vahjen W., Pieper R., Zentek J. 2014. Mol. Biol. Int. doi:10.1155/2014/548683.
Vanoni L. 2007. PhD thesis, University of Milan. Milan, Italy.

Reduction of histamine content in traditional raw milk cheeses

J. Haldemann*, P. Ascone, J. Maurer, S. Irmler, H. Berthoud, R. Portmann, M.-T. Fröhlich-Wyder, D. Wechsler*

AGROSCOPE, CH-3003 Bern, Switzerland
*john.haldemann@agroscope.admin.ch

Abstract
Mountain and alpine cheeses are part of the cultural heritage of several European countries. Most alpine cheeses are still made from raw milk and occasionally contain high concentrations of histamine. The objective of this work was to investigate the causes of consistently elevated histamine content in raw milk cheeses. Repeated screenings of milk samples from milk suppliers revealed that about 10 to 20% of the analysed raw milk samples were contaminated with histamine forming bacteria. Recently, *Lactobacillus parabuchneri* has been identified as the most important producer of histamine in cheeses by using a newly developed and species specific qPCR method. Moreover, genotyping of isolates provided a detailed insight into the diversity of *L. parabuchneri* in contaminated milks and cheeses and allowed to trace back the sources of contamination. Trace-back studies clearly demonstrated that milking systems frequently harbour persistent contamination sources of *L. parabuchneri.* Systematic elimination of contamination sources of *L. parabuchneri* at the farm level proved to be an important measure to prevent histamine formation during cheese ripening.

Keywords: Histamine, *Lactobacillus parabuchneri*, raw milk quality, cheese defects

Introduction
In raw milk cheeses, obligate heterofermentative lactobacilli, such as *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus parabuchneri* are occasionally found during the later stages of cheese ripening. Together with the facultative heterofermentative lactobacilli species *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* they constitute an important part of the non-starter lactic acid bacteria (NSLAB) community. The growth of NSLAB during cheese ripening influences the flavour and texture of cheeses or even can provoke cheese defects (Fröhlich-Wyder *et al*., 2015). In recent years, increasing attention has been paid to the presence of histamine in cheese, as the intake of this substance can result in health problems such as gastrointestinal disorders, diarrhoea, rhinorrhoea, headaches, and pruritus. People with histamine intolerance are particularly affected. In fermented foods, histamine is mainly produced by bacteria that display histidine decarboxylase activity (HDC, EC 4.1.1.22). The ability to decarboxylate the free amino acid histidine into the biogenic amine histamine is a strain-specific characteristic (Wüthrich *et al*., 2017). Recently, *Lactobacillus parabuchneri* has been identified as one of the most potent producers of histamine in cheeses of different origin and its presence has been documented in several traditional cheese varieties, such as Caciocavallo Pugliese (an aged pasta filata cheese), Berner Alpkäse (a Swiss alpine cheese), Parmigiano Reggiano, Gouda-type cheese, Pecorino Crotonese, Cheddar, Tête de Moine, Emmental, Cabrales (a Spanish blue cheese made from blends of raw cow's, sheep's and/or goat's milk), Gamoneu (a traditional Spanish smoked blue-veined cheese made from raw cow's, sheep's and goat's milk) Casín (a traditional Spanish, long-matured cheese made from raw cow's milk) and Manchego-type cheeses (Ascone *et al*., 2017; Berthoud *et al*., 2017; Diaz *et al*., 2016). Moreover, *L. parabuchneri* is frequently part of the indigenous microbial community of silages. The formation of histamine in cheese not only affects food safety, but also causes serious quality defects (burning flavour, formation of cracks or atypical openings, poor storage quality) and leads to high financial losses due to a downgrading of the contaminated cheeses.

Material and methods

Commercial cheese samples of different varieties and origin with an unpleasant burning taste were sampled and kept frozen until analysis. Case studies were performed in collaboration with four local cheese dairies producing Berner Alpkäse PDO, Raclette du Valais PDO, Tête de Moine PDO or Emmentaler PDO and that showed consistently elevated histamine content in their cheeses. The number of milk suppliers varied between 7 and 65 in the investigated cheese dairies. Determination of biogenic amines in cheese was performed by using a UPLC system as described by Ascone *et al*. (2017). In each of the case studies, raw milk samples of the involved milk suppliers were repeatedly collected and screened for the presence of histamine-forming bacteria as described by Ascone *et al*. (2017). In total, a number of about 1370 supplier milk samples were investigated. Milk samples that showed histamine formation after incubation were plated on modified decarboxylation agar containing histidine (MDA-H) to isolate histamine forming bacteria. The purified isolates were further analysed by species-specific qPCR analysis to identify isolates of *L. parabuchneri* as described by Berthoud *et al*. (2017). Similarly, *L. parabuchneri* isolates were collected from corresponding cheeses, which were processed of the sampled supplier milk samples. Quantitative detection of *L. parabuchneri* in raw milk and cheeses was performed by using a species-specific qPCR assay as described by Berthoud *et al*. (2017).

Results and discussion

The formation of histamine in cheese is associated with the development of an unpleasant burning taste that is easily perceptible at concentrations above 200 mg histamine kg^{-1} cheese. Table 1 shows the results of the analysis of eight commercial cheese samples of different origin with a burning flavour. The obtained results clearly indicate that this type of flavour defect is strongly associated with the growth of histamine forming strains of *L. parabuchneri* during cheese ripening.

Table 1: Histamine content and gene equivalents (GE) of Lactobacillus parabuchneri *in commercial cheese samples of different origin with a burning flavour. (Berthoud* et al.*, 2017)*

Cheese	Origin	Histamine $mg \cdot kg^{-1}$	*Lactobacillus parabuchneri* $GE \cdot g^{-1}$
Farmhouse cheese	Netherlands	957	$8.5x10^7$
Emmentaler PDO*	Switzerland	270	$1.3x10^7$
Tête de Moine PDO	Switzerland	330	$6.0x10^7$
Goat milk cheese	Switzerland	1012	$2.6x10^7$
Manchego PDO	Spain	749	$1.1x10^7$
Alpine cheese	Switzerland	774	$2.3x10^7$
Abondance de Savoie PDO	France	291	$8.0x10^7$
Queijo Sao Jorge PDO	Portugal	545	$1.7x10^7$

* PDO: Protected Designation of Origin

The repeated screening of supplier milks of several cheese dairies with elevated histamine content in the production revealed that *L. parabuchneri* contaminations occur quite frequently in practice. In individual cheese dairies, the share of contaminated supplier milks ranged typically between 10 - 20%. However, the population density of *L. parabuchneri* in contaminated supplier milks was usually below the limit of quantification of the applied qPCR method (125 gene equivalents per ml of milk). In about one third of the analysed samples the contamination level of *L. parabuchneri* was even below the limit of detection, indicating that a pre-enrichment step is necessary for the save identification of contaminated supplier milks. The genotyping *L. parabuchneri* isolates from contaminated raw milk and corresponding cheeses with multiplex qPCR allowed contaminations to be traced back to individual milk suppliers (Fig. 1). Furthermore, genotyping of isolates obtained from step-by-step controls (e.g. sampling of deposits within the installations by the use of cotton swabs) allowed to localize the contamination sources at the farm level (Fig. 2).

Figure 1: Example of multiplex-PCR genotyping of isolates of Lactobacillus parabuchneri *obtained from cheeses and corresponding supplier milks samples. Isolates from a downgraded cheese (Lane A and E), isolates form milk samples of an individual milk supplier (lane B, C, D and F, G, H) with permanent contaminations of* L. parabuchneri *in the delivered milk.*

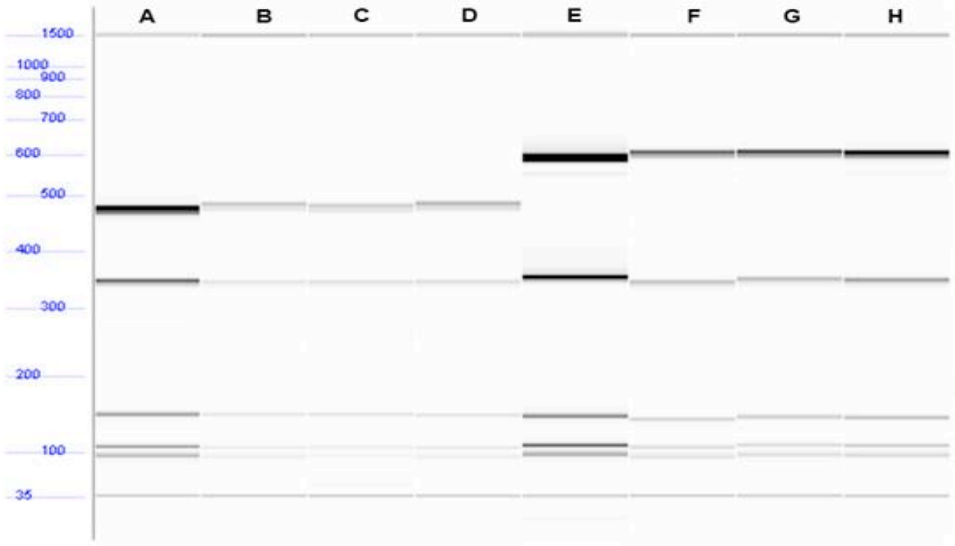


Figure 2: Examples of identified contamination sources of Lactobacillus parabuchneri *in milking systems. A contaminated tube of the end unit of a milking system with milk deposits (picture left) and a contaminated three-way valve of a pipe milking system (picture right) (Maurer* et al. *2016).*

Conclusions

The results of the present case studies showed that contaminations of *L. parabuchneri* in raw milk are mainly responsible for strongly elevated histamine content in raw milk cheeses.
Experience gained during the case studies indicate that periodical screening of supplier milks for histamine-forming bacteria would allow the recognition and exclusion of contaminated milk from being converted into raw milk cheeses, and thus could reduce the histamine content in such cheeses. However, the localisation and elimination of contamination sources of *L. parabuchneri* in cheese dairies and at the farm level remains a very demanding task.

Acknowledgements

The authors would like to thank the milk suppliers, and cheese dairy involved, and the project partners for their excellent collaboration and partial funding of this study.

References

Ascone P., Maurer J. Haldemann J., Irmler S., Berthoud H., Portmann R., Fröhlich-Wyder M.-T., Wechsler D. 2017. Prevalence and diversity of histamine-forming *Lactobacillus parabuchneri* strains in raw milk and cheese - a case study. Int. Dairy J. 70: 26-33.
Berthoud H. Wüthrich D., Bruggmann R., Wechsler D., Fröhlich-Wyder M.-T., Irmler S. 2017. Development of new methods for the quantitative detection and typing of *Lactobacillus parabuchneri* in dairy products. Int. Dairy J., 70: 65-71.
Diaz M., Ladero V., Redruello B., Sanchez-Llana E., del Rio B., Fernandez M., Cruz Martin M., Alvarez M.A. 2016. A PCR-DGGE method for the identification of histamine-producing bacteria in cheese. Food Control., 63: 216-223.
Fröhlich-Wyder M.-T., Bisig W., Guggisberg D., Irmler S., Jakob E., Wechsler D. 2015. Influence of low pH on the metabolic activity of *Lactobacillus buchneri* and *Lactobacillus parabuchneri* strains in Tilsit-type model cheese. Dairy Sci. & Technol. 95. 569585
Maurer, J., Haldemann, J., Ascone, P., Wechsler, D. 2016. Recommandations destinées aux producteurs de lait de fromagerie pour éviter les contaminations par des bactéries propioniques et Lactobacillus parabuchneri dans les installations de traite. Fiche technique Agroscope N° 1 / 2016, accessed at https://www.agroscope.admin.ch/agroscope/fr/home/publications/recherche-publications/fichestechniques/_jcr_content/par/externalcontent.external.exturl.pdf/aHR0cHM6Ly9pcmEuYWdyb3Njb3BlLmNoL2ZyLUNIL0VpbnplbH/B1Ymxpa2F0aW9uL0Rvd25sb2FkRXh0ZXJuP2VpbnplbHB1Ymxp/a2F0aW9uSWQ9MzY5Nzc=.pdf, 3 May 2017.
Wüthrich D., Berthoud H., Wechsler D., Eugster E., Irmler S., Bruggmann R. 2017. The histidine decarboxylase gene cluster of *Lactobacillus parabuchneri* was gained by horizontal gene transfer and is mobile within the species. Front. Microbiol., Vol. 8, Article 218.

Characterization and health-promoting properties of Traditional Mountain cheese microbiota

I. Carafa[1]* , F. Clementi[2] , K. Tuohy[1] , E. Franciosi[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige, Italy.
[2]Agricultural, Food and Enviromental Sciences-D3A, Università Politecnica delle Marche, 60131 Ancona, Italy.
*ilaria.carafa@fmach.it

Abstract
This study aimed to characterize the autochthonous lactic acid bacteria (LAB) established in Traditional Mountain (TM) cheese, and to select strains with technological or health-promoting properties for cheese production. The results showed a high biodiversity of wild LAB within TM-cheese and the presence of few strains with bioactive properties. The two best technological performing strains (a *Lactococcus lactis subsp. lactis* and a *Streptococcus thermophilus*) were tested as starter cultures in experimental TM-cheese production. They did not reduce the traditional high biodiversity of TM-cheese, neither standardized its sensory attributes, and suppressed the incidence of the pathogen *Streptococcus gallolyticus* (Carafa, 2016). Furthermore, a *Lactobacillus. brevis* strain showed *in vitro* a high production of γ-aminobutyric acid (GABA) and was tested as probiotic strain in two animal studies. In the first study, it survived during transit through the mouse gastro-intestinal tract; in the second study its therapeutic efficiency was assessed for treating induced metabolic obesity and type 2 diabetes mellitus (T2DM) in mice.

Introduction
Raw milk cheeses harbor a heterogeneous microbial composition characterized by adventitious microorganisms proceeding from raw milk, dairy environment and facilities (Eneroth et al., 1998). All those bacteria may be pathogens, spoilage or beneficial (Fox and McSweeney, 2004). Nowadays, lactic acid bacteria (LAB) are selected and used as starter or adjunct cultures during cheese manufacture owing to their fast acid production from the fermentation of lactose into lactic acid and their activity during cheese-ripening (Beresford and Williams, 2004). The use of health-promoting LAB strains as starter or adjunct cultures for dairy productions could facilitate the *in situ* bio-synthesis of bioactive molecules during the fermentation process, increasing the interest towards dairy products as multifunctional foods, which may have a regulatory activity in the human organism (Diplock et al., 1999; Leroy and de Vuyst. 2004; Settanni and Moschetti, 2011).
Metabolic syndrome is a collection of cardio-metabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia, which is unequivocally linked to an increased risk of developing T2DM and cardiovascular disease (Roberts et al., 2013). Around 3.4 million adults die each year as result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity (World Health Organization, 2014). Many medications are available for the management of hyperlipidemia and hyperglycemia but they have adverse effects. Therefore, the discovery and development of new substances that can safely inhibit obesity development and improve glucose metabolism will be of great benefit for slowing the development of T2DM and limiting its long-term complications (Tian et al., 2011).
The non-protein amino acid GABA has been reported to harbor an anti-obesity and anti-diabetogenic effect, as well as many other physiological functions in mammals including induction

of hypotension, diuretic and tranquilizer effects, and stimulation of immune cells (Hagiwara et al., 2004; Jakobs et al., 1993; Wong et al., 2003). Oral supplementation with GABA has previously demonstrated therapeutic effects against diet-induced type-2-diabetes in murine studies (Tian et al., 2011).
In this study, the autochthonous LAB from TM-cheese were characterized and screened for their technological and health-promoting activities. The most performing strains were tested in experimental cheese making production and *in vivo*. In particular, a *Lb. brevis* strain able convert monosodium glutamate (MSG) to GABA was tested in a mouse trial for treating induced metabolic obesity and T2DM.

Materials and Methods
Samples (n = 120) of milk, curd and cheese at different ripening times (24 hours, 1 month and 7 months) were enumerated in selective culture media. Six hundred and forty colonies were isolated from curd and cheese 24 hours following production, and 95 were isolated from cheese after 7 months of ripening. All isolates were genotypically characterized by Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) with two primers, species-specific PCR and partial sequencing of 16S rRNA gene. The phenotypic, technological and health-promoting activities of all strains were investigated. The best performing strains, were tested as starter, for the production of 9 experimental TM-cheese wheels in a Malga-farm, respectively. Three control (CTRL) cheeses were produced according to the tradition and any starter or adjunct culture was not added; three starter (STR) and three commercial starter (CMS) cheeses were produced inoculating the vat milk with both selected strains and a commercial *Sc. thermophilus* strain, respectively. After 24 hours, 1 month and 7 months of ripening the microbial content of all experimental cheeses was investigated. The total genomic DNA was extracted, and a fragment of the V1-V3 region was amplified and pyrosequenced.
The *Lb. brevis* strain harbouring the capacity to produce high concentrations of GABA *in vitro* was selected for being tested *in vivo* in healthy mice in order to evaluate its resistance to the gastro-intestinal digestion after one-week treatment. Furthermore, it was tested in mice suffering obesity-associated type-2-diabetes. The corresponding rifampicin resistant mutant (rif) was generated and the conversion rates of monosodium glutamate to GABA were investigated by next-generation amino acid analysis. The rif mutant strain was subjected to freeze-drying and tested for its ability to survive at different temperatures (+4°c, -20°C, room temperature).

Results and discussion
For the first time, the microbial population of TM-cheese has been characterized in order to select cocci and non-starter lactic acid bacteria (LAB) suitable for developing new starter or adjunct cultures, respectively. Mesophilic and thermophilic cocci dominated during the first 24 hours following production, and mesophilic lactobacilli were dominant at the end of ripening. Cocci clustered in 231 biotypes belonging to 16 different species, and non-starter LAB (NSLAB) clustered in 70 biotypes belonging to 13 different species. *Lc. lactis*, *Sc. thermophilus* and *Enterococcus faecalis* were dominant in curd and 24h-cheese; *Pediococcus pentosaceus* and *Lactobacillus paracasei* were the main species at the end of ripening. Some strains harbored very interesting health-promoting properties and produced bioactive substances. In particular, one *Lb. rhamnosus*, three *Lb. paracasei*, three *Pc. pentosaceus* produced between 70 and 130 mg/mL of total conjugated linoleic acid (CLA) *in vitro*. One *Lb. brevis* converted L-glutamate to a high concentration of GABA and showed bile salt hydrolysis (BSH) activity. These first results revealed that TM-cheese is a reservoir of a high microbial diversity, and the resident LAB could be exploited not only for the applicability in dairy production but also for the health-promoting properties.

The activity of the two best strains belonging to *Lc. lactis subsp. lactis* and *Sc. thermophilus* species as starter cultures was tested *in situ* through producing experimental Traditional Mountain (TM)-cheeses. Mesophylic cocci and lactobacilli dominated in cheese samples after 24 hours and 1 month of ripening, while cocci dominated in full-ripened cheese. The total genomic DNA was extracted, and a fragment of the V1-V3 region was amplified and pyrosequenced. Lactococci and streptococci were the most abundant species in CTRL and STR cheese, and the tested *Lc. lactis ssp. lactis* affected the proliferation of the (raw milk) indigenous *Lc. lactis ssp. cremoris* during the early fermentation. Moreover, the commercial *Sc. thermophilus* showed to be dominant towards *Lc. lactis supsp. lactis* and *cremoris* naturally present in raw milk and to be responsible in decreasing the abundance of *Lactobacillus sp.* and *Enterococcus sp.* The use of both autochthonous (*Lc. lactis subsp. lactis* and *Sc. thermophilus*) and commercial (*Sc. thermophilus) s*tarter strain reduced the presence of undesirable species, such as *Sc. gallolyticus, Sc. dysgalactiae* and *Lc. garviae.*
Our interest towards the GABA producing strains lead us to generate the *Lb. brevis* rifampicin resistant mutant strain, and test it *in vivo*. The conversion of monosodium glutamate to GABA by the rifampicin resistant mutant corresponded to 840.5 ± 266 μg/mL with about 73% of bioconversion. The strain showed to be resistant to freeze-drying, was stable at room temperature, +4 and -20 °C and survived transit through the mouse gastrointestinal tract. Its therapeutic efficiency was assessed *in vivo* to treat metabolic obesity and type-2-diabetes, and the data analysis is on-going.

Conclusions

The study confirmed that TM-cheese is a good reservoir of lactic acid bacteria with technological and health-promoting activity that could be used for improving cheese quality or producing multifunctional food.

References

Beresford T., & Williams A. 2004. The microbiology of cheese ripening. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 287–318.
Carafa I. 2016. In vitro and in vivo activities of lactic acid bacteria from Italian mountain cheese" and their exploitation in dairy production. PhD Thesis, http://hdl.handle.net/10449/32198
Diplock A. T., Aggett P. J., Ashwell M., Bornet F., Fern E. B., & Roberfroid M. B. 1999. Scientific concepts of functional foods in Europe - consensus document. Brazilian Journal of Nutrition, 81, 1–27.
Eneroth Å., Christiansson A., Brendehaug J., Molin G. 1998. Critical contamination sites in the production line of pasteurised milk, with reference to the psychrotrophic spoilage flora. International Dairy Journal, 8, 829–834.
Fox P. F. & McSweeney P. L. H. 2004. Cheese: an Overview. In Fox P. F., McSweeney P. L. H., Cogan,T. M. & Guinee T. P. (Eds.), Cheese: Chemistry, physics and microbiology. Third edition, Volume 1 (pp. 1–18). London: Elsevier.
Hagiwara H., Seki T., & Ariga T. 2004. The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. Bioscience, Biotechnology and Biochemistry, 68, 444–447.
Jakobs C., Jaeken J., & Gibson K. M. 1993. Inherited disorders of GABA metabolism. Journal of Inherited Metabolic Disease, 16, 704–715.
Leroy F., & de Vuyst L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science and Technology, 15, 67–78.

Roberts C. K., Hevener A. L., Barnard R. J. 2013. Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. Comprehensive Physiology, 3, 1–58.
Settanni L., & Moschetti G. 2010. Non starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiology, 27, 691–697.
Tian J., Dang H. N., Yong J., Chui W.S., Dizon M. P. G., Yaw C. K. Y., Kaufman D. L. 2011. Oral treatment with γ-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. PLoS ONE, 6, e25338.
Wong C. G., Bottiglieri T., &. Snead O. C 2003. GABA, γ-hydroxybutyric acid, and neurological disease. Annals of Neurology, 54 (6), 3–12.
World Health Organization 2014. Obesity and overweight—fact sheet N° 311. http://www.who.int/mediacentre/factsheets/fs311/en/.

The microbes, stowaways of milk

Geneviève Teil
SAD APT unit, INRA - Agroparistech, 16 rue Claude Bernard, Paris, France and French Institute of Pondichery, Pondichery, India

Abstract: Humans and natural or non-human economic actors enjoy very different statuses within the regulations aiming at framing the market interactions. Humans enjoy rights and responsibilities regarding the consequences of their actions while non humans, although essential participants to the production process are just seen as supports of external causes or biological processes. Therefore they have no responsibilities, but also no rights upon the production. More especially the status of microbes are outcast in the milk and cheese production organisation. In this communication we will get inspiration from the recent original interpretation of the Protected Denominations of Origin (PDO) terroir quality by vintners who want PDOs to protect the contribution of the vineyard to the production as if it were a co-author of the wine along with the vintner. This interpretation calls for resorting to PDOs and terroir protection to protect the milk microbes. Yet it also proposes a new and more efficient account of the artisanal-industrial divide.

Keywords: Microbes, terroir, human nature economical relationships, artisanal vs industrial production regime, denominations of Origin

Introduction

The Anthropocene crisis emphasizes the need for more sustainable governance. The award of legal statuses to some jeopardized or weak beings such as natural parks, endangered species does so by challenging the strong framing on human-nature relationships economic regulations impose on economic beings: the classification of the different market beings into "merchandise", "consumer", "producer"… determines property relationships and distributes rights of some beings over the other ones. This is what should be tackled in order to a more efficient interpretation of sustainability. In doing so, we can count with the work of great pioneers. Some researchers like Stone (1972, 1981), or Latour (1999) have pushed in this direction; yet more surprisingly maybe, vintners have also done so and in a very actual way. Decades after decades they have contributed to elaborate and feed the possible significations of the Protected Denominations of Origin and of the notion of terroir. One of these significations can be considered as achieving the embryo of a status for the nature they are working with and no more exploiting. Just like wine, milk and dairy products are issued from extremely complex production processes involving an endless list of contributions by a variety of beings, human or not. But, and this is part of the interest of the milk case, milk microbes are not only seen as weak beings requiring protection.
Grounding upon this wine example, I will discuss how we could elaborate a new governance regarding milk and its microbes.

Milk as the produce of a human and natural "process"

Within the market, milk is a commodity. This means, in France since the 1851 French law aiming at "a more efficient suppression of certain frauds in the sale of goods", that it must not be adulterated and enjoy an "authentic" quality. Part of this "authentic" quality is defined as the production of milch animals, and milk must therefore result from their milking. In the more recent European regulation 853/2004, milk is seen as the mix of a series of authentic ingredients, which can be removed, like fat, or on the contrary, added. Yet, in order to be called milk no foreign ingredient can be added to it.

Microbial and human unequal involvement in milk spoilage

Milk quality can be spoilt; humans may adulterate it. Therefore milk collect centres and milk buyers check for few current adulterations such as salt, sugar, urea, hydrogen peroxide, antibiotics, flour and starch… Thanks to Duclaux (1896), L. Pasteur and so many of his collaborators, the microbes have invaded, the wine, the milk, and all our life. Since they have identified the presence of microbes in the milk and accounted for their activity in the milk, not only men can spoil the milk but also microbes, by making the milk curdle, producing acidity, alcohol, toxins and generate diseases… Microbes are not ingredients of the milk, like its fat or its vitamins; they do no come out of the cow's udder. They are more or less considered as "stowaways" in the milk. As a source of spoilage, their presence is watched after and if there are too many coliforms, pseudomonas, or more simply to many microbes, the milk becomes unsuitable for human consumption and can't be sold anymore. In the CE regulation (2073/2005) the producer who wants to sell his milk must take care that the microbes do not invade it above the legal limits. But microbes are also the indispensable agents of cheese fermentation.

Biological mechanism, stowaways and commodities

Microbes endow a loose and plural status in the dairy process. As "stowaways" of the milk, their presence has to be closely watched by the producer of the milk who must comply with the microbial norms. Just like cows are the source cause of milk, microbes are the cause of cheese. But they don't produce it; they are biological mechanisms localised in a material support, inducing chemical transformations resulting in milk or cheese, under the vigilance and responsibility of the milk producer or cheese maker. Both humans and microbes can adulterate milk. However, if microbes are the "culprits" cause of bad or unacceptable quality, they have no responsibility for it; only humans are seen as liable for the milk adulteration. Since they are in the milk owned by the producer, he is responsible for their presence and allowed to more or less eradicate them by pasteurizing or sterilizing the milk.

However, next to milk stowaways, the microbes are also desirable living beings, agents of the cheese fermentation. Like cows, they are bred and sold to cheese-makers, although the commodification of these living beings raises concerns regarding their ownership.

The natural partners of human producers

From the market regulation point of view, microbes like cows are **natural living beings** bred by a producer in order to perform their expected transformation. Contrarily to producers, microbes and cows are not considered as performing any work. This can be seen as the consequence of their lack of will, intentionality or intelligence; the idea of work being reserved to intelligent, wilful, and intentional humans. But not all humans work in an intelligent, wilful, and intentional manner, and more importantly this divide perpetuates the divide between nature and humans and allows for the exploitation of nature by humans, until the natural supports of the living processes are declared endangered and may benefit from a special protection.

Let's turn now towards terroir wines where as we will see, intelligence, seen as peculiar to humans, is not necessarily required to grant living beings work activities.

AOC as a standing for terroir

In the 2000's AOCs were the source of a hot conflict regarding terroir quality. Some wines were denied the AOC label because they did not show the required typicity of their AOC. Since the 70's AOC include an agreement tasting. If it happened to a wine to fail, his producer was asked to doctor the wine so as to remove its defects and represent it. Yet this refuses crisis, as they soon called it brought a new problem forward. Most of the rejected wines were elaborated by famous vintners who categorically refused to modify their wines, as they described themselves as being committed to the highest respect of terroir expression. This attention paid to terroir induced a new interpretation of terroir. Instead of seeing it as a set of external causes influencing the taste of the wine, they saw it as a winemaking partner. Just as the musician respects the composer he is

interpreting so as not to distort his works, these vintners tried to respect the voice of terroir and not distort it. How did they respect terroir and make sure it was the case? They tried as much as possible to avoid the overworking of the vines and the covering or blurring of all the possible signs of terroir expression in the wine due to excessive intervention in the wine making process.
This new interpretation transformed the vine and the vineyard, formerly seen as natural resources and a natural source of raw material, the grapes, which quality they could have shaped to their desires or client tastes, into a producer, or better said a co-author of a wine. The difference between the author and the producers stands in the way they considered terroir typicity. For the colleagues who refused them, typicity was synonym of a definite identity that is, a list of criteria the wine had to match. For them, typicity was the unforeseeable result of practices respectful of terroir expression. Terroir was no more a set of predetermined factors but the expression produced every year by a being, terroir, and a vintner. Provided the vintner would correctly 'listen' to his terroir, the result of this partnership and mutual understanding and discovery could be said to resort to terroir quality although there was no reason for it to match predetermined criteria. AOC typicality had therefore to be considered as a very broad and possibly changing concept elaborated by more or less innovative authors whose work is protected by the AOC regulation from copies. Vine grapes are no more a raw material selected and chosen by the vintner, but the terroir contribution to the vintage wine (Teil, 2014).
This interpretation of terroir induces another consequence on terroir quality. Terroir quality is not to match any demand preferences. Moreover terroir quality means authenticity since it is the quality of terroir, which expression is protected by the vintner; and authenticity is to prevail on any other quality assessment such as high quality for instance. Terroir wine drinkers who look for authenticity answer that one cannot drink Chateau Petrus everyday. Every quality has its own usage. Should microbes, as well as milch animals, fields, local climate be considered as contributors to such a terroir works? What would qualify them as such?

From resources to partners
Terroir vintners suggest an original view on PDOs where vineyards are no more supports for biological processes suffering influence factors, but partners in a production process. They take their vineyards out of the realm of nature which don't have to bother with the consequences of their acts since they do not produce them. Then they introduce them in another world, where they are considered as authors of their partially unpredictable production. Production, as we use it now, differs from others achievements in a very specific way: it's the result of a production activity whose result is not completely known in advance and can be surprising.
The change started by the terroir vintners provides the vineyard with an embryo of status, not simply as a weak yet universally useful being entailing protection, but as a partner requiring respect and whose uncertain production is deemed valuable. It alters the way human beings may use, transform or interact with them. In this other world, all beings recognized as such are also responsible; they have to care about the consequences of their actions. So the next question is how can non-human beings take care of the consequences of their actions? Before considering this point, let's note that you don't need a will, a conscience, an intelligence to access this different world. Babies, mentally challenged people, national parks, endangered species… have legal statuses, which means that humans must respect them; they cannot behave as they wish with them. The interactions with these beings are framed and limited.
We can also note that all these beings are weak beings, which therefore require protection and were awarded a special status. Even terroir is considered by the vintners as an endangered being, which requires protection. In their interpretation, this is the aim of PDOs: they protects their work and demand that the vintners care of the terroir expression. However, what is the difference between the recognition of the weakness of a being and its right to respect? Weakness points a situation of exhaustion in a world of more or less extinguishable resources, while respect is what is due to any being contributing to a production processes. Yet microbes do not only make cheese. Their societies can host dangerous individuals? Should we award them the same respect as to the cheese workers?

Towards a new understanding of the "artisanal" vs "industrial" difference

Wine is not so different from cheese. Both are produced, in definite places by biological and microbiological beings. If microbes would be granted respect, they would also become accountable for the consequence of their actions, their responsibility in bad product quality, be it taste quality or sanitary quality. Yet what are cheese eaters ready to bear so as to enjoy the different microbe and cheese maker production? To what extend are cheese makers ready to help the microbes do their best to get the best cheeses?

With these last questions we enter a particular economic world, whose beings enjoy different relationships. This world is often said "artisanal" and differentiated from its opposite, the "industrial" world. Yet the opposition the names designate is difficult to explicit. Although the size of the production is definitely important, industries can be quite small and large production units still be artisanal. The difference is also often grabbed through raw or pasteurised milk use, again without great success. Artisan production is neither another word for traditional, preindustrial, old days production. Better than the size, the age or the milk, we suggest that it's the kind of relationship and most of all of respect allowed to the resources or contributors to the production process, which makes the difference.

Artisanal production, as we have accounted for, requires a particular consideration for the products, the producers and their partners, which not everyone is ready to provide. It has to coexist with other economic modes. However, its coexistence with other production regimes, may require specific tools to allow for its permanency, such as the particular AOCs the terroir vintners are inventing. These tools must ensure that respect can reign within the artisanal regime and stand the competition of the other production regimes. The question whether the norms should apply equally to all the production regimes or on the contrary if they should adapt to the specificity of the different economic regimes, has to be scrutinized. The proficiency of the artisans, a key point of the artisanal production regime, could be submitted to qualifications, while sanitary norms could better fit the particular needs of their production setting.

Acknowledgements

This work is issued from the Repastol research program, which has been funded by the INRA meta program GLOFOODS. We thank the whole Repastol team as well as the Glofoods Metaprogram for their support.

References

Duclaux, E.,1896. Imprimerie S. Charaire et Cie, Sceaux.
Latour, B., 1999. La Découverte, Paris.
Stone, C.D., 1972. Southern California Law Review 45, 450-501.
Stone, C.D., 1981. Environmental Practice News 10-11, 10.
Teil, G., 2014. Journal of World Intellectual Property 17, 96-113.

Mountain forage system management and dairy product quality

G. Borreani[1]*, E. Tabacco[1]

University of Turin, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, 10095, Grugliasco (Turin), Italy
*giorgio.borreani@unito.it

Abstract
The intensification of farming practices is putting at risk the efficiency and sustainability of mountain forage systems and their social, cultural and environmental values. This paper highlights how mountain dairy farms should be of low-intensity in terms of external inputs and should base their feeding strategies on local forage resources. The possibilities of utilizing grazing in summer and of producing high quality nutritional feed stock for the winter period (by cutting local forages at an early stage of growth and conserving them as wrapped haylage) have beneficial effects on production costs, animal health and productivity and quality of dairy products. Furthermore, using high quality forages all year round can contribute to reduce the use of purchased off-farm feeds, to link dairy products to their '*terroir*' origin and to preserve the high natural and biodiversity value of mountain dairy farms.
Keywords: forage systems, milk quality, grassland environmental values

Introduction

Following models of farming system intensification was one of the factors affecting the decline of mountain dairy systems in several areas of EU putting at risk a range of social, cultural and environmental values (Beaufoy, 2017). In the European Alps 40% of all farm holdings were abandoned within the past 20 years and almost 70% of the farms still operating are run as a secondary source of income (Tabacco et al., 2011). At a farm and local landscape level, the tendency reported in many regions in recent years is to abandon semi-natural pastures and to concentrate stock on more productive lowland, with increased intensification on this land (Beaufoy, 2017). These systems are found mainly in marginal areas where physical factors, and in some cases social factors, have prevented intensification of land-use. Specialization in agricultural systems has resulted in decoupling of cropping and grassland systems and livestock production disrupting within-farm nutrient cycling leading to large nutrient imbalances and excessive nutrient accumulation (Sulc and Franzluebbers, 2014).
A wide range of semi-natural habitats (with high species diversity and unique species communities), as well as habitats that are less natural, but nevertheless are the main refuge for a significant number of farmland species (Keenleyside et al., 2014). Several of these habitats, which are amongst the most important for biodiversity in Europe, are included and maintained by dairy farms in mountain areas (Van Dorland et al., 2008). These dairy farms are required to be of low-intensity in terms of external inputs and should be based on feeding strategies predominantly based on semi-natural forage resources produced on-farm (Borreani et al., 2007; Revello-Chion et al., 2010), and supplemented to a lesser extent by purchased fodder and feeds. Furthermore, the local forage based diets are part of the basic link between dairy products and their original '*terroir*', a notion at the basis of the PDO labeling and image of the product quality from sensory, nutritional, or healthy point of view (Coppa et al., 2015; Giaccone at al., 2016). In this context, maintaining environmental and economic sustainability of such dairy farms is a key factor for an efficient use of grassland resources and provision of their ecosystem services.

Linking quality traits to the production environment

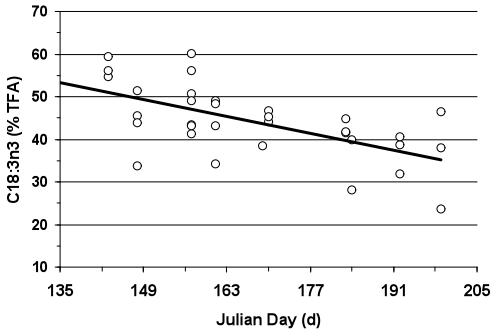
The forages are known to confer specific organoleptic and nutritional qualities to the milk products (Martin et al., 2005; Giaccone at al., 2016) and to provide a value added to the product, that could

justify its higher price and offer the consumers a healthy image of the mountain environment. Feeding animals with fresh herbage instead of conserved forages and/or concentrates induces a general improvement of nutritional properties of animal products (healthier fatty acids (FA) composition, higher antioxidant concentration), a difference in sensory properties (yellower and softer products, with richer sensory profile) and a potential increase in product shelf life (Coppa et al., 2017), while linking more strictly the product to their origin of production. Unfortunately, in mountain dairy farms of Italy extensive grazing could be only performed in the summer period (3 to 4 months), whereas confinement feeding are practiced over a large part of the year (8 to 9 months).

Increasing content of healthy FA in dairy products

The healthy image of grassland-based dairy products is confirmed by several studies, that have revealed high contents of beneficial functional FA in those products derived from Alpine grazing systems (Coppa et al., 2013). Among the fatty acids, studies reported that the conjugated linoleic acid (CLA) has a wide range of healthy effects, like anticarcinogenic and antiatherosclerotic effects (Parodi, 2004). The most beneficial FA profile to human health and the higher amounts of terpenes are obtained during summer season, when cows grazed mountain pastures (Revello-Chion et al., 2010). However, a large portion of the milk and cheese are produced in winter and early spring periods, when cow diets are mainly based on hay (locally produced or purchased) and concentrates. The concentration of healthy FA in milk and dairy products is mainly due to polyunsaturated FA (PUFA) concentrations in the diet. The forages, despite their low lipid concentration, are an important source of PUFA for dairy cow. Sources of variation in the FA concentration of forage are plant species, leaf-to-stem ratio, stage of maturity, weather, and fertilizer regime (Revello-Chion et al., 2011). The α-linolenic acid (C18:3 n-3), the main precursor of the beneficial FAs to human health present in milk fat, decreased during the growing stages in herbage samples of semi-natural meadow in Italian Alps (Figure 1; Revello-Chion et al., 2011), implying the need of an early utilization even when forages are used to produce winter feeding stock (Coppa et al., 2015).

Figure 1. Evolution of C18:3n3 in fresh herbage during first growing cycle of grassland at 1400 m a.s.l. in Italian Alps. Julian day: 135 = May 15 (from Revello-Chion et al., 2011).



Improving nutritional quality of conserved forage

Field-cured hay is currently the main preservation system used to produce conserved forages, and is normally harvested at a late stage of maturity. Due to the high mechanical losses and frequently rain damage, the hays resulted to be poor in quality and, consequently the winter milk production needs to be supported with concentrates purchased from outside the production areas (Borreani et al., 2007). Wrapped bale haylage has proved to be a good alternative to move from haymaking to silage technology on small-to-medium farms in the lowlands, since it can easily be mechanized and can be harvested with the same equipment that is used for field-cured hay, with the only addition of a plastic wrapper. For those production chain in which a ban on silages does not exist, wrapped bales at low moisture content (haylage) could provide high nutritional quality forages during the whole year and contribute to reducing feeding costs (Tabacco et al., 2011; Borreani et al., 2013), without

altering cheese-making technological aspects (e.g. late blowing) (Borreani et al., 2007). Cutting the forage at an earlier stage of growth than normally made for haymaking, wilting it in the field to a 50% DM and preserving it in wrapped bales allow to obtain a forage that have 50% more protein and 20% less NDF than traditional hay (Figure 2), without substantial reduction in annual DM yield (Table 1).

Figure 2. Evolution of crude protein (A) and NDF (B) during first growing cycle of grassland at 1400 m a.s.l. in Italian Alps (full line, black symbols) and relation with DM content at harvesting for an early (circle) and a traditional (triangle) cutting times (dotted lines).

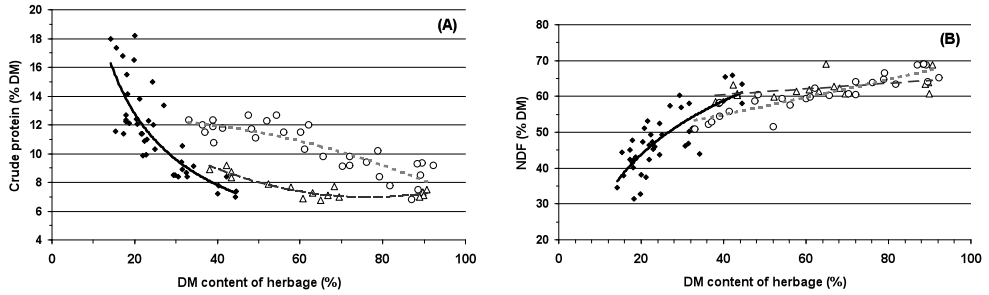


Table 1: Annual forage DM yield (t DM/ha), incidence on annual yield of first utilization of permanent grassland in relation of cutting time in Italian Alps (from Ciotti et al., 2000).

	Lowland (Samolaco, SO, 210 m asl)			Medium Alpine valley (Demonte, CN, 750 m asl)			Highland (Sauze d'Oulx, TO, 1500 m asl)		
Cutting time 1st growth	Annual DM yield	1st cut (%)	n. cuts	Annual DM yield	1st cut (%)	n. cuts	Annual DM yield	1st cut (%)	n. cuts
Early cut	11.8	26	4	9.0	52	3	4.6	61	2
Medium cut	13.1	34	4	9.1	54	3	5.2	77	2
Late cut	12.9	39	3	10.3	63	2.5	5.0	100	1

Table 2: Influence of nitrogen input on proportion of botanical families of permanent meadows in Valtellina (Italy) (Pers. Com. Fausto Gusmeroli – Ist. Fojanini, Sondrio).

	High input (200 kg N/ha)			Medium input (100 kg N/ha)			No-input		
Cut	*Poaceae*	*Fabaceae*	Other families	*Poaceae*	*Fabaceae*	Other families	*Poaceae*	*Fabaceae*	Other families
1st	71	3	26	52	13	35	39	13	48
2nd	70	4	26	44	16	40	28	20	52
3rd	53	5	42	35	13	52	20	18	62

Maintaining/increasing biodiversity of permanent grasslands

Low-intensity agricultural systems have consistently been shown to have higher biodiversity than more intensive systems, both in temperate regions and the tropics. Supporting such systems may therefore help stopping the decline of farmland biodiversity in terms of plants, mammals, bird and arthropod populations. At the field level, several management factors may affect biodiversity of grasslands interacting together in a large-scale temporal changes: use of organic and mineral fertilizers, grazing and cutting, drainage and ploughing, and the use of agrochemicals (Plantureux et al., 2005). When fertilizer are supplied at high level only a few fast growing plant species can compete for light (mainly *Poaceae*), eliminating less competitive plants and resulting in a decrease in the species richness (Table 2). From different studies, it appears that a significant reduction in plant diversity is generally observed even for fertilizer levels which are very low in comparison to the normal application rates in intensive grasslands. For nitrogen, a reduction of half of the total number of plant species can be observed for fertilizations greater than 50 kg N/ha per year (Plantureux et al., 2005).

Manage field margins and uncut strips for higher biodiversity

Semi-natural grasslands under extensive management typically have species rich communities, but their significance for agriculture has declined considerably, since most permanent grasslands have been turned into intensively managed grasslands (with several cuts per year and selected species) or crop fields (Lebeau et al., 2015), with a great reduction in related plant and animal biodiversity. Also the mowing process, especially with more frequent cutting at early stages of growth, is another important factor that has a direct and often substantial impact (in terms of mortality) on field invertebrates (Humbert et al., 2012), mammals and birds (Sargent et al., 2012) and reduction of plant biodiversity. In view of this, leaving uncut grass areas within meadows or uncut strips along field edges has been recommended as a mitigation measure to directly reduce mortality of beetles, orthopterans, spiders, lepidopteran caterpillars and other less mobile invertebrates (Humbert et al., 2012) and ground nesting birds and mammals. Furthermore uncut areas might also act as refuges to which invertebrates can move to and will provide foraging areas later in the season and maintain plant richness by allowing later-flowering plants to produce seeds.

Conclusions

Coupling summer grazing with the use of high nutritional forages during winter (obtained by cutting at an early stage of growth and conserving it as wrapped haylage) can contribute to a more efficient management of mountain grassland, a reduction in production costs and the possibility of a more strict link to the origin of production of mountain dairy products. Furthermore, some simple management aspects could contribute to maintain/increase the biodiversity value and the environmental importance of these high nature value farmlands.

References

Beaufoy G. 2017. Proc. 19th Symp. EGF. Wageningen Academic Publisher, 22: 251-267.
Borreani et al. 2007. J. Dairy Sci. 90: 5643-5650.
Borreani et al., 2013. J. Dairy Sci. 96: 6840-6855.
Ciotti A. et al. 2000. Riv. Agron. 34: 206-212.
Coppa M. et al. 2013. J. Dairy Sci. 96: 4197–4211.
Coppa M. et al. 2015. Anim. Feed Sci. Technol. 208: 66-78.
Coppa M. et al. 2017. Proc. 19th Symp. EGF. Wageningen Academic Publisher, 22: 251-267.
Giaccone D. et al. 2016. Int. Dairy J. 59: 10-19.
Humbert J.Y., Ghazoul J., Richner N., Walter T. 2012. Biol. Cons. 152: 96-101.
Lebeau J., Wesselingh R. A., Van Dyck H. 2015. PlosOne 1-17.
Keenleyside C. et al. 2014. Report Prepared for DG Environment, Contract No ENV B.1/ETU/2012/0035, Inst. Eur. Environ. Policy, London, UK.
Martin, B. et al. 2005. Anim. Sci. 81: 205-212.
Parodi P.W., 2004. Aust. J. Dairy Technol., 59: 3-59.
Plantureux S., Peeters A., McCracken D. 2005. Agron. Res. 3: 153-164.
Revello Chion A. et al. 2010. Food Chem. 121: 393-399.
Revello Chion A., Tabacco E., Peiretti P.G., Borreani G. 2011. Agron. J. 103:1072-1080.
Sargent et al. 2012 Michigan State University Extension, Publication No. E3190.
Sulc M.R., Franzluebbers A.J. 2014. Eur. J. Agron. 57: 21–30.
Tabacco E. et al. 2011. Proc. 16th Symp. EGF. Walling Ennst. Druckerei Verlag, 16:163-165.
Van Dorland et al., 2008. J. Sci. Food Agric. 88: 77-85.

Nutritive composition, carotenoid, tocopherol and tannin contents of cover crops used as forage plants for ruminants

G. Maxin[1]*, A. Le Morvan[1], S. Laverroux[1], B. Graulet[1]
[1]INRA UMR 1213 Herbivores, VetAgro Sup, Clermont Université, Université Lyon, F-63122 Saint-Genès-Champanelle, France
*gaelle.maxin@inra.fr

Abstract
The use of catch or cover crops provide agronomic and environmental benefits. These crops can also constitute forage resources for ruminants. They have potential high nutritive value, and also may offer some benefits on animal health and products quality as they contain bioactive secondary metabolites. The objective of this study was to determine the nutritive composition and the carotenoid, tocopherol and tannin contents of seven cover crops species harvested at vegetative and flowering stages. Nutritive composition and secondary metabolites contents varied both with species and growth stage, the differences between species being more important at flowering stage than at vegetative stage. The results showed that these cover crops species hold good nutritive value and carotenoid content especially at vegetative stage. In this study, sainfoin provided the most interesting nutritive composition and secondary metabolites contents.

Keywords: forages, cover crops, carotenoids, tannins, stage of growth

Introduction

Many agronomic and environmental benefits of growing catch or cover crops have been reported (Decourtye *et al.*, 2010; Vertes *et al.*, 2010) including improved soil fertility through atmospheric N fixation, increased soil conservation, reduction in weeds and improved habitat and feed resources for auxiliary and pollinators. These plants provide also fodders for ruminants. Their utilization is increasing in many French ruminant production systems particularly when drought conditions limit grass supply. Although the agronomic and environmental benefits are well described, the nutritive value of these novel forages (phacelia, forage rape, annual clovers…) has been poorly studied. These plants would have interesting nutritive value (Meslier *et al.*, 2014) and would also show other benefits for ruminants as they contain different secondary metabolites (tannins, carotenoids, vitamins, polyphenols…) that are likely to improve animal performances and health, to decrease nitrogen and methane losses (Leiber *et al.*, 2012) and to improve the quality of animal products. For instance, carotenoids and vitamin E as antioxidants, or some carotenoids as vitamin A precursors, influence animal health and the nutritional quality of animal products. Carotenoids are also able to influence the sensorial characteristics of products, directly by giving a yellow colour to fat (in meat, butter and cheese from bovines essentially) but also indirectly via their antioxidant properties (Nozière *et al.*, 2006). Elsewhere, tannins-containing plants never cause bloat and are effective against parasitic worms (Lüscher *et al.*, 2014). Concentrations of secondary metabolites in plants would vary first with species, but also with plant phenologic development. The first aim of this study was to determine the nutritive composition of several cover crops currently used as forages in French ruminant production systems, as well as their carotenoid, tocopherol and tannin contents. The changes between vegetative and flowering stages of these species were also examined for these parameters.

Material and methods

This study was carried out at the INRA Theix experimental site in France (45°430N, 03°010E; 890 m above sea level) on a deep silt loam soil. The growing conditions were

characterized by low rainfall (134 mm) and high temperatures (17°C on average) compared to historic annual averages. Pure stands of five leguminous species, sainfoin (*Onobrychis viciifolia,* cv. Ambra), berseem clover (*Trifolium Alexandrinum,* cv. Tigri), crimson clover, (*Trifolium incarnatum,* cv. Bolsena), vetch (*Vicia sativa,* cv. Marianna), alfalfa (*Medicago sativa,* cv. Timbale), and two non-leguminous species phacelia *(Phacelia tanacetifolia,* cv. Lilla), buckwheat (*Fagopyrum esculentum,* cv. Hajnalka) were sown on 22 April 2015 in plot of 3 × 1.35 m. Plants were harvested at two stages of growth: vegetative (VS) and flowering (FS), these stages being achieved at different dates by plants (Table 1). Plots were arranged in a split-plot design with three replications, where main plot was species and subplot was stage of growth, giving a total of 42 experimental plots (7 species × 2 stages × 3 replicates).

Table 1. Harvest dates, dry matter content (DM, %) at harvest and dry matter yield (DMY, kg ha^{-1}) for the seven cover crops species evaluated.

	Vegetative Stage (VS)			Flowering stage (FS)		
Species	Harvest date	DM, %	DMY, kg ha^{-1}	Harvest date	DM, %	DMY, kg ha^{-1}
Alfalfa	22-06-2015	22.0	1889	13-07-2015	35.4	2935
Phacelia	08-06-2015	13.2	2108	29-06-2015	18.3	7744
Sainfoin	22-06-2015	16.8	1565	09-07-2015	31.3	3478
Buckwheat	01-06-2015	13.2	757	18-06-2015	14.3	3114
Berseem Clover	29-06-2015	17.9	3615	09-07-2015	28.9	4413
Crimson Clover	22-06-2015	13.9	1720	06-02-2015	26.6	4787
Vetch	15-06-2015	16.5	1661	02-07-2015	22.7	5171

Forages were harvested using a precision chop forage harvester. All plant material in a plot was cut near to ground level (20 mm), and harvested material was weighed to determine fresh yield. Then, two subsamples were collected: one was used for DM determination (60°C for 72h) and nutritive value analyses, and the other was freeze-dried for secondary metabolites (condensed tannins, carotenoids and tocopherols) analyses. Dried samples were analysed for ash, crude protein (CP) content (Dumas method, Rapid N Cube, Elementar GmbH), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents using an Ankom fiber analyser (Ankom Technology, NY, USA) with sulphites. Condensed tannins (CT) content was determined by the colorimetric HCl-butanol method using an acetone-water solution (70:30, v/v) as solvent for extraction. Carotenoids and tocopherols were analysed as described by Chauveau-Duriot *et al.* (2010). Data were analysed as a split-plot design using the mixed procedure of SAS (version 9.4, SAS institute Inc., NC, USA). In the analysis, "species" (n = 7) was treated as main factor and "stage of growth" was nested factor within "species" as harvest dates were different according to species.

Results and discussion

The CP, NDF and ADF contents showed similar patterns for all species (Table 2), with higher CP and lower NDF and ADF contents for VS than for FS. These changes in nutritive value are commonly observed with increasing maturity of plants. However, these changes between both stages were higher for phacelia and buckwheat compared to the leguminous species. This could be explained by the leaf/stem ratio as phacelia and buckwheat had a lower proportion of leaves at FS (data not shown). Across both growth stages, leguminous species had higher CP content than phacelia and buckwheat. The CP values for alfalfa and vetch were lower than published references whereas they were comparable for sainfoin and berseem clover (www.feedipedia.org).

Table 2. Nutritive (in g kg^{-1} DM) composition, condensed tannin (in g kg^{-1} DM), carotenoid (in mg kg^{-1} DM) and tocopherol (in mg kg^{-1} DM) contents of seven cover crops species used as forages and harvested at vegetative (VS) and flowering stages (FS)

	Cover crop species																
	Alfalfa		Phacelia		Sainfoin		Buckwheat		Berseem		Crimson		Vetch			*P-values*	
	VS	VF	VS	VF	VS	VF	VS	VF	VS	VF	VS	VF	VS	VF	SEM	Specie	Stage(specie)
OM[1]	895	897	790	871	912	926	824	902	888	902	865	891	891	904	6.0	<0.001	<0.001
CP[1]	177	168	151	88	212	157	187	126	192	153	199	166	202	187	5.2	<0.001	<0.001
NDF[1]	291	331	208	344	215	311	203	326	371	355	263	374	276	301	9.1	<0.001	<0.001
ADF[1]	209	241	151	253	159	233	145	236	228	248	169	252	195	215	6.7	<0.001	<0.001
CT[1]	1.5	1.6	1.5	1.7	69.3	51.6	14.8	14.5	2.0	1.9	1.9	2.6	2.6	2.5	0.79	<0.001	<0.001
Antheraxanthin	35.6	59.0	74.2	22.2	85.5	73.0	118.8	14.1	55.4	54.0	64.5	26.9	64.6	49.5	7.3	<0.001	<0.001
β-Carotene	267.0	256.4	243.5	112.9	301.4	334.8	240.9	117.3	282.7	190.1	238.0	135.3	298.0	271.0	29.7	<0.001	<0.001
β-cryptoxanthin	2.1	2.0	2.1	1.1	2.9	2.8	2.6	1.6	2.0	1.3	2.9	1.2	2.3	1.3	0.3	<0.001	<0.001
Lutein	355.7	305.4	294.4	130.4	421.9	385.6	388.4	170.8	395.8	287.7	394.5	221.8	393.5	337.9	39.0	<0.001	<0.001
Lutein epoxyde	12.0	7.0	9.2	5.8	9.5	5.5	11.8	8.1	15.8	10.5	10.2	7.8	10.0	7.0	1.5	<0.001	0.007
Neoxanthin	101.3	90.0	83.2	38.3	97.3	118.4	88.6	38.7	118.5	89.1	99.7	66.3	106.2	104.8	13.4	<0.001	<0.001
Violaxanthin	252.3	150.4	198.5	88.4	236.5	142.5	238.6	21.8	246.4	127.1	205.8	76.7	267.5	160.7	24.3	0.01	<0.001
Zeaxanthin	15.1	24.7	23.0	8.9	35.6	54.0	53.4	28.1	18.5	22.5	28.2	14.5	28.6	20.3	4.7	<0.001	<0.001
Carotenoid sum	1041.1	894.9	928.1	407.8	1190.5	1116.6	1143.1	400.5	1135.1	782.2	1043.9	550.5	1170.8	952.5	111.7	<0.001	<0.001
Tocopherol[2]	61.4	102.4	92.5	74.5	80.1	176.3	106.3	80.1	77.5	94.9	52.8	40.8	80.9	72.8	14.4	0.008	<0.001

[1] OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre and CT: condensed tannin.

[2] tocopherol was the sum of α and γ isomers of tocopherol, the both forms being present in the plants.

Condensed tannin content was low (< 2.6 g kg^{-1} DM) in all species except sainfoin and buckwheat with respectively 69.3 and 14.8 g kg^{-1} DM at VS and 51.6 and 14.5 g kg^{-1} DM at FS. The CT content did not vary with stage for buckwheat whereas it decreased for sainfoin. This reduction may be related to both the leaf/stem ratio and the leaves age as CT in sainfoin would be mainly located in leaves and their concentration would decrease with leaves maturity (Malish *et al.*, 2015). Leiber *et al.* (2012) showed that both leaves and flowers contained important concentration of polyphenols in buckwheat, this could explained the unchanged concentration with maturity. Total carotenoids varied from 928 to 1191 mg kg^{-1} DM at VS and from 401 to 1116 mg kg^{-1} DM at FS. These results indicated that these species would be good suppliers of carotenoids to grazing ruminants compared to other forages (Noziere *et al.*, 2006; Graulet *et al.*, 2012), confirming also that lutein, β-carotene and violaxanthin are quantitatively the major carotenoids for all species. Total carotenoids decreased for all species between VS and FS, their composition not being truly affected. The carotenoid concentration is known to decrease with phenological stage due to the reduction in the leaf/stem ratio between vegetative and flowering stage (Noziere *et al.*, 2006; Graulet *et al.*, 2012). This reduction was especially important for phacelia (-56%), buckwheat (-65%) and crimson clover (-47%), may be due to a lower proportion of leaves in the total aerial part for these plants, whereas it was far more limited for the other plants like sainfoin, vetch or alfalfa. Concentrations of tocopherols were lower than values previously reported for natural grasslands (256 mg kg^{-1} DM, Graulet *et al.*, 2012) and varied highly with species from 40.8 mg kg^{-1} DM for crimson clover at FS to 176.3 mg kg^{-1} DM for sainfoin at FS stage. Within species, concentrations varied differently with stages since it decreased between VS and FS for phacelia, buckwheat, crimson clover and vetch whereas it increased for alfalfa, sainfoin and berseem clover. The regulation of tocopherol synthesis in plants is not well known but it would depend on the availability of the phytyl chemical group that is increased with chlorophyll breakdown following chloroplast senescence (Bramley *et al.*, 2000). Moreover, a positive correlation has been observed between tocopherol concentration and drought conditions (Kalinova *et al.*, 2006). In the present experiment, alfalfa, sainfoin and berseem clover were indeed the last plants harvested and suffered from the very high temperatures recorded in July 2015.

Conclusions

These results showed that nutritive composition and secondary metabolites contents were different between species and growth stages, the differences between species being more important at flowering stage than at vegetative stage. Overall, findings confirmed interests for ruminants of these cover crops especially at vegetative stage in term of nutritive value and vitamin concentrations. In this study, sainfoin was the most interesting species. The important variations in carotenoid and tocopherol contents observed for some species between VS and FS have to be investigated in less dry climatic conditions.

References

Bramley P.M., Elmadfa I., Kafatos A., Kelly F.J., Manios Y., Roxborough H.E., Shuch W., Sheehy P.J.A., Wagner K.H. 2000. J. Sci. Food Agric. 80:913-938.

Chauveau-Duriot B., Doreau M., Noziere P., Graulet B. 2010. Anal. Bioanal. Chem. 397: 777-790.

Decourtye A., Bouquet C. 2010, Fourrages 202, 117-124.

Graulet B., Piquet M., Duriot B., Pradel P., Hulin S., Cornu A., Portelli J., Martin B., Farruggia A.H. 2012. Fourrages 209:59-68.

Kalinov J., Triska J., Vrchotova N. 2006. J. Agric. Food Chem. 54:5330-5335.

Leiber F., Kunz C., Kreuzer M. 2012. Czeth J. Anim. Sci., 57:10-18.

Luscher A., Mueller-Harvey I., Soussana J.F., Rees R. M. Peyraud J.L. 2014. Grass Forage

Sci. 69:206-228
Malisch C.S., Lüscher A., Baert N., Engström M., Studer B.,Fryganas C., Suter D., Mueller-Harvey I., Salminen J.P. 2015. J. Agric. Food Chem. 63:10234-10242.
Meslier E., Férard A., Crocq G., Protin P.V., Labreuche J. 2014. Fourrages. 218:181-184.
Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. 2006. Anim. Feed Sci. Technol., 131:418-450
Vertès F., Jeuffroy M. H., Justes E., Thiébeau P., Corson M. 2010. Innov. Agr. 11, 25-44.

Effect of rapeseed or linseed supplements on the plasma and milk carotenoid concentrations in dairy cows fed grass-based diets

B. Graulet[1]*, D. Pomiès[1], A. Ferlay[1], B. Martin[1]

UMR1213 Herbivores, INRA, VetagroSup, Clermont Université, Université de Lyon, F–63122 Saint-Genès-Champanelle, France.

*benoit.graulet@inra.fr

Abstract

Dietary carotenoid availability could be limited by the low level of fat in the cow diet. The effect of different dietary oilseed supplements was observed on carotenoid concentrations in plasma and milk as well as milk colour in cows receiving high level of dietary carotenoids through a grass-based diet as grass silage during the indoor period or grazing during spring and summer time. Rapeseed supplements were more efficient than extruded linseed to increase the plasma carotenoid concentration by comparison to the control diet, whereas the form of rapeseed presentation (whole or extruded seeds or fat-rich meal) had no effect. The effect of oilseed supplements on milk carotenoid concentration didn't reach significance although the mean values were generally numerically higher than those for control cows. Moreover, the yellowness of milk was not modified by the dietary oilseed supplementation.

Keywords: carotenoids, cow milk, grass feeding, lipid supplementation

Introduction

Butter and cheese colour that are of primary interest for the appreciation by the consumers are under the dependency of carotenoid concentration in ruminant milk. Milk carotenoid concentration varies according to several main factors (Nozière et al., 2006) including the species, the breed, the diet composition and the animal physiology, performances and health. Whatever the species, the ability of carotenoid absorption by the intestine is usually known to be limited and dependent on the presence of lipids in the diet to favour absorption process. In the ruminant, the diet is usually rather poor in lipids which could be a limiting step of carotenoid absorption. Moreover, even though the extent of carotenoid degradation in the ruminant remains controversial (Nozière et al., 2006), it is likely to be low when carotenoids are supplied naturally from feedstuffs. Fresh grass is especially rich in carotenoids and its consumption by cows always induces a significant increase in milk β-carotene content (Nozière et al., 2006). However, this increase is not as high as it could be, considering the high carotenoid content in fresh grass. One hypothesis to explain this fact could be that the lipid content of the diet is not sufficient to let an efficient carotenoid absorption (Furr and Clark, 1997). The first aim of the present study was to explore if dietary oilseed supplements could induce an increase in the carotenoid status of dairy cows. A second aim was to test if a potential response was dependant of the form of lipid supplementation with feedstuffs.

Material and methods

The experimental design corresponded to the first experimental year of the study described in Lerch et al. (2012). It included sixty Holstein cows divided into 5 equivalent groups of 12 cows balanced for calving date, milk yield, fat content, protein content, lactation stage, and parity. Cows were fed for the entire lactating period either a control diet (C) based on 70% of grass forage (75% grass silage + 25 % hay) and 30% of concentrate (wheat and solvent extracted rapeseed meal), or the same diet with part of concentrate substituted by extruded linseeds (diet EL, rich in linoleic acid), extruded rapeseeds (diet ER), fat rapeseed meal (diet FRM) or whole rapeseeds (diet WR); these 3 latter diets being rich in oleic acid. These lipid

supplements provided an oil level of 3% of dry matter intake (DMI). During the outdoor period, the winter forage mix was removed and all the cows had access night and day to the same pasture; they received the same lipid supplement than during the winter period, but at constant amount (5 kg/d of concentrate). Individual blood (caudal vein just before the morning meal) and milk (two consecutive milkings) samples were collected at 4 periods: in calendar wk 46 (mid-November) of the Year 1, then in wks 15 (April, indoor), 23 (June, outdoor) and 36 (September, outdoor) of the Year 2. Carotenoid analysis in plasma and milk were performed as described by Chauveau-Duriot et al. (2010). Characteristics of milk colour were determined by spectrometry (Calderón et al., 2007). Data were analysed as repeated measures using the mixed procedure of SAS with corresponding data obtained in the pre-experimental period (wk 46 of the Year 1) as covariate (except for milk colour characteristics where no covariate was used). The model included covariate, treatment (i.e. lipid supplement), period, parity, and the complete interactions as fixed effects.

Results and discussion

In plasma or milk, six carotenoids were detected and quantified: zeaxanthin, lutein, β-cryptoxanthin, 13-*cis*-, 9-*cis*- and all-*trans* isomers of β-carotene, with all-*trans* β-carotene being the most abundant. Plasma carotenoid concentration increased between the indoor (5.01 µg/ml) and the outdoor periods (7.18 and 6.01 µg/ml in June and September, respectively) whatever the treatment (Table 1). This observation probably results mainly from variations in dietary carotenoid intakes since carotenoid contents are known to be higher in fresh grass in spring (June) than in September or in grass silage (main forage of the diet in April) (Nozière et al., 2006; Graulet et al., 2012).

Table 1: Variations of carotenoid concentrations (µg/ml) in plasma of cows fed a grass-based diet supplemented with extruded linseed (EL) or rapeseed (ER), fat rapeseed meal (FRM), whole rapeseed (WR), or not (C). Only the statistical significance of treatment (T), period (P) and their interaction (T×P) are presented.

		Treatments						P values		
		ER	WR	EL	FRM	C	se	T	P	T×P
Lutein	*April*	0.16	0.16	0.20	0.22	0.13				
	June	0.29	0.31	0.31	0.31	0.22	0.02	**	***	ns
	September	0.22	0.29	0.24	0.28	0.19				
Zeaxanthin	*April*	0.07	0.07	0.10	0.09	0.05				
	June	0.16	0.15	0.18	0.15	0.10	0.02	ns	***	ns
	September	0.12	0.15	0.12	0.13	0.08				
β-Cryptoxanthin	*April*	0.14	0.14	0.15	0.18	0.10				
	June	0.23	0.23	0.20	0.23	0.16	0.01	***	***	ns
	September	0.17	0.18	0.16	0.19	0.14				
13cis-β-Carotene	*April*	1.45	1.40	1.36	1.54	1.06				
	June	2.00	2.00	1.57	1.98	1.28	0.10	***	***	*
	September	1.63	1.74	1.49	1.60	1.23				
9cis-β-Carotene	*April*	0.14	0.13	0.12	0.17	0.12				
	June	0.19	0.21	0.13	0.19	0.14	0.02	*	***	ns
	September	0.21	0.23	0.16	0.18	0.14				
All-trans-β-Carotene	*April*	3.38	3.22	3.29	3.54	2.49				
	June	5.36	5.21	4.43	4.99	3.44	0.26	***	***	ns
	September	4.18	4.09	3.70	4.10	3.20				
Carotenoid sum	*April*	5.23	5.08	5.16	5.64	3.91				
	June	8.13	8.04	6.75	7.72	5.28	0.38	***	***	*
	September	6.38	6.60	5.75	6.37	4.94				

Dietary fat supplements increased plasma carotenoid concentrations (5.89 to 6.58 µg/ml) by comparison to C diet (4.70 µg/ml), but the effect was less pronounced for the EL group than for the 3 rapeseed-supplemented groups (ER, WR and FRM). This is especially observed during the grazing period, in June ($P_{T\times P}$=0.039). At this time, plasma carotenoid concentrations exceeded 8 µg/ml when cows received rapeseed supplements, whereas it was 6.75 for EL cows and only 5.27 µg/ml for C cows. Thus, considering similar carotenoid intakes between groups, lipids from oilseed supplementations could have effectively favoured the absorption of the dietary carotenoids by the intestine. Moreover, this effect seemed to be more pronounced with rapeseed than linseed as supplement but not different according to the form of rapeseed supply. It could consequently suggest that the fatty acid composition of the dietary lipid source had a regulatory impact on the underlying process. In human, a higher intestinal absorption of β-carotene has been observed when subjects consumed a meal containing beef tallow, rich in oleic acid, per comparison to a meal containing sunflower oil, rich in linoleic acid (Hu et al., 2000). Conversely, the very long chain n-3 fatty acid, eicosapentaenoic acid, has been shown to inhibit β-carotene intestinal absorption by cell lines, without effect of linolenic acid (Mashurabad et al., 2016). Thus, a possible explanation of the present results could be that the increase in dietary lipid content globally favoured carotenoid intestinal absorption but this effect could be minored by an inhibitory process on the intestinal transporter when these lipids were rich in n-3 fatty acids like in the case of linseed. Another hypothesis could be that plasma carotenoid enrichment would result from a change in the lipoprotein profile (HDL enrichment and enlargement), as previously observed when cows were supplemented with linoleic acid-rich supplements (Ashes et al., 1984). A differential efficiency of intestinal carotene conversion into retinol was unlikely since retinol concentrations were not affected by treatment (not shown) and because lutein, a non-provitamin-A carotenoid, was also affected.

The milk and fat yield of the cows were not different between groups but the fat content was significantly higher for WR (39.2 g/kg) cows than others (33.9 to 36.4 g/kg; P<0.01).

In this study, all-*trans* β-carotene concentrations in milk fat were higher than reported before for the Holstein breed but rather in the order of magnitude of concentrations reported for Anglo-Normand breeds, Jersey and Guernsey (Nozière et al., 2006). It probably reflects the carotenoid-rich forages supplied to cows all along the experiment (grass silage then fresh grass). For all-*trans* β-carotene, lutein, zeaxanthin and total carotenoids, time-dependent variations were similar, i.e. the mean values were higher in June than in the 2 other periods (Table 2). Total carotenoid concentration in milk fat was not significantly affected by the dietary treatment (P=0.141). Individual characteristics inducing variability to the treatment response could prevent to reach easily statistical significance. Lutein values tended to be higher for the EL cows, especially during the pasture grazing period, by comparison with ER or C cows, values being intermediate for WR and FRM. Similarly, zeaxanthin concentration for the FRM group was also lower than that for the EL group and the differences were significant. However, all-*trans* β-carotene concentration was higher for FRM cows (also EL in June) than for C cows, other groups being intermediate. Surprisingly, no variation among treatments was observed in the b index of the milk, probably due to the individual variability of response to treatments. However, other pigments found in milk (like riboflavin for example) could also interfere into the relationship between carotenoid concentration and yellow colour. Only the L index, corresponding to the positioning on the dark-light axis, was decreased by the supplementation with rapeseed provided to cows as RFM by comparison to others, especially ER (P = 0.028). This discrepancy was the most pronounced at the beginning of the grazing period in June. At the end of the summer, all the values were close, whatever the cow diet, and higher than those observed in the previous periods, indicating a brighter milk.

Table 2: Variations of colour (Arbitrary units) and carotenoid concentrations (mg/kg fat) in milk of cows fed a grass-based diet supplemented with extruded linseed (EL) or rapeseed (ER), fat rapeseed meal (FRM), whole rapeseed (WR), or not (C). Only the statistical significance of treatment (T), period (P) and their interaction (T×P) are presented.

		Treatments						P values	
		ER	WR	EL	FRM	C	se	T	P	T×P
L index	*April*	79.8	79.7	79.8	78.9	80.1	0.51	*	***	+
	June	79.4	78.8	78.5	76.4	77.7				
	September	82.4	81.2	81.8	81.3	81.7				
a index	*April*	-1.93	-2.09	-2.26	-2.08	-2.31	0.10	ns	***	ns
	June	-1.70	-1.59	-1.84	-1.81	-1.95				
	September	-1.57	-1.50	-1.53	-1.64	-1.59				
b index	*April*	6.55	6.92	6.74	6.32	6.99	0.31	ns	***	ns
	June	7.59	8.18	7.53	7.33	7.21				
	September	7.34	7.81	7.42	7.49	7.41				
Lutein	*April*	2.09	0.69	1.63	1.35	0.42	0.35	*	***	ns
	June	3.13	5.07	5.41	4.13	3.33				
	September	1.07	3.89	3.00	2.45	2.56				
Zeaxanthin	*April*	0.43	0.13	0.45	0.20	0.00	0.10	**	***	ns
	June	0.77	1.13	1.53	0.73	0.59				
	September	0.36	0.71	0.80	0.44	0.36				
β-Cryptoxanthin	*April*	0.25	0.23	0.34	0.31	0.36	0.12	ns	ns	ns
	June	0.11	0.13	0.72	0.00	0.00				
	September	0.11	0.35	0.31	0.00	0.22				
13cis-β-Carotene	*April*	3.00	3.23	2.16	2.87	2.40	0.33	ns	ns	ns
	June	3.86	3.37	3.14	3.71	2.34				
	September	2.66	2.50	2.26	3.23	2.20				
9cis-β-Carotene	*April*	0.15	0.02	0.23	0.13	0.00	0.11	ns	ns	ns
	June	0.44	0.38	0.49	0.27	0.20				
	September	0.14	0.33	0.18	0.70	0.14				
All-trans-β-Carotene	*April*	9.23	8.34	6.24	8.09	6.54	0.60	*	***	*
	June	9.24	9.35	12.08	12.42	7.57				
	September	8.37	8.30	7.16	9.46	7.21				
Carotenoid sum	*April*	15.2	12.7	10.6	13.0	10.0	1.3	ns	***	ns
	June	17.6	19.6	22.9	21.3	14.3				
	September	12.7	16.1	13.2	16.4	13.0				

Conclusions

The increase in plasma carotenoid concentration observed in cows supplemented with oilseeds, especially rapeseed, didn't lead either to a statistical significant increase in milk carotenoid secretion or to a change in milk colour. A limiting step in their transfer from plasma to milk could explain it.

Acknowledgements

This work was co-funded by the European Commission in the TrueFood project (6th FP). We acknowledge agents of INRA-Herbipole experimental facility for animal care.

References

Ashes J.R., Burley R.W., Sidhu G.S., Sleigh R.W. 1984. Biochim. Biophys. Acta 797:171-177.

Calderón S. F., Chauveau-Duriot B., Pradel P., et al., 2007. J. Dairy Sci. 90: 5651-5664.

Chauveau-Duriot B., Doreau M., Noziere P., Graulet B. 2010. Anal. Bioanal. Chem. 397: 777-790.
Furr H.C., Clark R.M. 1997. Nutr. Biochem. 8:364-377.
Graulet B., Piquet M., Duriot B., Pradel P., et al., 2012. Fourrages 209: 59-68.
Hu X., Jandacek R.J., White W.S. 2000. Am J. Clin Nutr. 71:1170-1180.
Lerch S., Ferlay, A., Pomiès D., Martin B., Pires J.A.A. Chilliard Y. 2012. J. Dairy Sci. 95:1956-1970.
Mashurabad P.C., Kondaiah P., Palika R., et al., 2016. Arch. Biochem. Biophys. 590:118-124.
Nozière P., Graulet B., Lucas A., Martin B., et al., 2006. Anim. Feed Sci. Technol. 131: 418-450.

Season and dairy cow breed influence milk composition, cheese yielding capacity and butter properties

C. Hurtaud[1]*, C. Rose[2], A. Valais[2], F. Launay[3], L. Delaby[1]
[1]PEGASE, INRA, Agrocampus Ouest, 35590, Saint-Gilles, France
[2]Organisme de Sélection en Race Normande, ZA Le Gué Thibout, 61700, Domfront, France
[3]Domaine Expérimental du Pin-au-Haras, INRA, 61310, Exmes, France
*Catherine.Hurtaud@inra.fr

Abstract
A trial was carried out to study the effects of 2 feeding systems (FS) (high and low inputs FS) with 2 breeds (Holstein and Normande) and 2 genetic types ("high milk yield" type and "high fat and protein contents" type) during 2 seasons: spring and autumn. Sixty-one cows were followed. Cows were divided into 8 groups according to their breed, their genetic type and their FS. The high FS consisted of a high energy diet and the low FS consisted of a low energy diet (no concentrate). Cheese and butter making aptitudes were tested. There were few effects of genetic types and feeding systems. Normande cows produced less milk but richer milk. Milk fat globules were larger and casein micelles were smaller. Laboratory cheese yield was better. Normande cows produced a more yellow butter. Season had an impact on milk yield and composition due to lactation stage.

Keywords: dairy cow, breed, feeding, butter

Introduction
In French dairy herds, the Holstein (Ho) cow has often replaced the less productive Normande (No) cow, but whose milk is richer in solid content (Lawless et al., 1999). The effect of No breed in terms of the quality of dairy products has never been clearly demonstrated, even if the milk from No appears to have a better coagulation capacity (Vertès et al., 1989). In Normandy, several types of PDO cheeses are manufactured with raw milk. Butter is also manufactured in Normandy. The aim of this trial was to study the effect of breed and season on milk composition, technological properties for cheese and butter making and on butter properties.

Material and methods
Experimental design
The experiment was carried out at INRA experimental farm of Le Pin-au-Haras in Normandy. Two breeds, Ho and No, 2 genetic types, "high milk yield" type (M) or "high fat and protein contents" type (C) and 2 feeding systems (FS), a High FS (HFS) with high energy diets and a Low FS (LFS) with low energy diets were used in this trial. These 2 systems corresponded to 2 different winter diets and 2 different pasture managements. Sixty one cows were divided into 8 groups according to their FS, their breed and their genetics. Calvings were compacted from January to March. Cows were milked every day at 0730 h and 1730 h.
Description of feeding systems and diets
We sampled milk of dairy cows during two periods: spring (all the dairy cows were grazing) and autumn (all the dairy cows were grazing but grass was supplemented with forage, the amount and nature depending on the feeding system).
Spring: HFS and LFS cows were grazing white clover (15-20%) - perennial ryegrass (80 - 85%) pastures. The total grassland area per cow allocated in spring was 0.22 ha. HFS cows received 4 kg/d of concentrates and LFS cows only received minerals and vitamins. Cows were managed on a simplified rotational grazing system (Delaby and Peyraud, 2003) based on

3 to 4 plots in spring and extended to 5 plots in autumn. Diets were formulated to meet cows' requirements for HFS and 80% for LFS (INRA, 2007).
Autumn: HFS and LFS cows were grazing white clover (15-20%) - perennial ryegrass (80 - 85%) pastures. HFS cows received 4 kg/d of concentrates. The total grassland area used in autumn was 0.33 ha per HFS cow and 0.55 ha per LFS cow. HFS cows received 5 kg of corn silage and 2 kg of grass silage and LFS cows received 5 kg of grass silage.
Milk sampling
Each sampling was related to a particular group of cows: Ho-M-HFS, Ho-C-HFS, Ho-M-LFS, Ho-C-LFS, No-M-HFS, No-C-HFS, No-M-LFS, or No-C-LFS. Each week, during eight weeks, two groups were sampled for determination of milk composition, cheese yielding aptitude and butter making. The milk of 4 successive milkings was transferred into specific tanks, which were then collected by the dairy and the laboratory. Finally, 2 series of manufacture by treatment were carried out for all the analyses (one in spring and the other one in autumn).
Milk composition. Individual analyses were performed at the Normandy Interprofessional Milk Analysis Laboratory (LILANO, Saint-Lô, France). Milk fat, protein and lactose contents were determined by mid-infrared spectrometry (MilkoScan FT6000, Foss, Hillerød, Denmark) and SCC by flow cytometry. A detailed analysis of milk samples was performed from milk of the groups of cows collected for butter making. Total nitrogen content, non-casein nitrogen (NCN), and casein were determined according to the Kjeldahl methods described by Alais (1984). Total calcium was analyzed by atomic spectrophotometry absorption on milk. A sample was collected and kept at room temperature with potassium dichromate (Merck, Darmstadt, Germany) to evaluate milk fat globule (MFG) and casein size distribution by laser light scattering (Mastersizer 3000, Malvern, UK).The average diameter $d_{4,3}= \Sigma(Ni \times di^4)/\Sigma(Ni \times di^3)$ was calculated by the Malvern software.
Milk coagulation and syneresis measurements. The coagulation capacity of these milks was measured by Berridge test. Laboratory curd yields were measured by centrifugation (2700×g, 10 min) of coagulated milk (0.25 mL of rennet containing 52 mg/L of chymosin). Curd and whey were weighted for calculation of fresh curd yield.
Butter making. Each week, the bulked milk of two groups was taken for the manufacture of butter. Milk was delivered to the dairy the day before manufacture of the butters. Milk from 4 milkings (2 morning and 2 evening) of cows of each group was put into individual tanks. This milk was cooled to 4°C as quickly as possible by the tank cooling system. The milk was sent by truck to Elvir private research laboratory (Savencia, Condé-sur- Vire, France) to be skimmed and processed into butter. Cream churning time (time elapsed from time zero, when the churn was started, to time the butter granules formed), butter yield and pH were measured during the butter production process. Color of butter was also measured after two weeks with a chromameter.
Sensory analysis. It was conducted at the ENILIA National School of Milk and Agrifoods Industries (ENILIA, Surgères, France) two weeks after the butters had been produced, at a tasting temperature of 14 ± 1°C per ISO 22935-2 (ISO, 2009) guidelines. The jury was a trained panel of 8 tasters formed per ISO 22935-1 (ISO, 2009) standard guidelines (16 training sessions of 1 h 30 min for jury before the trial and after; 40 trainings/ year). Data were collected and analyzed with FIZZ software (Biosystèmes, Couternon, France). At each tasting session, members of the tasting jury were asked to score spreadability at 4°C, color, sticking to the knife, friability, smell (overall intensity and the aroma tones double cream, milk, cut grass, hazelnut, hay, and rancid), flavor (overall intensity, acid taste, and the aroma tones cream, milk, cooked milk, hazelnut, cut grass, rancid, metallic, hay and persistence of the flavor), firmness, melt-in-the-mouth and fat texture. Scores were given on a 0-to-10 scale, with higher scores given to higher intensities.

Calculation and statistical analyses

All statistical analyses on the data set were performed using SAS software (SAS 9.2 Institute Inc., Cary, NC). The statistical significance threshold was set to $P < 0.05$. Trend was set to $P < 0.10$. The effects of breed, FS, season, genetic type, and their interactions on milk traits were assessed by period using the mixed procedure of SAS according to the statistical model: $Y_{ijklm} = \mu + Breed_i + Season_j + Genetics_k(Breed) + FS_l + Breed_j \times FS_l + Breed_i \times Season_j + \varepsilon_{ijklm}$ where μ is the mean and Y_{ijkl} is the variable dependent on the fixed effects of $Breed_i$ (Ho, No), FS_l (HFS, LFS), $Season_j$ (spring or autumn), $Genetics_k$ (L or C) and their interactions, and ε_{ijklm} is the residual error associated with each *ijklm* observation. Individual effects were treated as random effects.

Results and discussion

Genetic type (results not shown)

Genetic type had significant effects on few milk parameters (milk yield, fat and protein contents and yields). Genetic type "high fat and protein contents" numerically increased laboratory cheese yields (+7.0 units percent, P=0.115) and tended to decrease churning time (-13 min, P=0.085).

Breed

Milk yield was lower for No cows, but fat and protein contents were higher compared to Ho cows (respectively -5 kg/d, +3.1 and +1.9 g/kg, results not shown). Milk casein, total calcium, citrate contents were higher for No cows compared to Ho cows (Table 1). The increase in the casein and citrate contents is consistent with the literature (Vertès et al., 1989; Hurtaud et al, 2009). The increase in casein content appears to be related to the presence of casein variants, in particular the variant B of k-casein. Casein micelles were smaller and milk fat globule diameter was larger for No cows (Table 1). Remeuf et al (1991) showed that milks with small casein micelles coagulated more quickly and their curd was firmer. Breed reputed for butter making (Jersey, Guernsey, No) have milk fat globules larger than productive breed cows (Ho). The larger milk fat globules could result in a better butter-making capacity of the creams (Couvreur and Hurtaud, 2007). Laboratory cheese yield was higher with milk from No cows (Table 1). The improvement of the coagulation capacity with the milk from No cows results from its high content in coagulable proteins. This improvement in coagulation capacity could also be linked to the polymorphism of proteins, in particular k-casein. According to Grosclaude (1988), the milk of the homozygote k-casein BB (very prevalent in the No breed) has a shorter time of coagulation and a shorter firming time, as well as a firmer curd than the milk of the homozygote k-casein AA (very prevalent in the Ho breed). Breed had no effect on butter churning time. Butter yield tended to decrease with milk from No cows (P=0.109) (Table 1). No milks led to an increase in butter yellow color index perhaps due to larger milk fat globules (Michalski et al, 2003) (Table 1). Concerning sensory properties, globally, there were no marked breed effects on the smell, or flavor of the butters produced. The butters showed few specific sensory properties. Metallic aroma was lower in butters made with No milk (Table 2).

Feeding systems (results not shown)

Feeding systems had no effect on milk parameters, butter and cheese. In spring, the feeding systems were quite the same: all the cows were grazing, but some received 4 kg of concentrate. In autumn, they were also quite the same. All the cows were also grazing. To complete their diet, LFS cows only received grass silage and HFS cows received grass silage, maize silage and concentrate.

Table 1: Effect of breed and season on milk composition and technological properties

	Ho		No			Effect		
	Spring	Autumn	Spring	Autumn	RMSE	Season	Breed	Season* Breed
Casein, g/kg	24.0	26.0	27.9	28.2	1.77	0.229	0.009	0.393
Total Ca, mg/kg	1117	1335	1256	1337	60.2	0.001	0.045	0.051
Citrate, g/L	1.44	1.36	2.14	1.75	0.374	0.236	0.020	0.427
Fat globule diameter, µm	3.85	3.65	4.29	3.88	0.075	<0.001	<0.001	0.209
Casein diameter, µm	0.134	0.168	0.126	0.121	0.0193	0.181	0.021	0.024
Laboratory cheese yield, %	34.5	61.2	47.6	67.6	7.06	<0.001	0.024	0.381
Butter churning time, min	40.5	66.2	42.5	62.7	9.98	0.002	0.879	0.601
Butter yield, %	44.1	45.0	43.5	43.2	1.38	0.690	0.109	0.427
Butter color (b)	28.3	28.7	33.9	32.4	2.29	0.645	0.004	0.434

Table 2: Effect of breed and season on butter sensory properties.

	Ho		No			Effect		
	Spring	Autumn	Spring	Autumn	RMSE	Season	Breed	Season* Breed
Color	4.0	4.5	5.8	5.6	0.36	0.477	<0.001	0.138
Firmness in mouth	4.5	5.8	5.5	6.1	1.06	0.095	0.276	0.537
Fat in mouth	3.2	3.7	3.1	3.8	0.32	0.008	0.980	0.593
Global odor	4.3	5.0	4.5	4.9	0.47	0.049	0.802	0.553
Global flavor	4.4	4.6	4.3	4.8	0.30	0.026	0.646	0.429
Metallic aroma	0.3	0.4	0.2	0.2	0.11	0.100	0.019	0.239

Season

The two seasons were spring (May and June) and autumn (September and October). Milk yield was largely lower in autumn than in spring (-8.2 kg/d) and milk fat and protein contents were higher (respectively +7.9 and +4.2 g/kg) (results not shown). This difference was not due to seasons but to lactation stage. Calving period took place at the beginning of the year, from January to March. So in September and October, most of the cows were in late lactation. Season had no effect on casein and citrate contents. Milk calcium content was higher in autumn due to concentration associated with lower milk production. In autumn, milk fat globules diameters were larger (Table 1). MFG diameter was positively correlated with milk fat content (Couvreur and Hurtaud, 2017). Laboratory cheese yield was higher in autumn probably due to higher contents of milk solids. Butter churning time was longer in autumn (Table 1). This result is quite surprising because milk fat globules were larger in autumn. According to Hillbrick and Augustin (2003), the larger the milk fat globules, the shorter the churning time and the smaller the loss of fat in buttermilk. There was no effect of season of butter yield and color because at each season, cows were grazing. So the amount of ingested β-caroten responsible of yellow color (Nozière et al., 2006) was quite the same. Autumn tended to increase firmness of butter in mouth (P=0.095), increased fat in mouth, global odor and flavor (Table 2). The effect of season on butter firmness could be a consequence of modification of fatty acids. In autumn, dairy cows had a supplementary forage (maize silage or grass silage) and this forage could have increased saturated fatty acids and then increased

fat melting point. A comparative study undertaken on industrial butters produced in winter and summer in France (n = 480) agreed with this assumption (Guyonnet, 1989). Guyonnet (1989) characterized summer butters (pasture) by an average C16:0/C18:1 ratio of 1.00 and a share strength 45% lower, compared with winter butters (silage-based diets, C16:0/C18:1 = 1.50).

Conclusion

In this experiment, No breed had an important effect on milk composition and on laboratory cheese yield. The effect of genetic type was relatively low. In our sampling conditions (spring and autumn grazing), feeding system had no effect on milk composition and properties. In contrary, season had an impact partially due to stage of lactation and to supplemental forage in autumn. More precise investigations would be interesting to confirm these results, for instance making of Camembert cheeses in a dairy.

Acknowledgments

The authors thank all the staff of the experimental farm at le Pin-au-Haras (DEP, INRA, 61310, EXMES, France) and Elvir private research laboratory (Savencia, Condé-sur- Vire, France). We are also grateful to P. Debournoux, N. Huchet, T. Le Mouël, M. Lemarchand, and C. Perrier for technical assistance. This research received financial support from the Normande Genetics Association.

References

Alais C. 1984. Science du lait, principes et techniques laitières. 4ème édition, Sepale, Paris, France.
Couvreur S., Hurtaud C. 2007. INRA Prod. Anim. 20: 369-382
Couvreur S., Hurtaud C. 2017. Animal 11: 507-518
Delaby L., Peyraud J. L. 2003. Anim. Res. 52: 437–460
Grosclaude F 1988. INRA Prod. Anim. 1: 5–17.
Guyonnet, J.P. 1989. Les beurres français – tartinabilité et composition. Doc. Association pour le développement de la Recherche dans L'Industrie Laitière.
Hillbrick G., Augustin M.A. 2003. Aust. J. Dairy Technol. 57: 45-51.
Hurtaud C., Faucon F., Couvreur S., Peyraud J.L. 2010. J. Dairy Sci. 93: 1429-1443.
Hurtaud C., Peyraud J.L., Michel G., Berthelot D., Delaby L. 2009. Animal 3: 1327-1338.
INRA. 2007. Ruminant nutrition: Recommanded allowances and feed tables (ed. R. Jarrige), John Libbey, London, UK.
ISO. 2009. ISO 22935-1 and 2/IDF 099-2. ISO, Geneva, Switzerland.
Lawless F., Stanton C., L'Escop P., Devery R., Dillon P., Murphy J.J. 1999. Livest. Prod. Sci. 62: 43–49.
Michalski M.C., Gassi J.Y., Famelart M.H., Leconte N., Camier B., Michel F., Briard V. 2003. Lait 83: 131–143.
Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. 2006. Anim. Feed Sci. Technol. 131: 418–450.
Remeuf F., Cassin V., Dervin C., Lenoir J., Tomassone R. 1991. Lait 71: 397-421
Vertès C., Hoden A., Gallard Y. 1989. INRA Prod. Anim. 2: 89–96.

Once daily milking after calving: a practice to overcome reproduction problems in mountain low-input dairy systems

D. Pomiès[1]*, F. Fournier[2]
[1]UMR1213 Herbivores, INRA, VetagroSup, Clermont Université, Université de Lyon, F–63122 Saint-Genès-Champanelle, France
[2]UE1414 Herbipôle, INRA, F-63122, Saint-Genès-Champanelle, France
*dominique.pomies@inra.fr

Abstract
With the end of milk quotas, dairy mountain farmers can hardly compete with low-land farmers because of higher costs and lower productivity. So they have to maximize the use of local forages, reduce their inputs and make quality products like PDO cheeses. In 2011, we designed for 5 years two innovative low-input farming systems at an INRA experimental farm (1100 m asl), but those systems led to poor reproductive performances. To overcome the low proportion of pregnant cows (only 32% of the adult cows calved during the following season in 2014-2015), we tested in 2016 a 6-wk period of once daily milking at the beginning of the breeding season (n=28 cows). During this period the cows produced 22% less milk (4% on the total lactation) with higher fat and protein contents (+3.6 and +2.2 g/kg), compared to 28 cows from 2014-2015. Only 14% of the cows were not inseminated during the breeding season (*vs* 32% in 2014-2015), and among the inseminated cows 88% were diagnosed pregnant (*vs* 58% in 2014-2015). The benefit of once daily milking after calving on reproduction is obvious, but this practice will be further checked by an economic study in the context of PDO cheeses.

Keywords: dairy cow, low-input system, once daily milking, reproductive performances

Introduction
With the end of milk quotas in 2015, mountain areas can hardly compete with low-land because of the higher cost of inputs (cereals, fertilizers…) and their lower productivity. So, these farmers have to implement pasture-based milk production systems to reduce their inputs, reinforce their link to terroir and meet consumers' expectations with quality products like PDO cheeses (Horn *et al.*,2013). To study this new context, we conceived and implemented two low-input innovative farming systems called **BOTA** and **PEPI**, for a long term 'system experiment' at the INRA experimental farm Herbipôle (Marcenat, French Massif-central, 1100 m asl) from 2011 to 2015. These two systems were designed to be as self-sufficient as possible: 12 Holstein [**Ho**] and 12 Montbéliarde [**Mo**] cows each; a short breeding season of ~77 days; a calving season before turning-out to pasture to superimpose lactation curve on grass growth; no concentrate for BOTA and 4 kg/d at pasture for PEPI; >180 days at pasture on permanent grassland (Pomiès *et al.*, 2013). But during 5 years these systems led to poor reproductive performances: only 35% of the 136 adult cows calved during the following season, with no difference between systems nor breeds (Pomiès *et al.*, 2016). These results were probably related to the strong negative energy balance of the cows at pasture, with little concentrate but with a genetic profile oriented towards high milk production (32.8 kg/d of milk at the lactation peak for multiparous cows). Previous studies showed that once daily milking (**ODM**) for a few weeks leads to the same feed intake by cows than twice daily milking (**TDM**), despite an immediate lower dairy production from 19 to 46% (Pomiès *et al.*, 2008). During early lactation, ODM leads to a faster recovery of live weight and body condition score (Rémond *et al.*, 2004), and to a better energy balance of cows (Holmes *et al.*, 1992; Rémond and Pomiès, 2005). A consequence of this better energy

balance (related to the correlation between nutritional status and resumption of the ovarian cyclicity after calving) is often an earlier detection of oestrus in ODM cows (Patton *et al.*, 2006) and a reduction of the interval from calving to fertilising insemination (-24 days for primiparous, Pomiès *et al.*, unpublished). The aim of the present experiment was to test the potential of a 6-wk ODM period at the beginning of the breeding season to improve the reproductive performance of dairy cows in a mountain low-input system.

Material and methods

Animal management

In 2016, 28 cows (ODM group) (50% Ho, 50% Mo, and 36% primiparous) were followed individually from April 1 to July 31. They calved over a 69-d period, from February 26 to May 5. They were housed in the same free-stall barn until being turned to pasture on May 4, night and day. An individual feeding plan similar to the PEPI experiment was established for each cow, based on housing (inside or at pasture) and stage of pregnancy (before or after calving). We used the same rotational grazing system on permanent grassland as in PEPI in 2014-2015, with a total area of 11.2 ha divided in 4 plots (0.40 ha/cow). The 6-wk ODM period began the 1st Monday after the 6th day of lactation of the last calved cow (May 16). Cows were then taken to the milking parlour only once a day (at 6:30 AM) and spent the rest of their time at pasture. In order to maintain a calving period strictly identical to the 2 previous years, breeding season began on May 19. Artificial inseminations (**AI**) were performed after each observed heat, during 46 days. During this period, as in 2014-2015, a daily visit of the cows at pasture (at 1:00 PM) was implemented for heat detection, but nothing was done to overcome the lack of observation during the omitted evening milking. The AI period was followed by the introduction of a bull in the herd for 28 days, with a swap between a Ho bull and a Mo bull every week. The breeding season ended on July 31 and the ultrasounds for pregnancy diagnosis were performed on September 7. These cows were compared with the 28 cows of the PEPI system (TDM group) that calved from February 26 to May 12, in 2014 (n=10) and 2015 (n=18). Cows from the TDM group (54% Ho, 46% Mo, and 29% primiparous) had a similar dairy potential than ODM cows, estimated by the average production from Day 4 to Day 6 of lactation (21.6 *vs* 21.5 kg/d).

Data, calculations and statistical analyses

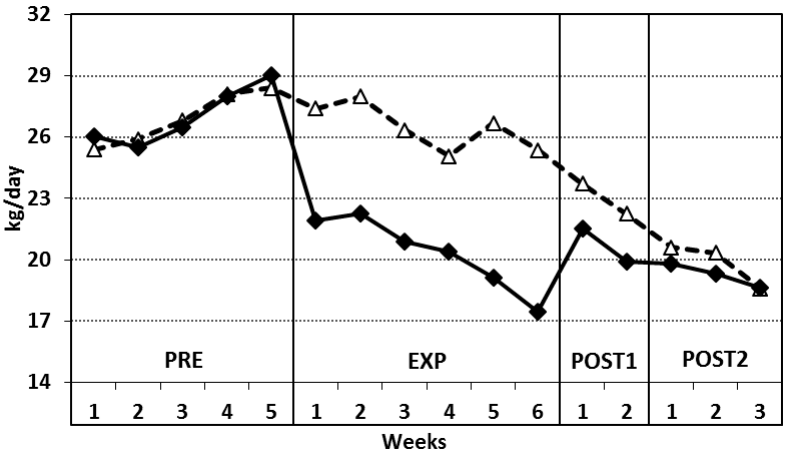
Milk production of each cow was recorded at every milking. Fat content, protein content and somatic cell count (**CCS**) were measured on individual milk samples from every milking, on two consecutive days per week. These measures allowed to calculate the individual milk production, daily, weekly and at the peak of lactation, as well as individual weekly milk composition. The dates of observed heat, AI, observed mating and the pregnancy results were recorded for each cow. The milk data were analysed using the mixed procedure of SAS software (SAS Institute Inc., 2013). The model took into account the effects of the cow (random factor), the milking group (ODM *vs* TDM), the breed (Ho *vs* Mo), the rank of lactation (1, 2, ≥3), the calving date and, for milk production parameters, the dairy potential as covariate. These analyses were performed on 16 weeks (April 11 to July 31), divided into 4 periods: 5 weeks before ODM (**PRE**); 6 experimental weeks with ODM (**EXP**); 2 weeks immediately after ODM (**POST1**); the 3 following weeks (**POST2**). The reproductive performance (number of cows inseminated, pregnant…) were compared by chi-square tests, according to the milking group, the breed and the rank of lactation, separately.

Results and discussion

From the very start of EXP, the milk yield of ODM cows declined dramatically (-7.1 kg/d, Fig. 1). During this period the difference between the two groups remained high (-22%, Table 1). At the beginning of POST1, the milk yield of ODM cows rose (+4.0 kg/d) but a small gap

still existed with the TDM group (-8%, *P*<0.10), before disappearing in POST2. ODM and TDM cows produced the same amount of milk at their lactation peak, suggesting that the practice tested did not affect the milking potential. The difference of milk fat content between the ODM and TDM groups was important during EXP (+3.6 g/kg, *P*<0.05), before rapidly disappearing in POST1. The same trend was observed with the ODM protein content in EXP (+2.2 g/kg, *P*<0.01), but return to the TDM level was slower (still +1.0 g/kg in POST2, *ns*).

Figure 1: Average daily milk yield of cows from ODM (—♦—) and TDM (--Δ--) groups, during 16 weeks divided in 4 periods (PRE, EXP, POST1, and POST2); cows from ODM group were milked once daily during EXP



Despite no significant difference between the two milking groups, higher values of SCC were also observed for the ODM group during EXP (+48,000 cells/mL), before gradually returning to the same level. The changes in milk yield and milk composition, observed during and after ODM, are classic and widely documented (Pomiès *et al.*, 2008; Rémond *et al.*, 1999). During the 6 weeks of EXP and the 5 following ones, each ODM cow produced on average 277 kg less milk (-15%) compared to TDM (-12% of energy corrected milk).

Table 1: Comparison of milk production and milk composition by period; mean values by milking group (ODM or TDM) adjusted by the mixed model, and effects of the factors

		Mean by group		Effect				
	Period	**ODM**	**TDM**	**Group**	Breed	Rank	Calving	Potential
Milk yield	PRE	25.8	26.2	**ns**	ns	ns	***	***
(kg/d)	EXP	20.6	26.5	***	ns	ns	ns	*
	POST1	21.1	22.8	+	ns	ns	ns	**
	POST2	19.4	19.5	**ns**	ns	ns	ns	*
	at peak	29.9	29.3	**ns**	ns	+	ns	**
Fat	PRE	41.9	41.4	**ns**	ns	ns	**	
content	EXP	41.2	37.6	*	ns	ns	ns	
(g/kg)	POST1	37.5	36.1	**ns**	ns	ns	ns	
	POST2	35.9	35.9	**ns**	ns	ns	ns	
Protein	PRE	32.3	32.2	**ns**	ns	ns	***	
content	EXP	32.0	29.8	**	ns	ns	ns	
(g/kg)	POST1	30.5	28.4	**	+	ns	*	
	POST2	29.1	28.1	**ns**	ns	ns	*	
SCC	PRE	4.98	4.87	**ns**	ns	ns	*	
(log_{10})	EXP	5.04	4.79	**ns**	+	ns	ns	
	POST1	5.07	4.89	**ns**	+	ns	ns	
	POST2	5.08	5.05	**ns**	ns	ns	ns	

*** *P*<0.001; ** *P*<0.01; * *P*<0.05; + *P*<0.10; *ns* (non-significant) *P*≥0.10

For the ODM group, the intervals "start of the breeding season–1st insemination" (AI or

mating; 26 d), "calving–1st insemination" (77 d), and "calving−fertilising insemination" (95 d) were not significantly different compared to TDM group (+1, +10, and -2 days, respectively). During the breeding season, 24 ODM cows out of 28 were detected in heat at least once (by the observer or the bull) and inseminated (AI or mating), against 19 TDM cows out of 28 (*ns*, Table 2). Among these cows, 88% of ODM ones were diagnosed pregnant compared to 58% of TDM ones ($P<0.01$). As a result, 21 out of the 28 ODM cows were diagnosed pregnant in September (75%) against only 11 out of the 28 TDM cows (39%), which represented a real breeding achievement. The success rate at first insemination (AI or mating) was higher for ODM cows compared to TDM ones (50% *vs* 21%, $P<0.10$), whereas the proportion of cows pregnant on a mating was similar (67% *vs* 64%, *ns*). This confirms the hypothesis that improving the energy balance of the cows by the ODM practice after calving has an immediate effect at the start of the breeding season.

Table 2: Reproduction parameters by milking group (ODM = once daily milking; TDM = twice daily milking), and effects of breed and rank of lactation

	Percentage by milking group		Effect		
	ODM *(n=28)*	**TDM** *(n=28)*	**Group**	Breed	Rank
Cows observed in heat and inseminated *(AI or mating)*	86 %	68 %	***ns***	*ns*	*ns*
Pregnant cows / cows observed in heat and inseminated	88 %	58 %	*	*ns*	*
Pregnant cows / 28 cows for breeding	75 %	39 %	**	*ns*	*ns*
Success rate at first insemination *(AI or mating)*	50 %	21 %	+	*ns*	*ns*
Cows pregnant on a mating / pregnant cows	67 %	64 %	***ns***	*ns*	*ns*

** $P<0.01$; * $P<0.05$; + $P<0.10$; *ns* (non-significant) $P\geq0.10$

Conclusions

Our results suggest that the use of ODM for 6 weeks after calving could be a solution to overcome problems of reproduction in seasonal low-input dairy systems, ensuring their sustainability without altering their production potential. In addition the loss of milk, that represent only 4% of the total milk produced during the lactation, is largely compensated by the higher protein and fat contents beneficial to PDO cheese-making. The benefit of this practice will be further checked by an economic study that takes into account specificities of the regional context. Another way to maintain those systems could be to use breeds that reproduce easily even with low inputs, such as Jersey or Holstein-Friesian from Ireland or New-Zealand (Piccand *et al.*, 2013).

Acknowledgements

The authors acknowledge O. Troquier and the staff of the INRA experimental farm of Marcenat for taking care of the animals, I. Veissier for reviewing the text, and the GALA Association for supporting financially the study.

References

Holmes C.W., Wilson G.F., Mackenzie D.D.S., Purchas J. 1992. Proc NZ Soc Anim Prod, 52: 13-16.

Horn M., Steinwidder A., Gasteiner J., Podstatzky L., Haiger A., Zollitsch W. 2013. Livest Sci, 153: 135-146.

Patton J., Kenny D.A., Mee J.F., O'Mara F.P., Wathes D.C., Cook M., Murphy J.J. 2006. J Dairy Sci, 89: 1478-1487.

Piccand V., Cutullic E., Meier S., Schori F., Kunz P.L., Roche J.R., Thomet P. 2013. J Dairy Sci, 96: 5352-5363.

Pomiès D., Fournier F., Farruggia A. 2016. In: 19th Meeting of the FAO-CIHEAM Mountain Pastures Subnetwork, 14-16 June 2016, Zaragoza, Spain. p 75-79.

Pomiès D., Marnet P.G., Cournut S., Barillet F., Guinard-Flament J., Rémond B. 2008. INRA Productions Animales, 21: 59-70.
Pomiès D., Martin B., Pradel P., Verdier-Metz I., Constant I., Delbès-Paus C., Troquier O., Fournier F., Montel M.C., Farruggia A. 2013. In: 17th Meeting of the FAO-CIHEAM Mountain Pasture Network, 5-7 June 2013, Trivero, Italy. p 22-26.
Rémond B., Coulon J.B., Nicloux M., Levieux D. 1999. Ann Zootech, 48: 341-352.
Rémond B., Pomiès D. 2005. Anim Res, 54: 427-442.
Rémond B., Pomiès D., Dupont D., Chilliard Y. 2004. Anim Res, 53: 201-212.

Alpine transhumance affects milk chemical composition and coagulation properties, and modifies cheese texture

M. Koczura1[2-3]*, G. Turille[3], M. Kreuzer[1], J. Berard[1], M. De Marchi[4], B. Martin[2]
[1]ETH Zurich, Institute of Agricultural Sciences – Zurich, Switzerland
[2]INRA, UMR1213 Herbivores – Saint-Genès-Champanelle, France
[3]Institut Agricole Régional – Aosta, Italy
[4]University of Padova, DAFNAE - Legnaro (Padova), Italy
*madeline.koczura@usys.ethz.ch

Abstract
The aim of the present study was to investigate the effects of transhumance of dual-purpose dairy cows to high altitude on the chemical composition and coagulation properties of the milk, and the consequences on texture and flavour of the cheese. Five transhumances of cows from three different farms were studied during summer 2016 in Aosta Valley, where cows had to walk for 1 h to 1.5 h between different highland pastures (altitude ranging from 1500 m a.s.l. to 2100 m a.s.l). Bulk milk and farmhouse Fontina PDO cheeses produced from milk obtained during the week of transhumance were collected. The milk fat content tended to increase right after transhumance. Protein content and somatic cell score were also higher. Milk coagulation properties evolved following the increase in protein content. The cheeses were more salty and less elastic the day after transhumance but no common trend for flavour was observed. Effects observed the day after the transhumance disappeared within five days. 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.

Key-words: alpine transhumance, fat content, somatic cells, Fontina cheese, texture

Introduction
In mountainous areas, transhumance is a common practice allowing an optimal valorisation of highland pastures during summer. Transhumance systems with dairy cows are often associated to the production of high quality farmhouse cheeses, particularly important to the maintenance of these typical farming systems. As an example, Fontina PDO (a semi-hard cheese from raw milk) is traditionally produced in the Aosta Valley region using local breeds grazing high alpine pastures in summer. However, farmers that are also cheesemakers, often report difficulties to produce Fontina during the actual time of transhumance, especially with the milk obtained directly following the walking transitions. The difficulties might be partly linked to the changing production areas but they could also result from compositional changes of the milk. Indeed, it has already been observed that walking can alter milk composition (D'Hour et al., 1994). However, these results were obtained under experimental conditions which corresponded to extreme walking, and the cheese-making ability of milk and cheese quality were not investigated. Therefore, bulk milk and cheeses from three local alpine farms were investigated in order to identify the changes in milk composition and coagulation properties and the prevalence of unfavourable cheese qualities.

Materials and Methods

Transhumances from medium to high altitude were practiced in three different farms located in Aosta Valley in Italy: (1) three transhumances namely from 1500 to 1800 m a.s.l., from 1800 to 2100 and from 2100 to 1800 m a.s.l. in Rhêmes-Notre-Dame, (2) one transhumance from 1800 to 2100 m a.s.l. in Vertosan and (3) one transhumance from 1500 to 1800 m a.s.l. in Cogne. In Rhême-Notre-Dame, cows were kept 24 h outside, whereas at both other sites they were milked and housed in the barn overnight. All transhumances took place between the end of June and beginning of September 2016, and animals had to walk between 1 h and 1.5 h. Bulk milk from the three herds was collected at 7.00 am and 6.00 pm 5 days before the transhumance took place, on the evening after (start of day 1) and during the 2 days after and finally 5 days after transhumance. This bulk milk of each herd was used to produce Fontina PDO cheese according to the official specifications (Disciplinare Fontina DOP) by the three different farmhouse cheesemakers. The dairies and cellars where the cheeses were manufactured changed along within the transitions. Contents of fat, protein, lactose, casein and urea in milk were measured by NIRS and somatic cell count (SCC) by a fluorimetric method (Fossomatic®, Foss Electric, Hillerod, DK). Somatic cell score (SCS, logarithmically transformed SCC) and the fat-to-protein ratio were calculated. Rennet coagulation time (RCT), curd-firming time (k_{20}), and curd firmness (A_{30}) were measured using a Formagraph (Foss Electric A/S). After 115 days of ripening, fat, protein and salt contents of cheeses were analysed by NIRS and sensory analyses were performed by a trained sensory panel of six assessors. The parameters studied were texture (elasticity, hardness and melting), taste (sweet, salty, acidic, bitter, fermented) and visual aspects (appearance of the eyes and colour of the core) of the cheeses. The mean value across panellists was used for statistical evaluation. Data were analysed using the MIXED procedure of SAS (version 9.1, Inst. Inc., Cary, NC). Data were pooled to "days after transhumance", starting from the evening just after transhumance: day 1 represents the evening of the day of transhumance and the morning after, and so on. Effects of the day after transhumance (1, 2, 3 or 5, "day"), and the morning or evening (M or E, "time") were integrated as fixed factors. As the interactions were not significant, they were removed from the model. For each investigated trait, mean values from the morning and evening obtained 5 days before transhumance were used as covariate. Day was considered as repeated factor, with transhumance as subject. $P < 0.05$ was considered significant.

Results and Discussion

Milk: The fat content of the milk tended ($P < 0.10$) to increase the day just after transhumance (+ 0.67 g/100g on day 1 compared to day 5; Figure 1.i). This is certainly due to the walk: it was previously observed at lower altitudes and with less difference in altitude by D'Hour et al. (1994) and Coulon et al. (1998). Body fat mobilisation was probably enhanced by the harsh alpine conditions (Leiber et al., 2006). Protein content slightly increased ($P < 0.01$) during day 1 (+ 0.07 g/100g compared to day 5; Figure 1.ii), right after transhumance, too. The fat-to-protein ratio was not significantly affected even if it varied from 1.32 on day 1 to 1.16 on day 5 (data not shown). Contents of lactose, casein and urea of milk were not modified neither (data not shown). The SCC was higher ($P < 0.05$) on day 1 than 2 days later (+ 156×10^3 cells/mL; Figure 1.iii). The A_{30} followed the evolution of protein content; it was higher on day 1 than on day 5 ($P < 0.01$) and the RCT on the contrary was lower on day 1 (P

< 0.01; Figure 1.iv). Lactose content was globally higher in the morning (+ 0.05 g/100g, $P <$ 0.01, data not shown). Other milk traits were not affected by time of milking (data not shown) certainly because the intervals between two consecutive milkings was regular enough to avoid these effects.

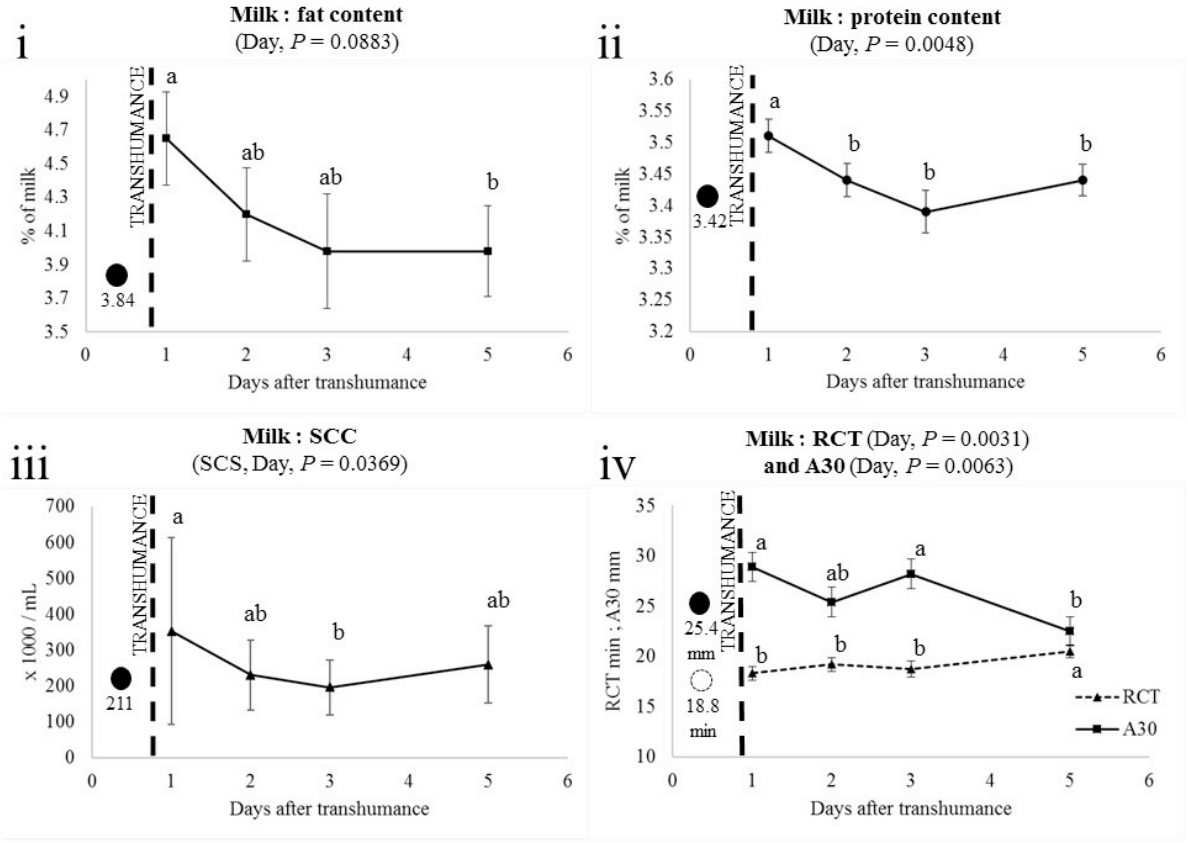


Figure 1: Effect of the days after transhumance on bulk milk characteristics (values with different letters are statistically different and mean values of the covariate are reported on the figures as points before transhumance; iii: values are arithmetic means of SCC whereas P-value was calculated for SCS)

Cheese: Unlike milk, fat in cheese dry matter was not affected by day possibly due to higher fat losses in the whey during the days after the transition. Cheeses tended ($P < 0.10$) to be more salty the day 1 compared to day 5 (+0.46 % NaCl in cheese dry matter). However, this difference was not perceived by the sensory panel. Attributes of taste or visual appearance of the cheeses were not affected by day (data not shown). Some defects in taste were globally observed in the cheeses but could not be directly associated with the day of transhumance, as their variability was high and could be due to the different environments of the three farms, too. The main trend observed concerns texture that was less elastic on day 1 (Figure 2.ii; $P <$ 0.05). This decrease could be due to a higher proteolysis linked to the increase of SCC (Bugaud et al., 2001). Fat in dry content tended ($P < 0.10$) to be higher in the evening cheeses and protein content was on the contrary higher ($P < 0.01$) in the morning cheeses (Table 1). The NaCl content tended to be higher in the evening (Table 1; $P < 0.10$). The effect of the factors tested on the salt content of cheeses was surprising because brining was made under the same conditions before and after transhumance. It could reflect differences in salt absorption linked to fresh cheese gross composition (not analysed in the present study). Cheeses produced from morning milk were harder and chalkier whereas in the evening they were softer and pastier (Figure 2.ii; $P < 0.05$). The number of defects observed tended ($P <$

0.10) to be higher in cheeses produced from the evening than the morning milk (2.05 defects vs. 1.37, respectively).

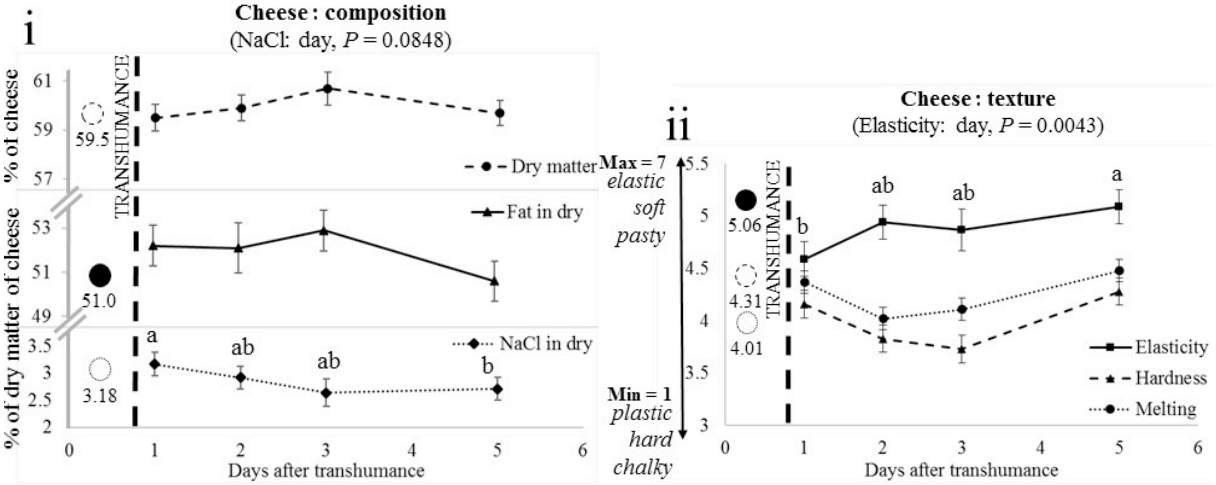


Figure 2: Effect of the days after transhumance on cheese composition and texture (values with different letters are statistically different and mean values of the covariate are reported on the figures as points before transhumance).

Table 1: Effect of the milking time (morning, « M », evening « E ») on cheese characteristics

	Fat (% DM)	Protein (%)	NaCl (% DM)	Elasticity (1 to 7)	Hardness (1 to 7)	Melting (1 to 7)
M	51.2 ± 0.260	25.3 ± 0.024	2.73 ± 0.203	4.88 ± 0.156	3.63 ± 0.291	3.85 ± 0.179
E	52.7 ± 0.242	24.4 ± 0.022	2.99 ± 0.192	4.87 ± 0.146	4.36 ± 0.266	4.65 ± 0.134
P	0.053	0.029	0.095	0.876	0.014	0.006

Conclusions

The present study confirmed that alpine transhumance clearly influences milk and cheese quality on the day after the walking. After five days, this effect was not observed anymore. Our results furnish first indications allowing to better characterise the changes during this transition. It seems that, by altering milk quality, walking transhumance may be indirectly responsible for an impaired cheese texture. The transformation process should be adapted to the altered milk quality of the days following transhumance to optimise production, keeping in mind that the changing production areas and microbial environments may also strongly affect cheese quality.

Acknowledgements

Thank you to ARAV laboratory (Padova) for analyses of MCP and Aosta regional panel for sensory analyses. Many thanks to the farmers and their families (A. Yeuilla and B. Jeantet).

References

Bugaud C., Buchin S., Noël Y., Tessier L., Pochet S., Martin B., Chamba J.-F. 2001. Lait 81: 593-607

Coulon J.-B., Pradel P., Cochard T., Poutrel B. 1998. J. Dairy Sci. 81: 994–1003

D'Hour P., Hauwuy A., Coulon J.-B., Garel J.-P. 1994. Ann Zootech 43: 369-378.

Disciplinare di Produzione della DOP "Fontina", Consorzio Produttori e Tutela della Fontina DOP, 1995.

Leiber F., Kreuzer M., Leuenberger H., Wettstein H.-R. 2006. Anim. Res. 55: 37–53

Prediction of cow diet composition from bulk milk spectra by Medium Infrared Reflectance Spectroscopy (MIRS)

M.Coppa[1*], A. Revello-Chion[2], D.Giaccone[2], E. Tabacco[1], G. Borreani [1]
[1]University of Turin, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, 10095, Grugliasco (Turin), Italy
[2]Associazione Regionale Allevatori del Piemonte, Via Livorno 60, 10144, Turin, Italy,
*mauro.coppa@unito.it

Abstract
The aim of this work was to develop equations using Medium Infrared Reflectance Spectroscopy (MIRS) spectra of bulk milk from commercial farms to predict dairy cow diet composition. A total of 429 bulk cow milk samples were collected from 176 commercial farms located in Northwest Italy from 2011 to 2017, and analysed by MIRS. Data on the production conditions were recorded on-farm at each sampling by compiling a detailed questionnaire. Calibrations for the predictions of cow diet composition were calculated with modified partial least square regression. Dairy cow feeding varied to a great extent.
The total forages calculated including the 60% of DM of maize silage and the total concentrates including the 40% of DM of maize silage were successfully predicted by MIRS ($R^2CV > 0.80$). The predictions for total concentrates (without part of DM of maize silage), the total forages (including the 100% of DM of maize silage), and the fresh herbage proposition in cow diet were found to be promising ($R^2CV > 0.75$). These results can have a great interest in the authentication milk, especially for those dairy products having feeding restriction in their specification protocols.

Keywords: milk, authentication, cow feeding, MIRS

Introduction
In Europe, dairy product labelling or certification strategies, such as protected designation of origin (PDO) or protected geographical indication (PGI) consist in a useful tool to support local producers, especially in marginal areas, giving an added value (and higher prices) to dairy products. These labels imply a specific protocol, which often include restriction in dairy cow feeding (i.e. interdiction of silage, limit to concentrate amount, minimum supply of fresh herbage, etc.), that result in dairy products with specific traits of composition or sensory profile (Martin et al., 2005; Giaccone et al., 2016). To guarantee the authenticity of such dairy products for consumers, reliable, rapid and cheap methods of authentication are required. Medium infrared reflectance spectroscopy (MIRS) is non-invasive, rapid and inexpensive and can be an alternative technique to reference analysis for a routine use in the collection of information on animal diet and milk authentication (Coppa et al., 2102).
The aim of this work was to develop equations using MIRS spectra of bulk milk from commercial farms to predict dairy cow diet composition.

Material and methods
A total of 429 bulk cow milk samples were collected from 176 commercial farms located in Northwest Italy from 2011 to 2017. To explore the maximum variability of milk composition, samples were collected in different seasons in farms ranging from the intensive systems of the plain to the extensive systems of the Alps. Milk samples were collected on each farm, transported at 4°C, without any added preservative, and analysed by MIRS (MilkoScan FT6000, Foss System, Hillerød, Denmark). Data on the production conditions were recorded on-farm at each sampling by compiling a detailed questionnaire drawn up by Borreani et al.

(2013). The total concentrate and forages were calculated both considering corn silage as forage and partially as a concentrate (40% of DM) and partially as a forage (60% of DM), as proposed by Mertens (2009). Calibrations for the predictions of cow diet composition were performed using WinISI II Project Manager, version 1.50 (Infrasoft International, South Atherton St. State College, PA, USA) and calculated with modified partial least square (MPLS) regression. The statistics used to develop and evaluate the calibration models included the standard error of calibration (SEC), the coefficient of determination for calibration (R^2C), standard error of cross-validation (SECV), the coefficient of determination for cross-validation (R2CV), and the ratio of the standard deviation of reference data to the SECV (RPD).

Results and discussion

The composition of the diet of dairy herds from which the milk was sampled are given in Table 1. Dairy cow feeding varied to a great extent and included full fresh herbage (pasture- or indoor-fed) or hay diets, corn silage- or grass or legume silage-based diets and diets in with more than 50% of the DM of concentrates. Feeding systems in northwest Italy are highly diversified because of the proximity of the Alps, whose pastures are grazed by dairy herds, and to the presence of the Po plain, which hosts intensive maize silage-based farming systems (Borreani et al., 2013).

Table 1: Descriptive statistics of cow diet composition in the calibration dataset

Feedstuffs (% diet DM)	Average	Min	Max	SD
Total forages (including 60% maize silage)	52	27	100	19.2
Total concentrates (including 40% maize silage)	48	0	73	19.2
Total forages (including 100% maize silage)	62	35	100	16.2
Total concentrates (exluding maize silage)	38	0	65	16.2
Hay	15	0	89	18.0
Total grass-derived forages	25	0	89	16.2
Maize silage	26	0	63	14.4
Fresh herbage	10	0	100	27.4

Table 2: MIRS prediction results for cow diet composition

Feedstuffs (% diet DM)	Calibration					
	N	SEC	R^2C	SECV	R^2CV	RPD
Forages (including 60% maize silage)	411	7.1	0.84	7.7	0.82	2.53
Concentrates (including 40% maize silage)	411	7.1	0.84	7.7	0.82	2.53
Forages (including 100% maize silage)	415	6.9	0.80	7.5	0.77	2.26
Concentrates (excluding maize silage)	415	6.9	0.80	7.5	0.77	2.26
Hay	397	8.8	0.49	9.6	0.41	1.40
Grass-derived forages	392	9.0	0.35	9.7	0.25	1.24
Maize silage	412	10.0	0.47	10.3	0.44	1.38
Fresh herbage	399	9.7	0.79	10.2	0.77	2.17

The MIRS calibration statistics obtained for the various feedstuffs are shown in Table 2. The total DM of forages calculated including 60% of maize silage (plant) and the total amount of concentrates including 40% of maize silage (grain) were successfully predicted by MIRS ($R^2CV > 0.80$). The predictions for total concentrates (without maize silage), the total forages

(including 100% of maize silage), and the fresh herbage proportion in cow diet were found to be promising ($R^2CV > 0.75$). The hay and the maize silage proportions in cow diet were poorly predicted and grass derived forages (hay and grass or legume silages) did not allow to obtain reliable models. The SECV were reasonably low, especially considering the method of estimation of reference data through in farm surveys that could imply a lower detail in information compared with controlled experiments (Coppa et al., 2013). This is the case for fresh herbage proportion in the cow diet that, however, showed a SECV similar or slightly higher than those of the other feedstuffs.

Conclusions

The presented model appear reliable for the estimation total forages, total concentrates and the fresh herbage proportions in cow diets. These results can have a great interest in the authentication of milk, especially for those dairy products having feeding restriction in their specification protocols (i.e. Protected Denomination of Origin). Further studies would be required to improve the equations and to individuate strategies for a careful use of those equations which have shown low performances.

Acknowledgements

This work was funded by Fondazione Cassa di Risparmio di Cuneo (CRC), Project "MIGLIORLAT".

References

Borreani G., Coppa M., Revello-Chion A., Comino L., Giaccone D., Ferlay A., Tabacco E. 2013. J. Dairy Sci. 96: 6840-6855.

Coppa M., Ferlay A., Chassaing C., Agabriel C., Glasser F., Chilliard Y., Borreani G., Barcarolo R., Baars T., Kusche D., Harstad O.M., Verbič J., Golecký J., Martin B. 2013. J. Dairy Sci. 96: 4197–4211.

Coppa M., Martin B., Agabriel C., Chassaing C., Sibra C., Constant I., Graulet B., Andueza D. 2012. J. Dairy Sci. 95: 5544–5551.

Coppa M., Revello-Chion A., Giaccone D., Ferlay A., Tabacco E., Borreani G. 2014. Food. Chem. 150: 49-57.

Giaccone D., Revello-Chion A., Galassi L., Bianchi P., Battelli G., Coppa M., Tabacco E., Borreani G. 2016. Int. Dairy J. 59: 10-19.

Martin B., Verdier-Metz I., Buchin S., Hurtaud C., Coulon J. B. 2005. Anim. Sci. 81: 205–212.

Mertens D.R. 2009. Maximizing forage use by dairy cows. WCDS Adv. Dairy Technol. 21: 303–319.

Multiple chemical markers to authenticate mountain PDO Idiazabal cheese

L.J. R. Barron[1*], M.A. Bustamante[1], N. Aldai[1], I. Valdivielso[1]
[1]Lactiker Research Group, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Paseo de la Universidad 7, E-01006, Vitoria-Gasteiz, Spain
*luisjavier.rbarron@ehu.eus

Abstract
The objective of this work was to investigate the usefulness of multiple chemical markers to authenticate mountain PDO Idiazabal cheese. Latxa sheep management in the Basque Country (northern Spain) follows a seasonal feeding strategy during lactation: indoor feeding in winter (early lactation), part-time grazing in early spring (mid lactation), and extensive grazing from late spring onwards (late lactation). Extensive grazing was traditionally done in mountain areas (around 1000 m of altitude) but this management has progressively decreased over the last decades. Cheeses made with milk from six commercial flocks were collected after 150 days of ripening throughout lactation period: indoor feeding, part-time grazing in valley, and mountain grazing. Fatty acids, tocopherols, retinoids and terpenoids were analysed, and multiple chemical markers were defined as combination of selected compounds found in cheese samples. Unsaturated and saturated fatty acid markers, vitamin marker, terpenoid marker, and preferably, a marker combination of the previous ones, were capable to effectively discriminate mountain cheeses from those of indoor feeding and part-time grazing. Therefore, multiple indices can be a useful approach to authenticate mountain cheese, and their effectiveness should be proved in a local context as is the case of mountain PDO Idiazabal cheese.

Keywords: multiple chemical markers, fatty acids, vitamins, terpenoids, seasonal feeding

Introduction
Many mountain cheeses in Europe are protected under quality labels such as Protected Denomination of Origin (PDO) or Protected Geographical Indication (PGI). At the same time, some PDO cheeses are produced either in mountain or in valley areas which is the case of Idiazabal cheese produced in Basque Country and Navarre (northern Spain). Latxa dairy sheep management follows a seasonal feeding strategy during lactation based on indoor feeding in winter (early lactation), part-time grazing in early spring (mid lactation), and extensive grazing from late spring onwards (late lactation). Extensive grazing was traditionally done in mountain grasslands (around 1000 m of altitude) but type of management together with cheesemaking in mountain farms have progressively decreased over the last decades. Limited on-farm facilities which imply manual milking, cheesemaking from raw milk twice a day (non-refrigerated milk tanks), non-automatic vats and chambers without temperature and humidity control are the main reasons for the abandonment. Cheese makers and institutions in the Basque Country are concerned about the disappearance of traditional mountain cheese which is greatly appreciated by consumers. In this regard, the Idiazabal Regulatory Board has recently approved a new quality label to promote and certificate the cheeses produced in mountain farms.
Most mountain cheeses in Europe are considered grassland products and major difficulties arise from the authentication of these products due to seasonal and geographic variation of the grassland composition and environmental conditions. Most studies are focused on identifying molecular markers in mountain cheeses such as fatty acids, terpenoids, tocopherols and other compounds (Prache *et al*., 2005; Valdivielso *et al*., 2015). In this respect, methodological approaches using information from different markers or ratios seem to be more effective in

order to authenticate mountain milk and cheese (Renna *et al*., 2012; Povolo *et al*., 2013; Valdivielso *et al*., 2017). The aim of this work was to investigate the usefulness of multiple chemical markers to authenticate mountain PDO Idiazabal cheese. In the present study, a holistic approach was undertaken considering the whole production system of commercial flocks as seasonal feeding and lactation stage were intrinsically linked.

Material and methods

Six commercial flocks of Latxa dairy sheep (200-500 lactating ewes) belonging to PDO Idiazabal cheese makers participated in this study. Same feeding regimen was followed by sheepherders: sheep fed concentrate and forage from February to mid March, sheep grazed part-time with concentrate and forage supplementation from late March to late April, and from May on sheep were moved to mountain grasslands in Aralar Natural Park (42º59'48" N and 2º06'51" W). Concentrates and conserved forages included in the feeding regimens were locally purchased while commercial flocks selected for the present study grazed in different pasture areas of Aralar Natural Park. During indoor feeding, the concentrate to forage ratio varied from 0.2 to 3.0 among farmhouses. During part-time grazing, ewes were allowed to graze up to 8 h/day depending on weather conditions in improved or community-owned non-improved valley grasslands. During mountain grazing, sheep flocks were kept outdoors and were allowed to graze all day long. Valley farms were located at 200 to 400 m of altitude whereas mountain farms were located at around 1000 m. Commercial cheeses (~ 1.5 kg) were manufactured according to the PDO Idiazabal guidelines (Ministerio de Agricultura, Pesca y Alimentación, 1993). Raw milk cheeses were manufactured in mid-February (indoor feeding), mid-April (part-time valley grazing), early June (early mountain grazing), and late-June (late mountain grazing) and after 150 days of ripening, one cheese from two replicated vats *per* type of feeding regimen was randomly collected (8 cheeses *per* flock).

Fatty acids (FA) were analysed by gas chromatography coupled to flame ionization detector, and tocopherols and retinoids by high performance liquid chromatography coupled to fluorescence detector, as previously described (Valdivielso *et al*., 2015). Terpenoids of cheese samples were extracted by solid-phase microextraction and analysed by gas chromatography coupled to mass spectrometry detector (Valdivielso *et al*., 2017). SPSS IBM Statistics software version 23.0 was used for statistical analysis. Mixed linear model of ANOVA including season (fixed effect) nested within flock (random effect) was used to investigate the differences in multiple chemical markers among cheeses. Fisher's Least Significance Difference test was used for pairwise comparisons and confidence intervals at 95% were estimated. Stepwise discriminant analysis was applied to selected compounds and multiple chemical markers to classify cheeses from different feeding regimens throughout lactation.

Results and discussion

As mentioned above, research efforts have been made to authenticate grassland and/or mountain dairy products. However, due to the high number of variability factors affecting milk and cheese composition, it is very unlikely that levels of individual FAs, vitamins, terpenoids, or other compounds, will be accurate enough to authenticate mountain cheeses. In this work, five multiple chemical markers were defined to differentiate mountain PDO Idiazabal cheeses from those made during indoor feeding and part-time valley grazing. The multiple markers were based on ratios between the content of selected compounds which strongly increased from indoor feeding to mountain grazing, and others that decreased or did not change throughout lactation. Two chemical markers were separately defined as ratios among the molar content (dry matter basis) of selected unsaturated and saturated FAs in cheese, respectively. Unsaturated FA marker (UFAM) was the sum of C18:1*t*11 (vaccenic acid), C18:2*c*9*t*11 (rumenic acid) and C18:3*c*9*c*12*c*15 (*α*-linolenic) contents divided by

C18:1*t*10 content. As previously reported, the content of vaccenic, rumenic and *α*-linolenic acid increased from indoor feeding to mountain grazing whereas C18:1*t*10 did not significantly change (Valdivielso *et al*., 2015). Other authors reported that C18:1*t*10 is accumulated in milk fat of ruminants fed diets with an increasing level of concentrate and/or diets rich in oils which are usually fed indoors (Aldai *et al*., 2013). Saturated FA marker (SFAM) was the content of C14:0 (myristic acid) multiplied by 10 and divided by the sum of C10:0 (capric acid) and C12:0 (lauric acid). The myristic acid content of cheese samples did not change from indoor feeding to mountain grazing but capric and lauric acids strongly decreased (Valdivielso *et al*., 2015). The vitamin marker (VITM) was the ratio between the molar content (dry matter basis) of *α*-tocopherol and retinol. The highest content of *α*-tocopherol was found in mountain cheese whereas the retinol did not vary throughout lactation (Valdivielso *et al*., 2015). The terpenoid marker (TERM) was the sum of *α*-caryophyllene and *γ*-cadinene divided by the relative abundance of *α*-muurolene. This last sesquiterpenoid was detected at the same abundance in all cheese samples whereas the abundance of *α*-caryophyllene and *γ*-cadinene strongly increased in mountain cheeses (Valdivielso *et al*., 2017). Table 1 shows changes in the UFAM, SFAM, VITM and TERM values of cheeses from indoor feeding to late mountain grazing. In all cases, the values for these multiple chemical markers increased significantly ($P \leq 0.05$) from indoor feeding to mountain grazing, and cheeses from part-time valley grazing were significantly different from those from early mountain grazing. With the exception of SFAM, non significant ($P < 0.05$) differences were found between early and late mountain grazing. Furthermore, a new multiple marker named mountain Idiazabal cheese marker (MICM) was defined as the sum of UFAM, SFAM, VITM and TERM. According to confidence intervals at 95%, MICM together with SFAM showed the greatest differences between the upper limit for part-time valley grazing and the lower limit for early mountain grazing (Table 1).

Table 1: Chemical marker values (mean ± standard deviation) and confidence intervals at 95%) of PDO Idiazabal raw milk cheeses from commercial sheep flocks (n=6) managed under different feeding regimens. Confidence intervals at 95% are shown in brackets.

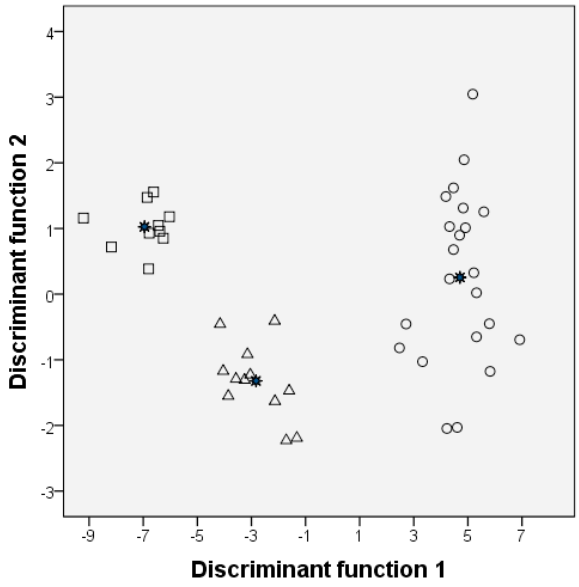
	Milk sampling dates and feeding regimens			
	Mid-February	Mid-April	Early-June	Late-June
Chemical markers	Indoor feeding	Part-time grazing in valley	Early mountain grazing	Late mountain grazing
UFAM[1]	12.1 ± 4.14c [10.7-13.4]	18.2 ± 4.21b [16.8-19.9]	25.2 ± 4.91a [23.7-26.7]	26.1 ± 4.70a [24.7-27.4]
SFAM[2]	66.7 ± 5.00d [65.4-68.1]	75.3 ± 4.64c [73.9-76.6]	91.4 ± 4.06b [89.9-92.8]	94.8 ± 3.92a [93.5-96.2]
VITM[3]	1.35 ± 0.190b [1.07-1.63]	1.87 ± 0.570b [1.59-2.15]	2.77 ± 0.464a [2.46-3.07]	2.81 ± 0.794a [2.53-3.07]
TERM[4]	1.01 ± 0.831b [0.00-4.07]	4.06 ± 2.31b [1.90-6.22]	15.9 ± 7.31a [13.7-18.5]	20.1 ± 4.61a [17.5-21.6]
MICM[5]	80.1 ± 0.726d [75.6-84.6]	101 ± 6.12c [97.4-103]	134 ± 12.5b [132-139]	144 ± 6.33a [140-146]

[1]Unsaturated fatty acid marker = (C18:1*t*11+C18:2*c*9*t*11+C18:3*c*9*c*12*c*15)/C18:1*t*10
[2]Saturated fatty acid marker = (C14:0x10)/(C10:0+C12:0)
[3]Vitamin marker = *α*-tocopherol/retinol
[4]Terpenoid marker = (*α*-caryophyllene+ *γ*-cadinene)/*α*-muurolene
[5]Mountain Idiazabal cheese marker = UFAM+SFAM+VITM+TERM
a,b,c,d Means followed by a different letter in the same row are significantly different ($P \leq 0.05$)

A stepwise discriminant analysis was also applied to the selected chemical compounds and multiple markers to classify cheese samples from the different feeding regimens. Figure 1shows cheese sample distribution displayed in the two discriminant functions. As observed, cheese samples from indoor feeding and part-time valley grazing were correctly classified but

cheese samples from early and late mountain grazing formed a single group. Therefore, this multivariate approach using selected compounds and multiple markers was able to clearly discriminate mountain cheeses from those produced indoor and under part-time valley grazing.

*Figure 1: Discriminant analysis on selected compounds and multiple chemical markers of cheese samples from different seasonal feeding regimens throughout lactation. □, indoor feeding; ∆, part-time grazing; ○, mountain grazing; *, group centroid*



Conclusions

Unsaturated and saturated fatty acid markers, vitamin marker, terpenoid marker, and preferably, a combination of previous markers, were capable to effectively discriminate mountain cheeses from those produced from indoor feeding and part-time grazing in valley. Therefore, multiple indices can be a useful approach to authenticate mountain cheese, and their effectiveness should be proved in local context as is the case of mountain PDO Idiazabal cheese.

Acknowledgements

This work was funded by the Spanish (AGL2013-48361-C2-1-R) and Basque (IT944-16) Governments.

References

Aldai N., de Renobales M., Barron L.J.R., Kramer J.K.G. 2013. Eur. J. Lipid Sci. Technol. 115: 1378-1401.
Ministerio de Agricultura, Pesca y Alimentación. 1993. B.O.E. 289: 34591-34596.
Prache S., Cornu A., Berdagué J.L., Priolo A. 2005. Small. Rumin. Res. 59: 157-168.
Povolo M., Pelizzola V., Passolungo L., Biazzi E., Tava A., Contarini G. 2013. J. Agric. Food Chem. 61: 447-455.
Renna M.P., Cornale P., Lussiana C., Giordano M., Belviso S., Zeppa G., Battaglini L.M. 2012. Dairy Sci. Technol. 92: 203-218.
Valdivielso I., Bustamante M.A., Buccioni A., Franci O., Ruiz de Gordoa J.C., de Renobales M., Barron L.J.R. 2015. J. Dairy Res. 82: 334-343.
Valdivielso I., de Renobales M., Aldai N., Barron L.J.R. 2017. J. Dairy Sci. 100: 96-105.

Characterization of detailed fatty acid profile of 18 categories of cheeses from mountains and plains

G. Stocco*, C. Cipolat-Gotet, M. Bergamaschi, G. Bittante

Dep. of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 45020 Legnaro (PD)
*giorgia.stocco.1@studenti.unipd.it

Abstract

The aim of this study was to characterize the fatty acid (FA) profile of 258 cheese samples of 18 different categories collected in the mountains and on the plains of Veneto region (Italy). From the results it was evident that, beyond the very peculiar FA profile of goat cheeses (more short-chain FAs and less MUFAs), the 3 categories of *Formaggi di malga*, (artisanal cheeses produced in the temporary summer farms during transhumance to Alpine pastures), were very different than the other cheese categories in relation of the much higher CLA and Omega-3 content. Two other categories of cheeses produced by permanent farms located in the mountains (*Morlacco del Grappa* and *Monte Veronese PDO*) were intermediate and two other categories of cheeses, in the past produced in the mountain areas (*Asiago PDO* and *Montasio PDO*) but nowadays produced mainly in the plains, were not distinguishable from the other cheese categories.

Keywords: fatty acid profile, CLA, omega-3, mountain cheese, Alpine pastures

Introduction

The discussion of dietary fatty acids (FAs) and their impact on health has largely dominated the scientific field, and it is still opened to major changes in food policy in several countries. There are still many doubts about the role of SFA, MUFA and PUFA in increasing or decreasing the risk of developing cardiovascular diseases. However, with regard to dairy products, beneficial effects have been paradoxically attributed to the cheeses from mountain origin, mainly because of the high α-linoleic acid (ALA) and CLA content. Dairy products are the main source of SFA, *cis*-MUFA and TFA intake in many countries (Hulshof et al., 1999; (Schiavon et al., 2016; Pegolo et al., 2016). Since Italian cheese production is characterized by a wide and heterogeneous variety, it would be interesting to assess the FA profile of different type of cheeses, and to study the differences in FA profile among cheeses produced in the mountain and in lowland environment. For these reasons, on a total of 258 cheeses belonging to 18 different cheese categories, we analyzed 74 single and 17 groups and indices of FAs, among which some are of proven dietary benefit (i.e., CLA), while others are of recent nutritional interest for their questionable effects on human health (i.e., short- and long-chain Omega-3 and -6, odd-chain FAs).

Material and methods

Origin of samples

A total of 258 samples of different cheeses belonging to 18 categories (Table 1) were collected between 2012 and 2014 at the *Caseus Veneti* cheese contest, promoted and organized by Veneto region (North-East of Italy), and at two cheese expositions in Trento Province (Valsugana and Val di Non).

Analysis of fatty acids

The lipid extraction procedure was performed according to ISO 14156 (2001) method. Cheese fat was methylated with basic esterification (Christie, 1982). The FA profile was analyzed

using a GC×GC instrument (Agilent Technologies 7890A, CA, USA) with two columns in series. The FA identification was completed by comparison of the cone position in the chromatogram with the cone position of FA contained in the reference standards, and by considering the elution order and the position of each cone in the two-dimensional chromatogram using the GC Imagine Software (Zoex Corporation, TX, USA).

Statistical Analysis

The individual fatty acids (g/g total FA × 100) and various related groups were analyzed using the following linear mixed model SAS (SAS Inst. Inc., Cary, NC):

$$y_{ij} = \mu + category_i + e_{ij},$$

where: y_{ij} = is the observed trait (fatty acids); μ = is the overall intercept of the model; $category_i$ = is the fixed effect of class category (1 to 18); e_{ij} = residual random error ~ N (0, σ2e).

For each fatty acid, orthogonal contrasts were also estimated between each category *vs* all others. Principal Component Analysis (PCA) was performed using Statistica 7.1 (StatSoft, Paris, France), to analyze relationships among the FA profile of the 18 cheese categories.

Results and discussion

As expected, the goat cheese category (*Formaggi di capra*) was the richest in SFA, mainly due to the contribution of short-chain fatty acids (SCFA), with particular regard to caproic, caprilic, capric and lauric acids (Table 1).

Table 1. Lsmeans of Dry Matter and main fatty acids groups of the 18 cheese categories analyzed (in bold if higher, in italics if lower).

	N	Dry Matter	SFA[1]	MUFA	PUFA	TFA	BCFA
Mean	-	59.7	65.42	29.08	5.50	4.03	2.48
Sd	-	10.0	4.53	3.90	0.97	1.57	0.34
Min	-	40.4	55.04	10.36	3.54	1.48	1.70
Max	-	71.4	83.54	39.50	7.95	7.82	3.70
[2]F-*value*	-	30.3***	25.0***	21.2***	16.3***	26.4***	8.8***
Cheese category							
Formaggi di capra	14	**44.0*****	**70.70*****	*23.81****	5.49	3.01	*2.07****
Casatella Trevigiana DOP e altri freschi	20	**43.7*****	66.69	28.39	4.92	*2.80***	2.38
Morlacco del Grappa	7	**47.3*****	*63.67***	30.40	5.93	4.40	2.64
Caciotta & latteria	12	**54.7*****	67.15	28.21	*4.64***	3.11	2.32
Mozzarella STG e altri pasta filata molle	7	**40.4*****	68.53	27.00	*4.47***	2.90	2.15
Provolone DOP e altri pasta filata dura	9	60.8	68.23	27.09	*4.69***	2.87	2.28
Formaggi affinati nelle vinacce	7	*65.9****	66.88	27.87	5.25	3.48	2.56
Formaggi aromatizzati	14	*64.3****	68.39	26.92	*4.69***	3.06	2.28
Asiago DOP	20	*66.5****	67.00	27.81	5.19	*2.92*	2.44
Montasio DOP	12	*66.6****	68.21	26.85	4.94	*2.84*	2.44
Monte Veronese DOP	14	*65.0****	68.10	*26.15***	**5.75**	**4.27****	**2.67*****
Formaggi a pasta semidura	8	*68.3****	68.85	26.41	4.73	3.16	*2.17*
Piave DOP e altri formaggi a pasta dura	9	*71.4****	68.44	26.35	5.20	3.41	2.37
Grana Padano DOP	10	*68.0****	67.87	27.04	5.09	2.80	2.29
Formaggi di malga freschi	42	61.3	*60.73****	**33.02*****	**6.25*****	**5.34*****	**2.68*****
Formaggi di malga vecchi	19	*66.2****	*60.15****	**33.30*****	**6.54*****	**5.79*****	**2.69*****
Formaggi di malga trentini	24	*65.5****	*60.78****	**32.79*****	**6.43*****	**6.22*****	**2.80*****
Others[3]	10	**53.9*****	68.61	27.00	*4.38****	*2.84***	2.38

[1]SFA= Satured Fatty Acid; MUFA= mono unsatured fatty acid; PUFA= poli unsatured fatty acids; TFA= trans fatty acids ; BCFA= branched-chain fatty acids. [2]F-*value*: refers to the effect of the category on residual variance; [3]Others= blue-marbled-, washed- and mold-soft cheeses; $P < 0.001$ = *** , $P < 0.01$ = **.

Particular was the case of the branched-chain (BCFA) C11:0 *iso*, exclusively present in this category (data not shown). Differently, all the 3 categories (*Formaggi di malga*) of artisanal cheeses produced in the temporary farms during transhumance to Alpine pastures (Bergamaschi et al., 2016) had higher MUFA and PUFA content, and lower SCFA.
On the contrary, the concentration of long-chain (LCFA), in particular margaric and stearic, was very high. These 3 categories, together with *Monte Veronese PDO*, were characterized also by higher content of BCFA and TFA. It worth noting that the impact of TFA on health depends on the single *trans*-FA, and it is directly correlated with the fatty source and the pathway originating it (Haug et al., 2007). For example, vaccenic acid (C18:1 *trans*-11) seems not to be correlated with cardiovascular diseases, and by the way, it is precursor of rumenic acid (C18:2 *cis*-9, *trans*-11 CLA), beneficial to humans.
Collomb et al. (2002) observed differences of cheese CLA content in relation of pasture altitude, showing average values of 0.9, 1.6 and 2.4% in cheeses produced with lowland, mountain, and highland pastures milk, respectively. These differences are confirmed also by our study, in which CLA content of *Formaggi di malga* is significantly higher compared to the other categories. Although any statistical significance was found, we observed high CLA content for *Monte Veronese PDO* and for *Morlacco del Grappa* cheeses. This could be explained by the fact that the PDO cheese is produced also in the Prealps and Alps (Lessinia and Monte Baldo areas), and *Morlacco* is produced especially in the Grappa Massif, a mountainous group of Prealps. On the other hand, *Montasio PDO* showed the highest content of C18:2 *cis*-11, *trans*-13 CLA, maybe because part of this production is also made in mountainous areas. The categories including *pasta filata* cheeses as well as blue-marbled-, washed- and mold-soft cheeses were particularly poor in both CLA and Omega-3 FAs.

Table 2. Lsmeans of CLA, omega-3 and -6 of the 18 cheese categories (in bold if higher, in italics if lower).

Cheese category	Sum of CLA	C18:2 *cis*-9, *cis*-11	C18:2 *cis*-9, *trans*-11	C18:2 *cis*-11, *trans*-13	Omega 3	Omega 6
Formaggi di capra	0.705	0.043	0.645	0.028	0.867	*2.725****
Casatella Trevigiana DOP e altri freschi	0.711	0.033	0.654	0.024	*0.664***	**2.445*****
Morlacco del Grappa	1.124	0.083	1.047	0.025	0.965	2.311
Caciotta & latteria	0.687	0.043	0.632	0.019	0.650	2.173
Mozzarella STG e altri pasta filata molle	*0.514***	0.022	*0.491***	0.018	*0.621***	2.222
Provolone DOP e altri pasta filata dura	*0.526***	0.021	0.498	0.018	*0.658***	2.395
Formaggi affinati nelle vinacce	0.875	0.065	0.786	0.024	0.931	2.186
Formaggi aromatizzati	0.580	0.053	*0.543***	0.016	0.704	2.213
Asiago DOP	0.706	0.036	0.661	0.015	0.882	**2.453*****
Montasio DOP	0.655	0.031	0.622	**0.059*****	0.766	**2.396*****
Monte Veronese DOP	1.055	0.056	**0.986****	0.020	**0.990****	**2.389****
Formaggi a pasta semidura	0.574	0.051	0.550	0.019	0.716	2.299
Piave DOP e altri formaggi a pasta dura	0.727	0.050	0.679	0.016	0.893	2.333
Grana Padano DOP	0.612	*0.023***	0.575	0.018	0.834	**2.507*****
Formaggi di malga freschi	**1.399*****	**0.129*****	**1.263*****	0.025	**1.072*****	*2.143****
Formaggi di malga vecchi	**1.584*****	**0.132*****	**1.426*****	0.028	**1.118*****	*2.124****
Formaggi di malga trentini	**1.627*****	**0.144*****	**1.464*****	0.027	**1.207*****	*1.836****
Others[1]	*0.575***	0.026	*0.541***	0.026	*0.652***	2.071

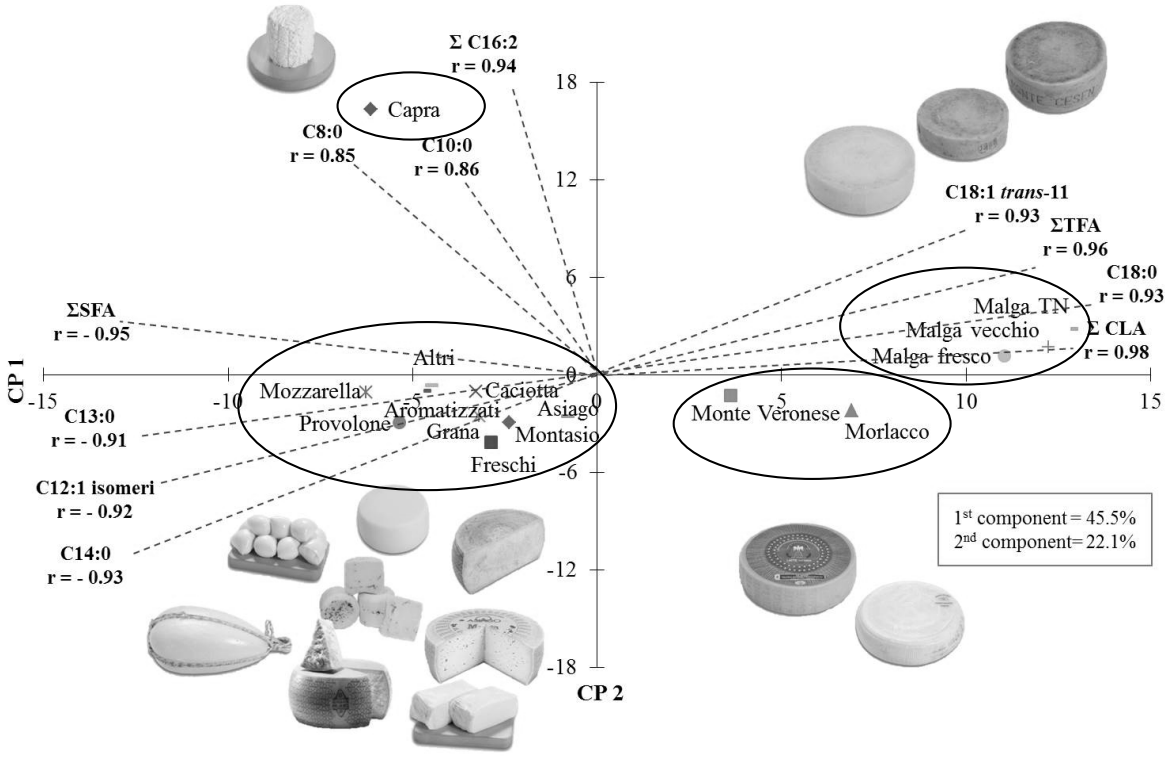
[1]Others= blue-marbled-, washed- and mold-soft cheeses ; $P < 0.001$ = *** , $P < 0.01$ = **.

It has been generally known that cows fed with fresh forage produce milk richer in Omega-3, compared to milk produced by cows fed with corn diets, or rich in concentrates (Chilliard and Ferlay, 2004). This difference is confirmed by the present study, where all the 3 categories of cheese produced with milk from cows reared during summer on Alpine pastures (*Formaggi di malga*) showed a significant higher content of Omega-3. As well as for *Monte Veronese PDO*,

supporting the assumption presented with regard to CLA profile. Moreover, ALA, being a FA contained in fresh grass till 75% of total lipids, was found particularly higher in *Formaggi di malga*, and lower in fresh cheese categories (*Casatella Trevigiana DOP e altri freschi* and *Caciotta & latteria*). The differences in single Omega-6 FAs are even more accentuated for *Formaggi di malga* and *Formaggi di capra*, which had the lowest Omega-6 content when compared to the other categories. It is interesting to note that most of the PDO labels showed significant higher content of Omega-6.

Results from PCA showed that the most important difference was between *Formaggi di malga* and the other cheeses, with *Monte Veronese DOP* and *Morlacco del Grappa* intermediate, and where the highest correlations resulted for the sum of CLA and TFA, followed by vaccenic (C18:2 *cis*-9, *trans*-11 CLA) and stearic acid (C18:0) (Figure 1). The highest correlation within the 2nd component was for ∑C16:2, C10:0 and C8:0, which discriminated neatly the goats cheeses *(Formaggi di capra*) *vs* the other categories.

Figure 1: Principal Component Analysis (PCA) for FA profile of 258 cheeses of 18 categories from Veneto region in Italy.



Conclusions

These results confirmed that the FAs content can be explained in part by the production area, and in part by the production technology, as well as by the species involved. This study confirms the favourable nutritional value of artisanal mountain cheeses (produced in the temporary summer farms during transhumance to Alpine pastures or in permanent farms located in the mountains) especially when compared to cheeses in the past recognised to have a strong link with the mountain territory but nowadays produced mainly in the intensive farms of lowlands.

References

Bergamaschi M., Cipolat-Gotet C., Stocco G., Valorz C., Bazzoli I., Sturaro E., Ramanzin M., Bittante G. 2016. J. Dairy Sci. 99: 9631-9646.

Chilliard Y., Ferlay A. 2004. Reprod. Nutr. Dev. 44: 467-492.
Collomb M., Bütikofer U., Sieber R., Jeangros B., Bosset J.O. 2002. Int. Dairy J. 12:649- 659.
Haug A., Høstmark A.T., Harstad O.M. 2007. Lipids Health Dis. 6: 25.
Hulshof K.F., van Erp-Baart M.A., Anttolainen M., Becker W., Church S.M., Couet C., Hermann-Kunz E., Kesteloot H., Leth T., et al. 1999. Eur J Clin Nutr., 53:143-57.
Pegolo S., Cecchinato A., Casellas J., Conte G., Mele M., Schiavon S., and Bittante G. 2016. J. Dairy Sci. 99: 1315-1330.
Schiavon S., Cesaro G., Cecchinato A., Cipolat-Gotet C., Tagliapietra F., Bittante G. 2016. J. Dairy Sci. 99: 8759-8778.

Multicriteria evaluation for conjoint assessment of milk quality and environmental performances of dairy farms

R. Botreau[1]*, B. Martin[1], S. Hulin[2], C. Kanyarushoki[3], A. Farruggia[1], F. Monsallier[4], A. Loisel[4], R. Hérisset[5], C. Laurent[1]

[1]UMR1213 Herbivores, INRA, VetAgro Sup, 63122 Saint-Genès-Champanelle, France
[2]Pôle Fromager AOP Massif Central, 15000 Aurillac, France
[3]Ecole Supérieure d'Agriculture (ESA), 49000 Angers, France
[4]Chambre d'agriculture du Cantal, 15100 Saint-Flour, France
[5]Chambre régionale d'agriculture de Bretagne, 35000 Rennes, France
*raphaelle.botreau@inra.fr

Abstract

Within the French research project Qualenvic, an evaluation method to conjointly assess environmental performances and milk quality at dairy farm level has been developed to produce a diagnostic. For the environmental assessment, pre-existent tools (EDEN and IBEA) were used and combined, whereas for quality assessment a new method had to be developed. The evaluation of milk quality depends on the targeted final product. Two different products were considered in the project: cheese and drinking milk, only cheese evaluation is presented here. Milk quality is assessed twice a year (in winter and in summer) to consider seasonal variability. For both environment and quality, evaluation criteria were identified and feasible and relevant indicators were selected. The two multicriteria evaluation models were parameterized according to expert opinion, databases and literature. All criteria and overall assessments are expressed on the same 0 (bad) – 10 (excellent) scale. The evaluation method was then tested on a set of 30 diverse French dairy farms. Environment scores ranged almost over the whole scale, from 1.5 to 8.3 (average score at 4.9) whereas quality scores appeared severe, ranging from 1.7 to 4.7 (average score at 3.2). No correlations were obtained between environment and quality scores. However, and despite the reduced range of scores used, significant differences were observed between winter and summer quality criteria scores.

Keywords: multicriteria evaluation, environment, milk quality, cheese

Introduction

Dairy farms are more and more concerned by additional issues to economic sustainability, such as the respect of the environment and the quality of the milk they produce. For the farmer to know how far his management practices are performant on these issues, an evaluation method has to be designed. The French research project Qualenvic (2013-2016) developed an evaluation method to conjointly assess environmental performances and milk quality at dairy farm level and at year scale with the aim to produce a diagnostic tool useful for the farmer or his technical advisor.

Material and methods

To develop the method to assess the environmental performances and the milk quality at farm level we followed a multicriteria evaluation process in several steps (Botreau et al, 2014):

Step 1 – Define the object to be assessed;
Step 2 – State the objective(s) of the assessment;
Step 3 – Identify the specifications of the method deriving from the objective(s);
Step 4 – Define the evaluation criteria;
Step 5 – Choose or develop indicators to check the conformity with the criteria;
Step 6 – Construct the evaluation model: interpret and aggregate the indicators and criteria.

Steps 1 to 3 were ran by members of the research project, whereas Steps 4 to 6 mainly relied

on experts' consultation, with the help of literature and databases. At Step 6, indicators and criteria were aggregated using the CONTRA tool (Bockstaller et al., submitted) relying on the definition of decision trees with fuzzy logic. To be parameterized this tool needs the definition of: favourable and unfavourable threshold values for each indicator, weights to be assigned to the different elements (indicators or criteria) to be aggregated together, and a set of parameters modulating the level of compensation of a bad score on one element by a good one on another. To define these parameters the expert opinion was modelled using conjointly CONTRA and Excel Solver.
The evaluation models were then tested on a set of 30 French dairy farms selected to cover a wide range of system's diversity (Table 1). Environmental indicators were obtained through a questionnaire (one farm missing, n=29), whereas the quality indicators were obtained through analyses performed on samples of bulk milk (n=30). Collected data were then analysed using XLSTAT to run Pearson's correlations and Student's t-test for paired samples.

Table 1: Farms' characteristics.

	No. of dairy cows	Milk production (L/cow/year)	Surfaces' use				No. of grazing days / year
			UAA (ha)	Permanent grasslands (ha)	Temporary grasslands (ha)	Cultures (ha)	
Mean ± *SD*	55 ± *20*	6313 ± *1154*	75 ± *27*	32 ± *35*	29 ± *26*	14 ± *17*	160 ± *73*
Min	24	4479	29	0	0	0	0
Max	100	9535	129	118	87	78	231

Results and discussion

Evaluation method's development

Step 1. The 'object' to be evaluated is the dairy farm over a year, taking into account seasonal variability of milk quality.
Step 2. The 'objective' is to produce a diagnostic tool based on the conjoint evaluation of both environmental performances and milk quality, pointing out strengths and weaknesses.
Step 3. The method must thus remain transparent, avoiding "black boxes", and standardised. To be applied on commercial farms, the method should be performed with limited time and money resources: less than one day on the farm to fulfil environmental questionnaire, only two visits to collect bulk milk samples for quality evaluation (in winter and in summer), and routinely feasible milk analyses for less than 100€/year.
Step 4. Criteria were defined and hierarchically organized with experts to design two decision-trees, one for environment and one for quality. Thus, each issue (environment and quality), is subdivided into several components (5 and 4, respectively), subdivided into sub-components, and themselves subdivided into criteria (Table 2). Since bulk milk quality evaluation depends on the final product, one decision tree must be designed per type of product. The bulk milk quality evaluation model presented here is dedicated to uncooked pressed cheese made from whole raw milk (e.g. Cantal cheese).
Step 5. Indicators were defined for each criteria. For environment, two pre-existent tools were used: EDEN (van der Werf et al., 2009) for the 7 Life-Cycle Analysis (LCA) indicators used in Air, Water, Soil and Resources components, and IBEA (ibea.portea.fr/) for the 33 indicators used in Biodiversity component. For milk quality, a total of 36 indicators were selected to match previously identified specifications, some of them appearing several times in the decision tree.
Step 6. CONTRA needs the user to define a set of parameters to aggregate the different elements. First of all, we defined the threshold values to define the favourable situation (10/10) and the unfavourable one (0/10) for each indicator. For environment, biodiversity

indicators were already interpreted and aggregated up to the sub-component levels by IBEA. For the 7 LCA indicators, thresholds were defined using a database and considering percentile 0.025 as the favourable threshold and percentile 0.975 as the unfavourable one. For quality, thresholds were defined by experts for all the indicators. A given indicator may be used to assess different criteria, and its threshold values may thus vary accordingly. Experts weighed all the elements to be aggregated (see Table 2 for components and sub-components' weights). For quality, the two components linked to cheese organoleptic properties (organoleptic quality) and cheese making (technologic quality) were considered by experts as the most important ones, with weights of 35% and 30%, respectively, far ahead of components linked to nutrition and health. This reflects the fact that cheese remains a "pure-pleasure product" for the consumer. Finally, concerning management of compensations during the aggregation process, experts decided to limit them rather strongly. For instance, when two equally weighted criteria score 0 and 10, the aggregated score assigned by experts is only 3 (instead of 5 with the mean). Experts opinion was successfully modelled by optimising several parameters, resulting in a root mean square error of only 0.33.

Table 2: Environment and quality decision trees: components and sub-components considered, number of indicators required, and weights used for the aggregation of sub-components and components (expressed as %).

Issue	**Components**		**Sub-components**		**No. of indicators**	
	Weights	Name	Weights	Name		
Environmental performance	30	Air quality	-	Climatic change	1	40 ind.
	20	Water quality	-	Eutrophication	1	
	15	Soil quality	60	Acidification	1	
			40	Terrestrial ecotoxicity	1	
	15	Preservation of resources and energy	30	Agricultural surface used	1	
			30	Water consumption	1	
			40	Non-renewable energy consumption	1	
	20	Biodiversity	40	Domestic biodiversity	4	
			60	Wild biodiversity	29	
Milk quality for cheese	35	Organoleptic quality	25	Texture	6	50 ind.
			25	Appearance	3	
			50	Flavour	7	
	30	Technologic quality	20	Aptitude for acidification	5	
			45	Cheese yield	5	
			35	Aptitude for draining	5	
	20	'Health' quality	20	Living organisms	1	
			30	Toxicology	1	
			50	Bioactive constituents	11	
	15	Nutritional quality	75	Fat and nitrogenous matter	4	
			25	Mineral matter	2	

Test on dairy farms

The two evaluation models (i.e. for environment and milk quality), were tested on 30 dairy farms. Environment and quality scores are not correlated at all ($p=0.99$, $R^2=0.00$). All situations are thus possible, including farms rather good or rather bad on both.

Environment scores ranged from 1.5 to 8.3, with an average at 4.9, ranging over almost the whole 0-10 scale (Figure 1). Air and Resources components obtained significantly lower scores (average at 4.71 and 4.88, respectively) than Biodiversity (average at6.60).

Quality evaluation appeared severe ranging only from 1.7 to 4.7, with an average at 3.2 (Figure 1). Despite these low scores, significant differences appeared between components (see Table 4) and periods (summer *vs.* winter). At component level, periods are significantly ($p<0.05$) different, with organoleptic quality and health quality better in summer, and

technologic quality and nutritional quality better in winter. As a consequence, no difference is observed between summer and winter overall quality scores.

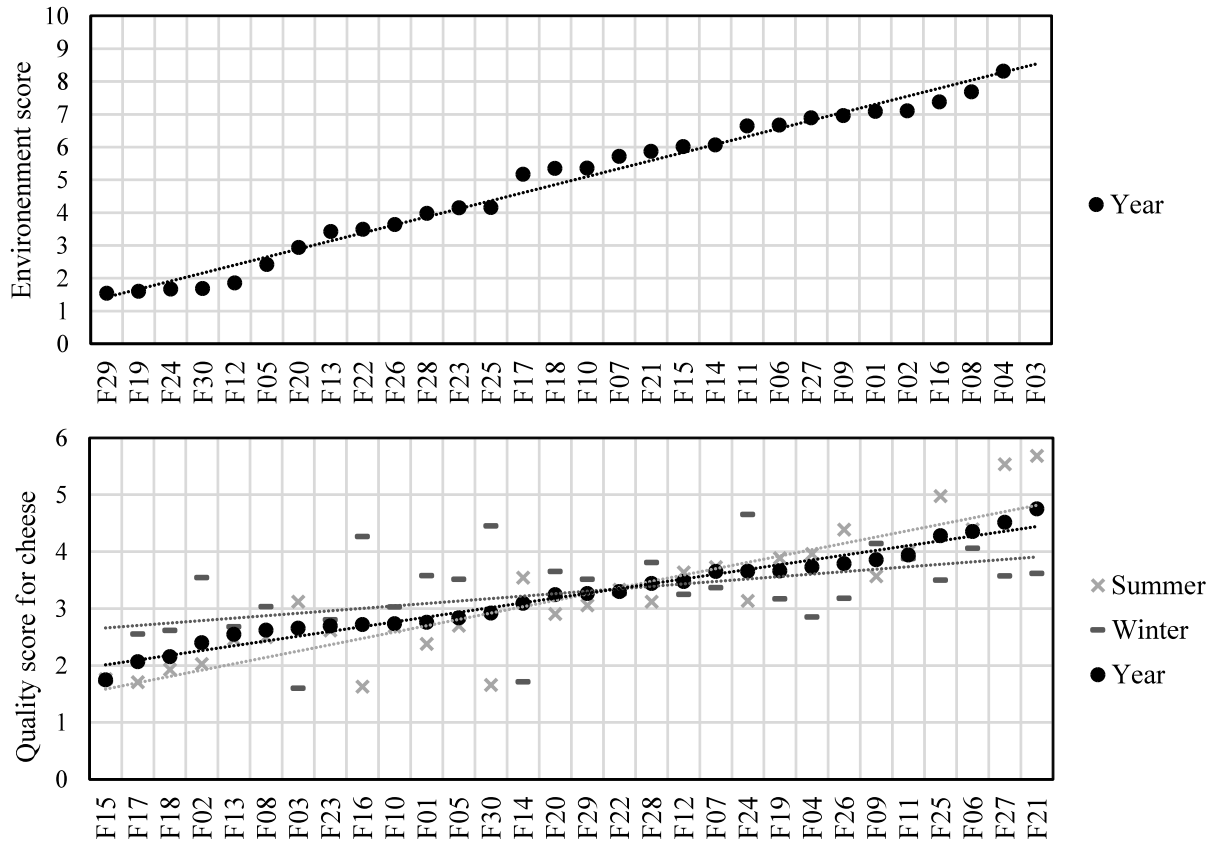
Conclusions

The innovative evaluation method developed here allows to join environmental assessment to milk quality by producing only two synthetic scores that can be disaggregated in a second step to identify strengths and weaknesses. However, the method needs to be refined, especially for quality assessment that results in severe scores. The first test on 30 farms illustrates anyway the method's potential with preliminary results that needs be more deeply investigated and linked to farmers' practices to identify improvement lever.

Table 4: Average scores obtained on the farms for environmental performance (n=29) and milk quality for cheese (n=30), and their components, including statistical comparison between components (p-value < 0.05, with a better than b better than c).

	Components' score					**Overall score**
ENVIRONMENT	Air	Water	Soil	Resources	Biodiversity	
Year	4.71 ± *3.07* c	5.77 ± *2.02* abc	5.67 ± *2.44* ab	4.88 ± *1.92* bc	6.60 ± *2.15* a	**4.85 ± *2.10***
QUALITY	Organoleptic	Technologic	Health	Nutritional		
Summer	4.14 ± *1.16* b	3.33 ± *1.20* c	6.44 ± *0.98* a	2.97 ± *1.42* c		**3.20 ± *1.10***
Winter	3.48 ± *0.74* b	4.38 ± *1.25* a	4.26 ± *0.78* a	4.60 ± *1.68* a		**3.28 ± *0.75***
Year	3.81 ± *1.02* b	3.86 ± *1.32* b	5.35 ± *1.40* a	3.78 ± *1.75* a		**3.24 ± *0.93***

Figure 1*: Scores obtained on the farms for environmental performances (n=29), milk quality for cheese (n=30), including summer and winter scores for quality.*



Acknowledgements
The authors would like to thank the French Ministry of Agriculture w for providing the Casdar funds for QUALENVIC. Thanks are also due to all partners of the project, especially to F. Jourjon, S. Beauchet, M. Thiollet-Scholtus and R. Hérisset.

References
Bockstaller C., Beauchet S., Maneville V., Amiaud B., Botreau R. *Submitted*. Environmental Modelling and Software.
Botreau R., Farruggia A., Martin B., Pomiès D., Dumont B. 2014. Animal 8: 1349-1360.
van der Werf H.M.G., Kanyarushoki C., Corson M. 2009. Journ. Envir. Man. 90: 3643-652.

Socio-economic study of dairy farms in a semi-mountain PDO cheese area (St Flour, Cantal, France): Ways and strategies to improve production system robustness

L. Rey-Cadilhac[1]*, Y. Le Roux[1], H. Rapey[2], J.B. Fanjul[3], F. Monsallier[4], S. Lerch[1*]
[1]UR AFPA / ENSAIA, Université de Lorraine / INRA, Vandœuvre-lès-Nancy, France
[2]UMR Territoires, IRSTEA / INRA / AgroParisTech / VetAgroSup / UCA, Aubière, France
[3]Saint-Flour Communauté, Saint-Flour, France
[4]Chambre d'agriculture du Cantal, Saint-Flour, France
*lrey-cad@hotmail.fr; sylvain.lerch@univ-lorraine.fr

Abstract
In response to the decreasing number of dairy farms and associated workers, municipalities of the Saint-Flour area (Cantal, France) commissioned a study which aimed to better understand and describe the structures and common strategies to putatively robust dairy farms. Fifteen farms were selected and surveyed for their putative robustness. Four main successful strategies associated with improved robustness towards economic, social and environmental risks were highlighted: i) to prioritize forage autonomy through a maximization of grass use and parsimonious concentrate feeding and purchase, ii) to enhance milk paid in link with its intrinsic quality (increases of milk solids contents…) and/or through the milk delivery to dairy plants oriented to high value-added markets, iii) to diversify the income sources with enhancement of the meat byproduct from the dairy herd and/or with the introduction of a suckling cow/ewe herd, or a farmhouse cheese plant, and iv) to reduce structural expenses by subcontracting tasks or by cooperative use of agricultural equipment and/or by very limited investments in equipment and building. Based on this assessment, a panel of proposals will be proposed to stakeholders and decision makers in order to promote those successful strategies via extension programs.

Keywords: systemic assessment, robustness, dairy farm

Introduction
In the semi-mountain Saint-Flour area (average altitude: 1 000 m asl), the dominant agricultural activity is grassland base livestock production systems, and dairy farming is linked to Protected Denomination of Origin (PDO) cheeses (Cantal, Bleu d'Auvergne, Saint Nectaire…) promoting associated touristic activities as well as patrimonial (customs and practices) and landscapes (biodiversity, grassland open area…) conservation. Therefore, it is necessary to maintain dairy farms to guarantee social, economic and environmental dynamics of the area. Nonetheless, recurring hazards have threatened dairy farm sustainability and associated farm jobs over the last 20 years. Thus, municipalities (merged on the name of "Saint-Flour Communauté") were concerned by reduction of dairy farms and commissioned a study to the "Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires" (ENSAIA, Nancy, France). The aim was to better understand the organization and functioning of the complex dairy sector of the area (diversities of farm and factory types) and to bring out drawbacks and strengths for farms robustness in order to present a panel of proposals to decision makers and stakeholders which could be applied via extension programs. Study was performed in two steps: at the sector scale and at the dairy production system scale. This paper reports the results of the second axis, which aims to understand the farming systems structure and functioning, and the characteristics improving their robustness.

Material and methods
Selection of dairy farm
Fifteen dairy farms were identified from a previous study examining the diversity of dairy farming systems in the Saint-Flour area (ENSAIA, 2015), and based on advice from local

experts (INRA, IRSTEA and "Chambre d'Agriculture du Cantal"). The selection was oriented to be representative of the diversity of dairy farms systems of the area in terms of production scale, work organization and feeding systems, and to select farms putatively robust towards climate and economic constraints. Robustness is defined as the ability of the system to face hazards. To do so, the system can react through different types of adaptive processes in response to constraint as resistance, elasticity or plasticity (Blanc *et al*., 2013).

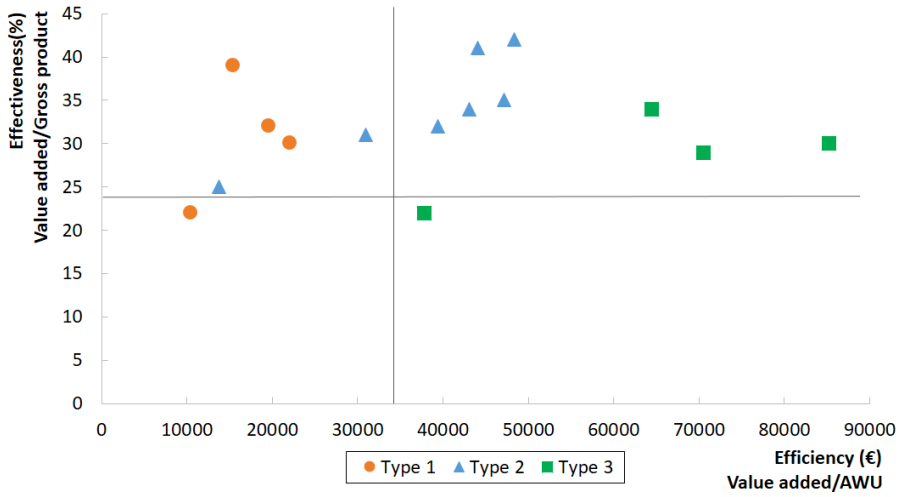
Semi-directive survey

Farmers answered a survey during a 2 to 4-hour farm visit in February 2016 by five students. The semi-directive survey included questions on global organization of farming systems and on five specific topics: technical-economic management (from general accountancy data), work force management, land management, socio-economic environment, and planned future of the farm.

Data Analyses

Each farming system was firstly individually analysed and synthetically characterized regarding: i) land management paying attention on fields diversity and organization [cf. Bathfield *et al*. (2016) method], ii) workforce management paying attention on workload, paid and non-paid workers and work organization through talk from farmers, and iii) an annual economic situation paying attention on balance between receipts and costs and their origins (method of the "Institut de l'élevage" and "Chambre d'agriculture" for the "Réseau Inosys" farms). Three groups of farms were compared through these three topics: i) type 1: farms with less than 130 000 L of milk/annual worker unit (AWU, equivalent to the work of one full-time familial or salaried person), ii) type 2: farms from 130 000 to 250 000 L/AWU; iii) type 3: farms with more than 250 000 L/AWU, each group being supposed to work differently in terms of robustness and ways to improve it. Common assets, drawbacks and overall strategies in order to improve robustness were highlighted for each types based on this transversal analysis. Then, to compare the economic robustness of each farm, we selected and evaluated two economic indicators: one about labour efficiency (value added divided by AWU), one about production effectiveness (value added divided by gross product). Both indicators were calculated based on the general accountancy data (CER France, 2013). Value added (value added = animal, vegetal and other products – operating costs – external cost) was used because it measures the wealth created by the farm without taking subsidies income, workforce, land and investments costs into account. Therefore, farms are evaluated independently of nature of lands (owned or rented) or nature of workers (head of the farm or employee). According to regional economic references (CER, 2013; Velay, 2016), we considered that work efficiency was high when it was more than 34 000€ (limit to be able to earn incomes from the activity), whereas effectiveness of the systems was high when the ratio was more than 24%, as proposed by Velay (2016, Figure 1).

Figure 1: Representation of the surveyed farms in function of the effectiveness of the system (limit at 24%) and the efficiency of labour force (limit at 34 000 €) by type. Type 1: farms with less than 130 000 L/Annual worker unit (AWU); type 2: from 130 000 to 250 000 L/AWU; type 3: more than 250 000 L/AWU.



Results and discussion

As expected based on farm selection criteria, all surveyed farms can be classified as highly effective (Figure 1) and potentially robust at the farmers lifetime scale, even if the incomes obtained by the system are quite modest in case of type 1.

Nonetheless, only nine farms have high work-efficiency, a characteristic which is influenced by dairy production level (i.e., 5 of the 7 farms of type 2 and all the 4 farms of type 3 are very efficient).

Basically, two main ways coexist to achieve robust economic performances: to raise and/or to stabilize inter-annually the gross product, and limit exposure to milk price volatility, and/or to reduce or stabilize both operating and structural costs.

Farmers followed different ways/strategies to improve [i) and ii)] or stabilize [iii)] the gross product:

i) **To enhance the milk value in link with its intrinsic** (milk solid contents, somatic cells and microbial counts) **or extrinsic qualities** by following specific production constraints linked to the milk delivery to dairy plants oriented on high value-added markets (organic milk, raw milk and/or non-fermented grass forages traditional cheese), or through the farmhouse cheese transformation. Almost all the type 1 farms and a part of types 2 and 3 farms followed this strategy raising the milk value (see milk price in Table 1).

ii) **To enhance the meat outcome from the dairy herd** (sales of non-reproductive calves and culled cows), linked with the breed choice and/or a fine reproduction management [e.g., artificial inseminations with sex-sorted semen in order to obtain dairy heifers from the expected reproductive cows, and with meat-breed semen for the rest of the non-reproductive cows (types 2 and 3)].

iii) **To diversify the production with mixed systems** (introduction of a suckling cow or ewe herd which can optimise the use of available buildings/facilities and agricultural surfaces) **or with a farmhouse transformation and commercialization of milk**.

*Table 1: Surveyed farms features by type (mean ± standard deviation). [a]AWU: Annual worker unit; [b]Surfaces covered by permanent or temporary grasslands; [c]Receipts from beef sold minus purchased (byproduct from dairy activity); [d]Gross operating profit minus structural costs, depreciation costs and financial costs; *n=5 because expenses could not be allocated between meat and dairy activities in two diversified farms.*

	Type 1 < 130 000 L/AWU[a]	Type 2 from 130 000 to 250 000 L/AWU[a]	Type 3 > 250 000 L/AWU[a]
Number of farms	4	7	4
Structural features			
Milk production (L)	157 250 ± 41 031	295 000 ± 82 912	471 250 ± 150 686
Number of cows	35 ± 4	48 ± 12	69 ± 15
Number of workers (AWU[a])	2.0 ± 0.8	1.6 ± 0.5	1.3 ± 0.6
Total cultivated area (ha)	56 ± 10	92 ± 51	106 ± 39
Grass area[c] (%)	89 ± 9	94 ± 6	85 ± 14
Technical features			
Milk production by AWU[a] (L)	86 125 ± 30 931	194 786 ± 37 434	378 194 ± 89 578
Milk price (€/1000L)	383 ± 39	381 ± 55	366 ± 43
Meat byproduct[b] (€/1000L)	77 ± 29	68 ± 18	77 ± 23
Cows productivity (L/cow/year)	4 850 ± 1 034	6 200 ± 1 204	7 200 ± 1 249
Distributed concentrates (T/cow/yr)	0.7 ± 0.5	1.4 ± 0.3	1.7 ± 0.3
Economic features			
Operating costs (€/1000L)	129 ± 52	165 ± 29*	206 ± 34
Income[d] / AWU[a] (€)	11 640 ± 3923	28 403 ± 21 332	28 804 ± 6 774
Gross product (€)	108 084 ± 52 486	179 834 ± 54 467	281 747 ± 106 833
Value added (€)	33 199 ± 17 684	63 313 ± 25 535	84 654 ± 44 907
Effectiveness (%)	31 ± 7	34 ± 6	29 ± 5
Efficiency (€)	16 907 ± 5 092	40 071 ± 7 982	64 502 ± 19 823

Conversely, strategies to reduce expenses are:
i1) **To promote feed self-sufficiency through maximization of grass use** (all types have more than 85% of the area covered by grass) **especially grazing** which allows balanced nitrogen/energy diet and reduced grass harvest costs (most of type 1 lands) **and/or through raising the productivity by surface unit with an intensification of the forage areas** (type 2 and 3). Adaptations to increase grazing include the use of mobile milking equipment if grasslands are far from the barn (one farm of type 1, two of type 2 and one of type 3).
i2) **To promote feed self-sufficiency through limited feed purchases** (often linked with a limited cow productivity, type 1, Table 1) **and/or the introduction of crops production** to increase concentrate self-sufficiency (all types). A key factor is the coherence between the amount of distributed concentrate and the cow productivity.
ii) **To reduce structural expenses by subcontracting tasks or by cooperative use of agricultural equipment and/or by very limited investments in equipment and building.**
These results are in accordance with a previous study (Velay, 2016) summarizing year-2014-economic data of 1 673 farms of the Massif Central area. Nonetheless, man should remember than the 2014 year in Massif-Central was quite good for dairy production in regards to both milk price and climate. To really assess robustness, it would be interesting to follow farms over several years to see how they react and modulate their trajectories in face of constraints. This could highlight differences between farm strategies that are not obvious in an economically-profitable year.

Conclusions
Type 1 farms are based on a greater self-sufficiency from the input markets (feed, fertilizers) in order to reduce operating costs as much as possible. This low-input strategy brings them a certain robustness against input price fluctuations, even if it allows only limited income due to

limited dairy cow productivity and then work-efficiency. Therefore, those farms are viable and robust at the generational scale, but seem hardly transmissible at the intergeneration scale because of the low income level. Conversely, type 3 farms showed comfortable incomes in 2014 but are more exposed to input price fluctuations. Building and equipment are also more modern than in type 1 due to a greater investment capacity. Nonetheless, farmers have a heavier workload, which is counterbalanced by overworking or by an essential non-paid labour force (family members). Type 2 farms show mixed strategies: they earn comfortable incomes while keeping a fair feed self-sufficiency. However most of these farms also restrain investments.

Acknowledgements
This work was founded by "Saint-Flour Communauté". We thank L. Richard, general director and P. Jarlier, president of "Saint-Flour Communauté"; ENSAIA students for the field work, notably A. Masson and J. Tizon; ENSAIA teachers for supervision of the study, notably C. Ducoulombier; J. A. A. Pires and C. Mosnier (UMR Herbivores, INRA, Theix, France) for revising the manuscript regarding English language fluency; and the surveyed farmers.

References
Bathfield B., Rapey H., Ingrand S. 2015. Fourrages. 222: 149-156.
Blanc F., Ollion E., Puillet L., Delaby L., Ingrand S., Tichit M., Friggens N. C. 2013. Pag. 265-272 in proc. 20ème Rencontres autour des Recherches sur les Ruminants. 4-5 December, Paris, France.
Brunschwig G., Josien E., Bernhard C. 2006. Fourrages. 185: 83-95.
ENSAIA. 2015. Etude commanditée par Saint-Flour Communauté. 9 pp.
CER France. 2013. Lettre veille économique. 7 pp.
Velay N. 2016. Colloque scientifique SFER LML. 9-10 June, Lempdes, France.

Preliminary study for a new approach to decide the qualitative level of the cheese controlling the animal diet

S. Carpino[1]*, V.M. Marino[1], T. Rapisarda[1], R. Rubino[2]
[1]CoRFiLaC, s.p. 25 Ragusa Mare km 5, 97100, Ragusa, Italy
[2]ANFOSC, Viale Basento, 108, 85100, Potenza Italy
* carpino@corfilac.it

Abstract
The aim of the present study was to analyse 11 cheeses using other quality indicators such as Degree Antioxidant Protection (DAP) and volatile profile other than fat and protein standard parameters. Cheeses from different feeding diets and cheese making technology were analysed. Cheeses were classified by diet in Indoor cheeses derived from cows fed hay and concentrate and Outdoor cheeses from pasture. Two of the cheeses were produced with milk from farms following "Nobile" model where the diet ration was made up of hay polyphyte meadow in percentage of 70% of dry matter. Cheese samples were analysed for gross composition, fat soluble vitamins and volatile profile. Fat and protein values in cheese samples analysed were overall included in range expected for that cheese typology. However, it was observed that the cheese samples were different in antioxidant fat-soluble vitamin contents and aroma profiles. Alpha-tocopherol and β-carotene amount was higher in Outdoor cheeses compared to "Nobile" and Indoor cheeses. Some cheeses had aldehydes indicating ongoing lipid peroxidation. DAP calculation, in fact, supported by aroma profile determination could be useful to show the influence of animal diet on cheese quality. In conclusion, it would be realized a new model to evaluate the quality of a dairy product.

Keywords: cheese quality, pasture, volatile compounds, degree antioxidant protection

Introduction
Several studies show the relationship between animal diet and cheese quality. It is known that fresh forage significantly affects cheese antioxidant fat-soluble vitamin content. Higher α-tocopherol and β-carotene levels are found in cheese in response to fresh pasture intake (La Terra et al., 2010, 2012; Marino et al., 2012). In this context, Pizzoferrato et al (2007) introduced the new parameter Degree Antioxidant Protection (DAP), in order to discriminate pasture from no pasture cheeses. Furthermore, cheeses from animals fed pasture show a richer aroma profile than those from stable (Rapisarda et al., 2013, 2014). Although there is a conspicuous literature regarding factors affecting cheese quality, a great economic value is still exclusively attributed to milk fat and protein content. Indeed, the "quality concept" should be wider extendible also to other chemical parameters. Thus, on the basis of all acquired scientific notions, a new dairy model, like occurs for winery field, would be implemented in order to predict milk and cheese quality.
In this study, several cheeses from different animal diets and cheese making technology were screened. DAP and volatile compounds analysis were used as quality indicators, valid for all cheese typologies.

Material and methods
In this study a total of 11 traditional cheeses including Caciocavallo and Monte Veronese were analysed. Cheeses were classified by diet in Indoor cheeses derived from cows fed hay and concentrate and Outdoor cheeses from pasture. In detail, 6 cheeses (4 Cacicavallo and 2 Monte Veronese) were manufactured with milk from cows reared Indoor in winter season (ID1, ID2, ID3, ID4, IDN1 and IDN2). Forage and concentrate proportions fed were different

within the Indoor cheese group. Only two cheeses, IDN1 and IDN2, were manufactured with milk from farms following "Nobile" model where the diet ration was made up of 70% of hay polyphyte meadow and 30% of concentrate. In addition, 5 cheeses (2 Caciocavallo and 3 Monte Veronese) were produced in summer season from cows reared Outdoor when pasture was available with a little concentrate supplementation (OD1, OD2, OD3, OD4, OD5). Cheese samples were analysed for dry matter and fat using the APHA (2004) method and the IDF (2008) method (ISO 3433), respectively.

Fat-soluble vitamins and volatile profile were determined in all cheese samples. The determination of cheese fat-soluble vitamin contents was as described by Marino et al (2012). The DAP (Degree Antioxidant Protection) index was calculated as indicated by Pizzoferrato et al (2007), a molar ratio between antioxidant components (AC) and oxidation target (OT):

$$DAP = \sum_{i=1}^{n} AC_i (n° \text{ moles}) / OT (n° \text{ moles})$$

Alpha-tocopherol and β-carotene compounds are considered as AC and cholesterol compound as OT.

Volatile profile and odour active compounds (OACs) were determined using Gas Chromatography Olfactometry (GCO) as reported by Carpino et al., 2004.

Results and discussion

This trial, without a rigorously designed experimental setup, is considered just a screening of different cheeses. In fact, no details about farm management were reported. Considering different moisture content in cheese samples, for commodity, all results are discussed on dry matter basis (Tab. 1). Alpha-tocopherol and β-carotene amount was higher in Outdoor cheeses compared to Nobile and the other Indoor cheeses (720 vs. 513 vs. 380; 331 vs. 265 vs. 110 mg/kg dry matter, respectively). .Higher α-tocopherol and β-carotene levels in Outdoor compared to Indoor cheeses agreed with Butler et al. (2008) and Marino et al. (2012) who emphasised the importance of fresh forage on milk antioxidant fat-soluble vitamin content. Moreover, these results were confirmed with the DAP data, on average 9.5 in Outdoor and 5.1 in Indoor cheeses (Tab.1). In accordance with Pizzoferrato et al (2007), when DAP values are ≥7.0 in cheese, the pasture feeding is predominant in the animal diet. However, despite Nobile cheese samples derived from animals fed with hay and concentrate without fresh pasture, DAP values were on average higher than other Indoor samples (6.5 vs. 4.3, respectively), suggesting a high-quality of hay and a good farm management (Tab.1). This result could be explained by Nobile milk disciplinary regulation implicating hay feeding includes not less than five plants in composition.

Table 1: Antioxidants and DAP values in INDOOR and OUTDOOR cheeses

	INDOOR						OUTDOOR				
	ID1	ID2	ID3	ID4	IDN1	IDN2	OD1	OD2	OD3	OD4	OD5
Antioxidants											
α-tocoferolo (μg/100g)	243.3	269.9	373.8	146.7	263.3	353.0	447.7	443.8	403.8	661.9	687.1
β-carotene (μg/100g)	97.8	41.2	62.9	82.7	145.3	171.0	258.0	219.3	255.2	259.6	207.7
α-tocoferolo/DM (%)	401.4	375.5	505.8	238.2	481.9	543.5	603.5	678.0	591.1	862.6	863.2
β-carotene/DM (%)	161.4	57.3	85.1	134.2	265.9	263.3	347.8	335.0	373.6	338.3	260.9
DAP	3.8	3.9	4.7	5.0	5.8	7.1	8.3	8.5	9.1	10.0	11.9

ID = Indoor cheese; IDN = Indoor cheese f rom "Nobile milk" ; OD = Outdoor cheese; DAP = Degree Antioxidant Protection

Within Indoor group the ID1 cheese had the lowest DAP value (3.8) probably due to the presence of corn silage in the diet. In the Nobile group the IDN1 compared to the IDN2 cheese had lower DAP value (5.8 vs. 7.1) confirmed by lowest α-tocopherol content and oxidation products like pentanal, ethoxy propanol, nonanone, octenhydroperoxide, nonanol,

detected by GCO (Tab.2). Thus, the literature (Pizzoferrato et al, 2007) suggests that DAP parameter is useful to overall discriminate cheeses from different animal feeding regimen and to estimate the oxidative stability of cheese fat. Moreover, DAP value could give further information about forage quality. However, also other factors could affect antioxidant fat-soluble vitamin content in cheese such as the milk thermal treatment and cheese making technology (Marino et al., 2016). In general, odour active compounds (OACs) profile was not very rich in all cheese samples analysed. Within the same cheese typology, the results relative to Nobile cheese were interesting with a high number of OACs compared to other Indoor cheeses Monte Veronese Outdoor cheeses showed the highest odour active molecules number among all cheeses, in particular ester compounds giving to cheese samples fruity flavour. Moreover, higher DAP values were found in Outdoor Monte Veronese compared to Indoor cheese samples. However, in Monte Veronese including surprisingly also Outdoor cheeses, markers of lipid oxidation were found. In fact, 2-hexanal and heptanal molecules were present in Indoor and Outdoor samples, respectively, assuming a probable consumption of milk antioxidants.

Table 2: Odor active compounds in INDOOR and OUTDOOR cheeses

					INDOOR						OUTDOOR				
Compound	Chemical class	Descriptor	LRI	Ident	ID1	ID2	ID3	ID4	IDN1	IDN2	OD1	OD2	OD3	OD4	OD5
ethyl acetate	ester	apple	568	PI/MS											*
dimethyl sulfide	sulphur	garlic	580	PI/MS				*							
methyl ethyl sulfide	sulphur	sulphur, garlic	616	PI/MS	*			*	*	*	*	*		*	*
isobutanol	alcohol	yeast, solvent	634	PI/MS	*					*					
diacetyl	ketone	butter	640	PI/MS	*		*	*	*	*	*	*		*	*
thiophene	sulphur	rancid, garlic	665	PI/MS	*		*	*	*	*	*	*		*	*
pentanal	aldehyde	garlic	729	PI/MS					*						
methyl methylbutanoate	ester	apple	745	PI/MS		*	*				*			*	*
propyl propanoate	ester	peel apple up,pineapple	793	PI/MS		*					*				*
ethyl butyrate	ester	apple	797	PI/MS	*	*	*	*		*	*	*	*	*	
ethoxypropanol	alcohol	apple	828	PI/MS					*						
butyric acid	acid	butyric	843	PI/MS								*	*		
2-hexanal	aldehyde	orange	849	PI/MS		*	*				*				
methylbutyric acid	acid	rancid	866	PI/MS					*						
3-isothiocyanato-1-propene	thiocyanato	garlic	885	PI/MS										*	
ethyl valerate /ethyl pentanone)	ester	apple	896	PI/MS		*	*				*			*	*
methyl-2-(methylthio)acetate	ester	potato	900	PI/MS										*	*
heptanal	aldehyde	butyric,rancid	903	PI/MS							*				*
methyl hexanoate	ester	apple	931	PI/MS						*					
ethyl 3-hydroxybutanoate	ester	apple,marshmallow	935	PI/MS							*				*
methional	sulphur	potato	926	PI/MS	*			*	*	*		*	*		
methionol	sulphur	potato up	940	PI/MS							*				*
3-mercaptothiophene	sulphur	cooked meat, garlic	977	PI/MS						*		*	*		
2-octanol	alcohol	sulphur/mushroom	987	PI/MS							*			*	*
3-carene	terpene	orange up	995	PI/MS		*	*				*			*	*
limonene	terpene	orange	1011	PI/MS						*		*	*		
ethyl dimethyl pyrazine	pyrazine	bread	1068	PI/MS			*								
nonanone	ketone	sweet, hot milk	1093	PI/MS					*						
limonene oxide	terpene	fruity/orange	1125	PI/MS		*	*							*	*
octenhydroperoxide	peroxide	mushroom	1127	PI/MS					*						
nonanol	alcohol	floral, green	1153	PI/MS					*						
ethyl octanoate	ester	wine	1188	PI/MS		*	*				*			*	*
ethyl octenoate	ester	wine	1192	PI/MS				*		*		*	*		
Total					6	8	10	7	10	10	14	9	6	12	15

LRI = Linear retention Index; PI = identif ication by f lavournet internet database; MS = identif ication by mass spectrometer ; ID = Indoor cheese; IDN =

Conclusion

This screening, although cheeses were different in cheese-making technology, confirm one more time the importance of feeding diet on cheese quality. Fat, protein values in cheese samples analysed were overall included in range expected for that cheese typology. However it was observed that these samples were different in antioxidant fat-soluble vitamin contents and aroma profiles. Moreover, volatile aldehydes identified in some cheese samples showed ongoing lipid peroxidation. These results demonstrated that fat and protein are not enough to define the quality of the cheese. DAP calculation supported by aroma profile determination could be either useful to show the influence of animal diet on cheese quality and to estimate the oxidative stability of cheese fat. In conclusion a new model of evaluation should be implemented to define the quality of a dairy product.

References

APHA 2004.

Butler G., Nielsen J.H., Slots T., Seal C., Eyre M.D., Sanderson R., Leifert C. 2008. J. Sci. and Food Agric. 88: 1431-1441.
Carpino S., Horne J., Melilli C., Licitra G., Barbano D.M., Van Soest P.J. 2004. J. Dairy Sci. 87: 308-315.
International Standard - ISO 3433. 2008. pp.1-12.
IDF. 2008. Bulletin N° 222. ISO 3433.
La Terra S., Marino V.M., Manenti M., Licitra G. 2010. Dairy Sci. Technol. 90: 687-698.
La Terra S., Marino V.M., Schadt I., Caccamo M., Azzaro G., Carpino S., Licitra G. 2012. Dairy Sci. Technol. 93: 1-10.
Marino V. M, Schadt I, La Terra S., Manenti M., Caccamo M., Licitra G., Carpino S. 2012. Dairy Sci. Technol. 92: 501-513.
Marino V. M., Belbeldi A., La Terra S., Licitra G., Carpino S. 2012. J. Food Agri Env. 10: 186-190.
Marino V.M, Schadt I., 2016. Int. Dairy J. 56: 29-32.
ISO 8968-1. 2014.
Pizzoferrato, L., Manzi, P.,Marconi S., Fedele V., Claps S., Rubino R. 2007. J Dairy Sci. 90: 4569-4574.
Rapisarda T., Pasta C., Belvedere G., Schadt I., La Terra F., Licitra G., Carpino S. 2013. Dairy Sci. Technol. 93: 117-134.
Rapisarda T., Pasta C., Carpino S., Caccamo M., Ottaviano M., Licitra G. 2014. Anim. Feed Sci. Technol. 191: 39-46.

The link with the Terroir in PDOs (Protected Designations of Origin), a fundamental element to establish a suitable specification

A. Ognov[1]*, Ch. Marzin[1],E. Vergnol[2], G. Flutet[3]
[1]INAO, Agricultural products and agro-food department – 12 rue Henri Rol-Tanguy TSA 30003 – 93555 Montreuil Sous Bois cedex - France
[2]INAO, Territorial Delegation Auvergne Limousin - Village d'entreprises - 14, avenue du Garric 15000 Aurillac - France
[3]INAO, Territories and delimitation department – la jasse de Maurin – 34970 Lattes - France
*a.ognov@inao.gouv.fr

Abstract
In France, the designation of origin concept is largely based on the "terroir" idea.
This notion has been defined in 2006, by a working group gathering INRA (Institut National de la recherche Agronomique) and INAO (Institut National de l'Origine et de la Qualité) experts. The "terroir" is based on an historical construction of a system of interactions between human factors (the know-how) and natural factors (physical and biological environment) (Casabianca *et al*, 2006).
This terroir system can be described in the "link with the geographical area" as required by the European rules for PDO registration.
In France, for 80 years, INAO* examines the application for PDO registrations. To do so, INAO assesses if the project reveals a real link between the product and the "terroir", and if all the key elements are detailed in the product specification.
The INAO assessment relies on studies by scientific experts. As far as cheeses are concerned, and especially mountain cheeses, the link with the geographical area reveals practices adapted to an area, both on its natural environment (breed, feed) and on its social aspects (cheese size, producers organization, traditional practices…).

Keywords: Protected Designations of Origin, terroir, cheese

Introduction
In France, 45 cheeses are protected as PDO. A national Institute, INAO, is in charge of applying this European policy, by recognizing these PDO products, especially through the "terroir" idea. To do so, INAO, has defined the "terroir" (in collaboration with INRA) and evaluates if there is a link between the product and its terroir. As far as cheeses are concerned, and especially mountain cheeses, the link with the geographical area reveals practices adapted to an area, both on its natural environment (breed, feed) and on its social aspects (producers organization, traditional practices, cheese size, …).
After explaining the registration procedure of PDO cheese by the INAO, we will present the "terroir" idea, through a definition and some examples of PDO cheese specifications.

Registration procedure
The originality of French policy in PDO is INAO. INAO, the French Institute for Origin and Quality is a public-sector body, which, among other assignments, examines applications for new GI (Geographical Indications) products or for amendments to a PDO specification. It also supervises controls on all the signs and protects products against illegal use of their names.
INAO is composed of 5 national committees which are deliberative body responsible for recommending the recognition of products likely to benefit from PDO and for revising existing specifications.

National committees are mainly composed of representatives of industry professionals engaged in production, processing and distribution of quality and origin products, and representatives of French administration (Ministry of agriculture and ministry of economy).
The application for PDO registration or for amendment of PDO specification is subject to a validation process.
Commission of inquiry is appointed by the relevant national committee to accompany the applicant group to write the product specification and to fulfill legal and technical requirements.
Alongside the work carried out by the Commission of Inquiry, national committee can be consulted to give directions or to take decision step by step.
Besides national committees and its Commission of Inquiry, the INAO assessment relies on expert works which are specialized in natural environment (geology, climatology, …), in technical practices (agronomy, livestock science, …) or in social science (history, ethnology,…).
Their main expertise is to define the geographical area and to help writing down the link between the product and the geographical area.
During this process, INAO assesses if the project reveals a real link between the product and the terroir (PDO can't be reduced to a mere geographical origin), and if all the key elements are detailed in the product specification.

A definition of "terroir": a tool to define the link with origin
In 2006, a working group composed of INRA researchers and INAO proposed a definition of "terroir ». This definition is:
« A terroir is a delimited geographical area, in which a human community achieved a collective knowledge of production, based on a system of interactions between a physical and biological environment, and human factors, in which the socio-technical processes involved, reveal an originality, confer typicity, and generate a reputation, for a product native of this terroir ».(Casabianca et al., 2006)

For the characterization of the link with the geographical area, key points must systematically be analyzed, such as:

- human community
- collective knowledge of production,
- system of interactions between human factors and natural factors,
- socio-technical processes,
- originality, typicity and reputation of product.

Many of these elements can be used to define the geographical area of production (Berard et al., 2004)
After its approval by national committee, the draft specification is then subject to public consultation so that all the actors potentially involved in such a project can express their views thereon.
In PDO specifications, the technical process must be consistent with the link with the geographical area.

Table 1:4 examples of PDO mountain cheeses

PDO Cheese	production specification					
	Type of cheese	Area	Breed	Feeding	Manufacturing process	Min. Ripening
LAGUIOLE	Pressed uncooked	Aubrac	Aubrac French simmental	Grazing 120 days and hay	Manufacturing process with raw milk	4 months
SALERS	Pressed uncooked	Massif central	Salers (for "Traditions Salers" cheeses)	Only grazing, from April, 15th to November, 15th	Milking in a wooden tank ("gerle")	3 months
ABONDANCE	Pressed cooked	Alpes	Tarine and Abondance	Grazing and hay	Milking conditions which respect the natural microflora of the milk Manufacturing process with raw milk Use of traditional materials (copper vats, wooden board for ripening)	100 days
COMTE	Pressed cooked	Jura	Montbelliarde and French simmental	Grazing and hay	Milking conditions which respect the natural microflora of the milk Manufacturing process with raw milk Use of traditional materials (copper vats, wooden board for ripening) "fruitiéres "	120 days

The breeds are adapted to the environment. For example, the "Aubrac" is the historical breed from the Aubrac area which was defined as the geographical area of the Laguiole PDO. "Abondance" and "Tarine" breed are also adapted to the geographical area because of their ability to graze in high altitude.

The feeding with grazing and hay is a traditional practice in theses mountain areas, and it influences the characteristics of milk and therefore cheeses (Coulon *et al,* 2005).

The process takes into account traditional elements:

- "gerle" for Salers PDO which was identified as an important "terroir" factor during the registration process
- milk must be brought quickly to milk processing plants for Comté PDO, so that a social organisation in cooperatives called "fruitiéres " has been maintained until now.

Grassland based livestock systems also provide ecosystem services in mountain areas. In PDO cheeses specifications, feeding shall be sourced within the defined geographical area and other rules, for example, limitations of livestock density and a minimum amount of grazing days can help to maintain the specific grassland biodiversity of this area. These systems also contribute to landscape's quality and helping maintaining livestock farming they sustain employment in mountain areas.

Conclusions

The analysis of the link with the geographical area leads to a specification which includes practices adapted to a geographical area.

Nowadays, consumers/citizens have high expectations for products which respect the territory they come from and PDO products fully respond to this demand.

Although PDO registration is based on historical and common practices, amendments to a product specification may occur to take into account, for example, technical evolutions. Once again, INAO scrutiny aims at maintaining the "link with the terroir". Sometimes, at this stage, some past production methods, abandoned or forgotten but now responding to social or local expectations, may arise again.

References

Casabianca F., Sylvander B., Noel Y., Beranger C., Coulon JB., Giraud G., Flutet G., Roncin F., Vincent E. 2006. Pag 544-551 in proc. 5th International Terroir congress, Bordeaux and Montpellier, France.

Berard L., Marchenay P. 2004. *Les produits de terroir, entre cultures et règlements* CNRS édition, Paris, 2004, 229 p., 72-77.

Coulon J.B., Delacroix-Buchet A., Martin B., Pirisi A. 2005. INRA Prod. Anim. 18(1): 49-62

Regulation (UE) n°1151/2012 of the European Parliement and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs.

Salers, Comté, Abondance and Laguiole specifications: http://www.inao.gouv.fr/.

Reimagining British Mountain Cheese

Bronwen Percival[1]*
[1]Technical Manager, NEAL'S YARD DAIRY, 108 Druid Street, London, SE1 9AB, United Kingdom
*bronwen.percival@nealsyarddairy.co.uk

Abstract
Over the course of the late-nineteenth and early-twentieth centuries, subsistence hill farmers in Britain gradually abandoned cheesemaking as improved transportation, an eager market for liquid milk, and competition from inexpensive imported factory cheeses made farmhouse production increasingly unviable. As a result, 'dales-style' cheeses made on marginal land either became extinct or evolved to suit factory methods of production. What were once soft and supple seasonal cheeses made entirely from grass-based forage have been transformed into the crumbly acid cheeses that we know today. Since the deregulation of the British milk market in 1994, prices have fallen and the sale of liquid milk no longer provides a comfortable income for small-scale dairy farmers; at the same time, the market for British farmhouse cheese is growing and becoming more sophisticated. Adding value at the farm presents an opportunity for these farmers to resurrect forgotten modes of production and explore their gustatory, environmental, and social implications.

Keywords: Britain, Wensleydale, Northern Dairy Shorthorn, invented tradition

Introduction
It might seem peculiar to include a presentation on British cheese in a mountain cheese conference: the highest peak in England and Wales has a height of 1085 metres, far lower than the *average* elevation of all the land in Switzerland, at 1350 metres. Rather, today Britain is best known for its cheeses that evolved on lush, wet lowlands, such as Somerset Cheddar.
But Britain was once home to a very different family of cheeses, which were made seasonally on marginal smallholdings in the remote hills of the Yorkshire Dales, County Durham, and Wales. The production systems for cheeses such as Wensleydale, Cotherstone, and Caerphilly once shared many attributes with modern continental mountain cheeses. The small number of upland hay meadows of the Yorkshire Dales that have been conserved in their native form are host to a diverse community of terpene-rich dicotyledons, insects, birds, and small mammals; hay was produced from these meadows to feed the animals through the winter, when no cheese was made. The breeds of these regions were similar to robust modern mountain cows: multi-purpose animals that provided both meat and milk for cheese, and whose low yields were counterbalanced by hardiness, suitability for rugged terrain, and long lifespan.
The cheeses, too, bore little resemblance to their modern counterparts. While in contrast to Alpine-style cheeses, these original British hill cheeses would have been made by women, their slow acidity development (due to use of preripened milk and/or the microbial biofilms on porous equipment rather than added whey or commercial starter cultures) gave them a supple, pliant texture, often punctuated by naturally-occurring blue mould, possibly not dissimilar to Savoyard Bleu de Termignon. An entry in the *Journal of the Royal Agricultural Society of England* from 1861 stated that Cotherstone cheese "more than any other English cheese…resembles the foreign Rochefort." In 1935, writer Osburt Burdett stated, "Wensleydale…should be creamy, rich, subtle in flavour, and be soft enough to spread." Due to their slow acidification and lack of exogenous starter cultures, these cheeses would have tasted of the unique raw materials used to make them, and their long keeping quality befitted their remote areas of production, allowing them to be collected just a few times over the

course of a season by cheese merchants for sale at distant cheese fairs.
Prior to the advent of railway access to the Yorkshire Dales in the late 1870s, all the milk produced on farms was transformed into butter and cheese. However, as better transportation infrastructure became available, it became possible for small farms to sell liquid milk for direct consumption to markets as far away as London, 250 miles distant. Furthermore, as early as the 1890s, progressive cheese merchants in the Yorkshire Dales, frustrated by the variable quality of the cheeses made on small farms, began to introduce the practice of buying milk rather than cheese, centralising production in creameries under their direct control. Both these innovations, along with the high market price of liquid milk, served as an enticement to smallholders to lighten their workload and improve their cash flow. By 1957, farmhouse production of Wensleydale had ceased entirely.
As production has moved from farm to factory, the cheeses themselves have been physically transformed. Where the process on the farm once depended on variables such as the microbial load of the milk and the ambient temperature of the kitchen, within the factory it has been brought under the discipline of the clock. Furthermore, the use of large amounts of starter culture and mechanised production lines has allowed the process to be sped up, leading to crumbly, demineralised textures and pronounced acid flavours in the finished cheese. The ripening time has decreased as well; where once, "as befit[ted] its quality, Wensleydale require[d] about six months to ripen," modern factory Wensleydale derives its acidic flavour from the primary fermentation and can be sold when it is only a few weeks old.

Discussion
Wensleydale, with its illustrious but largely forgotten history as one of the "finest of all blue cheeses," is one of the great lost cheeses of the British Isles. Today, interest in raw milk, farmhouse cheeses is growing within the United Kingdom, but most new entrants to the market have chosen to produce cheeses modelled upon established styles of soft-ripened cheese from France, Italy, or Switzerland, which are better understood by many consumers in search of 'artisan' cheese for their tables. Meanwhile, the few remaining producers of historic British cheeses have begun to plumb the historical records for inspiration and insights into the ways their own cheeses have evolved over the past hundred years, while specialist retailers are beginning to recognise that a resurgence of diverse farmhouse British cheeses that take their inspiration not from factory templates but from earlier methods of production is overdue. The situation presents an opportunity for a new generation of farmhouse cheese producers focusing on the original styles of their regions.
This presentation takes as its case study the example of Andrew and Sally Hattan of Low Riggs Farm, a remote 186-hectare hill farm at an altitude of 250-430 metres above sea level in the Nidderdale Area of Outstanding Natural Beauty near Harrogate, Yorkshire. The Hattans are not struggling liquid milk producers, but rather took on Low Riggs as a sheep farm in 2007 with the intention of practicing environmental stewardship and restoring the natural biodiversity of its upland hay meadows and the condition of its crumbling dry stone walls.
Andrew, who holds a PhD in energy utilisation in high-yielding dairy cows, was intrigued to explore the potential for the farm's marginal land to support cattle at the opposite end of the spectrum, and acquired a small herd of original population Northern Dairy Shorthorn (NDS) cattle, which are classified as 'critical' on the Rare Breed Survival Trust's watchlist (i.e., the population consists of less than 150 registered breeding females). The dual-purpose NDS breed originated in the area and was bred to suit the demands of the landscape and the needs of the local smallholders. With so many farms in the area reliant on European agricultural subsidies for survival, and with the spectre of Brexit calling into question the longevity of any agricultural subsidy whatsoever, the Hattans' plan is to revive the old production methods of farmhouse Wensleydale within an extensive farming system, tapping into the demand for

'natural' and 'authentic' British farmhouse cheese and thus securing the future of the farm.
The project faces significant challenges. First and foremost amongst these is recreating the physical process. The methods for making farmhouse cheeses evolved slowly over centuries through a process of trial and error, with many of the most important elements of the process relying on tactile judgments that were never recorded. In communities with hundreds or thousands of producers and a living tradition, questions can be answered and observations made; without this infrastructure and with the technical knowledge of the cheese now firmly rooted in factory production, the Hattans have been forced to rely on sparse descriptions gleaned from a few contemporary chronicles that were written by observers who were not intimately familiar with the cheesemaking process. They have also looked to the very few living members of their community who can remember the farmhouse cheese, now centenarians, for context and hints as to the way the process was carried out.
No less a challenge is interfacing with regulators. Starting a business that expressly looks to historical methods for inspiration in a region that destroyed its farmhouse cheesemaking traditions fifty years ago involves recreating clusters of technical competency from scratch. The local Environmental Health Officer assigned to inspect and approve the Hattans' business is not a cheese specialist, and her only previous encounter with cheesemaking has been at a large factory making pasteurised, high-acid Wensleydale for supermarkets. Even though the methods the Hattans propose to use for their farmhouse Wensleydale (such as the use of raw milk, low doses of liquid starter cultures, and wooden boards for ripening) are employed perfectly legally and successfully by other cheesemakers elsewhere in the UK, and will be fully supported by a HACCP-based quality system by the time they begin commercial production, the need to satisfy a nervous and sceptical inspector for whom factory production represents the norm is yet one more obstacle that will require a significant investment to overcome.
Finally, the system of production proposed at Low Riggs Farm, which aims to respect biodiversity and the extensive nature of the original subsistence farms of the Yorkshire Dales, is—by definition—one of high labour inputs and low yields. While the cows' diet will be almost exclusively grass-based, minimising the cost of bought-in feed, the total yield may be as low as 1800 litres over a 152-day lactation. In addition, the requirement for highly-skilled labour means that the Hattans will be tied to the farm throughout the cheesemaking season. To be viable (let alone attractive as a business proposition), the wholesale price of the cheese ex-farm will need to be high, in the range of £19/kg, which will translate to a retail price of approximately £50-£60/kg.
From the perspective of a retailer/exporter of British farmhouse cheeses, the initial trial batches made by the Hattans have been extremely exciting, particularly for their supple texture, which is completely unlike the crumbly roughness of factory Wensleydale. Several specialist independent retailers are already mobilising to support the project. Implicit in this is starting a conversation about a next wave of British farmhouse cheese production characterised by the utilization of autochthonous resources at every level, from microbes to diverse pastures to rare breeds of cow. If the conversation can be framed in such a way as to further stratify the market, the future looks very bright indeed.
The Low Riggs Wensleydale project is currently in its development phase, having received in December of 2016 an EU LEADER grant towards the cost of the cheesemaking infrastructure. The summer of 2017 will see the Hattans visiting the Auvergne to research options for mobile milking parlours and making further trial batches of cheese to refine their method. The cheese's prospective commercial launch date is late-summer 2017 or early 2018.

Acknowledgements
The author wishes to thank Neal's Yard Dairy for the support they have provided for research

and participation in the Mountain Cheese Network Conference, Andrew and Sally Hattan of Low Riggs Farm, and Andy Swinscoe of The Courtyard Dairy for their collaboration.

References

Calvert K. 1977. *Wensleydale Cheese.* (Clapham: The Dalesman Publishing Company Ltd.) 12-15.
Hallas C. 1986. Agric. Hist. Rev. 34: 38-39.
Hartley M., Ingilby J., 1997. *Life and Tradition in the Yorkshire Dales.* (Otley: Smith Settle) 18.
Hattan, A., Hattan, S. 2016. Reviving the Northern Dairy Shorthorn: A Model for Using Low-Yielding Breeds Within Extensive Dairy Systems." Presentation at the Conference on the Science of Artisan Cheese, North Cadbury, Somerset. 23 August 2016.
Squire J., ed. 1937. *Cheddar Gorge: A Book of English Cheeses.* (London and Glasgow: Collins Clear-Type Press) 105.
Various. 2011. *A Guide to English Cheeses – A Collection of Historical Articles on Varieties of English Cheese.* (United Kingdom: Read Books Ltd.) 16.
Voelcker, A. 1861. "On the Composition of Cheese, And On Practical Mistakes in Cheese-Making." *JRASE* 22:47.

Terroir Cheese network: an original tool to support Research and Development for PDO cheese sector

C. Spelle*
CNAOL coordinator
*cspelle@cniel.com

Abstract
Terroir Cheese network is a network of diverse actors gathered from Training, Research, technical centres and terroir cheese sectors. It works on strategic issues for the Terroir cheese sector. The aim of the network is to develop projects that correspond to the need of the field and to assure the transfer of the results. Since 2009, the network has worked on several themes specific to the Terroir Cheese sectors: Product quality, natural resource enhancement, farm and sectors sustainability. Productions include summary books on a strategic subject but also tools and methodological approaches which allowed change in practice (on a technical or organisational level).
The activity of the network is currently focused on 4 key subjects: microbial ecosystem of terroir cheeses, capitalisation and transmission of cheesemaking know-how, forage management and ecosystem services and performance of the PDO farms.

Keywords: terroir cheese, research and development network

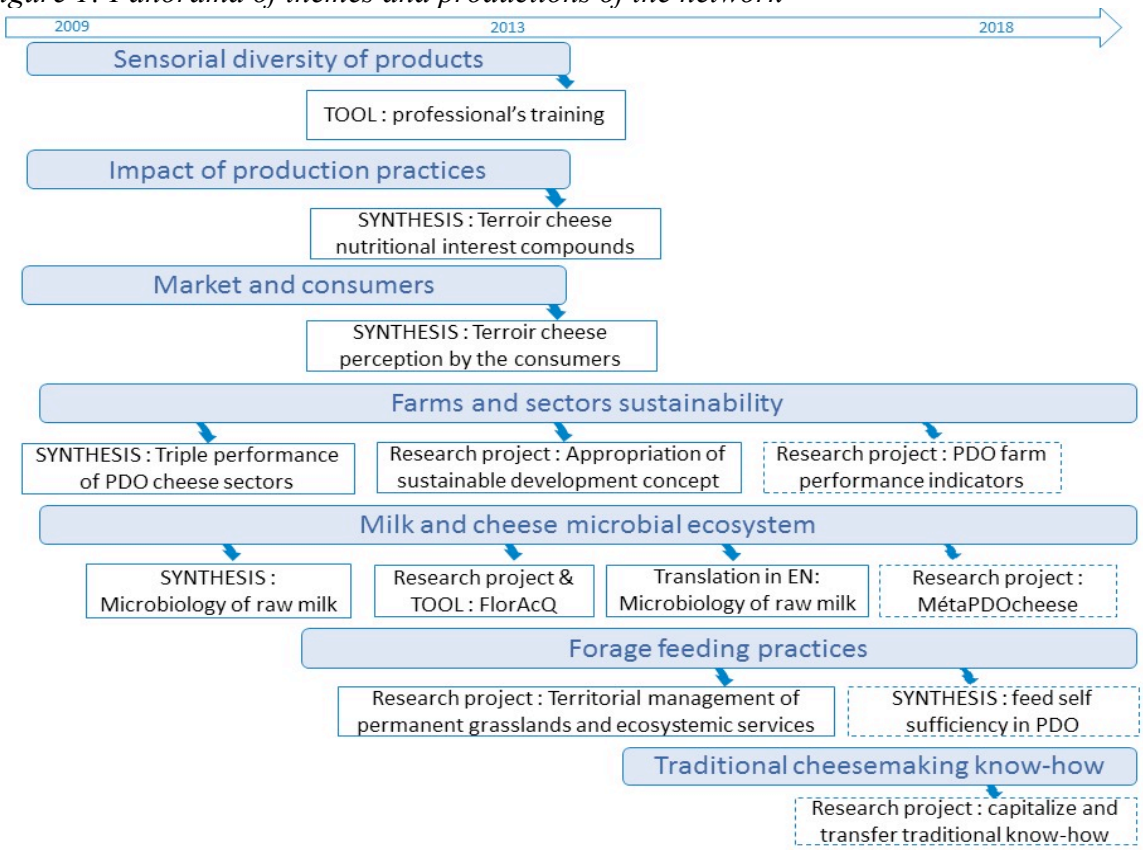
Introduction
In France, an agricultural sector as strategic as the one of Terroir Cheeses, needs strong support from Research & Development to assure a harmonious progression. Different PDO cheese territories had organised their own Research & Development (in the Alps, in Franche-Comté or in Massif Central for example). Ten years ago, the need for a national structure that would encourage communication and collaboration between the different regions was felt. Furthermore, a national network would help to strengthen the links with academic research in order to carry out ambitious programs. This is how the Terroir Cheese Network emerged, in 2009.

Organisation of the network
Terroir Cheese Network is a “Réseau Mixte Technologique (RMT)”: a tool funded by the French Minister of Agriculture. It finances the coordination of a network gathering actors from Research, Development and Education around a specific subject. The goals are to consolidate a pool of competences on a strategic subject and to boost interactive innovation in agricultural research. There are currently 20 agricultural RMTs in France. They are labelled for a 5 year period. A project labelled by an RMT has direct access to some public calls for proposals.
The Terroir Cheese Network is coordinated by the CNAOL (National Dairy Designation of Origin Committee) and gathers three national structures of Research (INRA, Actalia, Idele), four regional development centres (Pôle AOP Massif Central, GIS iD64, Ceraq, CTFC) and three educational establishments (ENIL’s network, CFAA64, VetagroSup).

Production and impact of the network
Regarding its organisation, the Terroir Cheese network ensures a strong link between professionals, regional development centres and Research. The coordination by a Professional Association ensures the link with the sectors. The structuration of multi-actors working groups allows for the meeting of different structures and approaches.

The RMT doesn't provide funds for the carrying out of projects. However, it consolidates the major steps before and after a project: to identify the relevant problems, to build the project and the partnerships and afterwards, to transfer continuously the results.
Those steps are essential for the management of projects which match the needs of the field and also which have an appropriate transfer of results.
The network has worked on diverse subjects specific to the Terroir Cheese sector.
Figure 1 presents the themes and the productions of the network.

Figure 1: Panorama of themes and productions of the network



Major axes of works focused on Terroir Show (enhancement of local resources, maintenance of traditional know-how) and on territorial anchoring of cheese sectors.
For example, concerning natural resources enhancement, the network has been working for a long time on the microbial ecosystem of raw milk cheese. The research project FlorAcQ (2011-2014) aimed at studying the diversity of raw milk microflora and its factors of variation at the farm level. It developed an innovative tool to assist producers in their management of raw milk microbial ecosystem in order to produce a cheese of quality. This was innovative for two reasons. First, due the technical tool developed to study and represent microflora diversity of raw milk and second, for the new approach towards management of raw milk quality it proposes. Currently, the network structures a program to capitalize on genomic tools to study, understand and manage microbial ecosystem in cheese production. The first step is the project MetaPDOcheese (2017-2020) which will make the inventory of the microbial population of French PDO cheeses. It will study this biodiversity but also will help to understand the structuring of the microbial communities better.

Conclusions

The Terroir Cheese network coordinates and contributes to research projects specific to PDO cheeses. Its originality relies on its links with the professional sector. Studying functional and

structural microbial communities in cheese is one of its major subjects. We believe that it would specifically benefits from European partnerships.

More information on : http://www.rmtfromagesdeterroirs.com/

Properties of lactic acid bacteria strains isolated from raw ewe's milk produced at three mountainous areas of Greece

D. Bozoudi, N. Tzanetakis, E. Litopoulou-Tzanetaki*
Laboratory of Food Microbiology and Hygiene, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
*ganet@agro.auth.gr

Abstract
The objective of this study was to characterize the 24 NSLAB isolated from raw ewes' milk produced at three mountainous areas of Greece where traditional Feta cheese is made, and study the heterogeneity of their technological properties. Results obtained by SDS-PAGE indicated that the microbiota was composed mainly of lactococci (areas 1, 2), or lactococci and enterococci (area 3). NSLAB from area 2 exhibited higher ($P<0.05$) mean acidifying activity after 24h than those from area 1. The isolates also differed in respect of their caseinolytic activity, with area's 1 strains showing stronger ($P<0.05$) degradation of both caseins. Mean proteolytic and mean aminopeptidase activity was the same for all strains, while rate and extent of autolysis at pH 5.1 was higher ($P<0.05$) for NSLAB from area 1. NSLAB from area 1 exhibited a narrow spectrum of antibacterial activity compared to isolates from areas 2 and 3. Typing data indicated intraspecies genetic heterogeneity and specificity to the production area. Results point to the conclusion that there's maybe a link among the area of production and the ecology of the LAB community, as well as its technological characteristics. Selected strains could be tried as starters for cheese manufacture in each area.

Keywords: raw ewe's milk, NSLAB, SDS-PAGE, technological properties, PDO Feta cheese

Introduction
LAB naturally present in the milk, usually serve as starters to milk fermentation. It is well known that the typicality of traditional dairy products is linked mainly to the microbes originating from the milk (Berthier *et al*., 2001). The biodiversity of these microorganisms could therefore be considered as a fundamental factor for the features and quality of artisanal products (Morandi *et al*., 2011). Raw milk's microbiota is known to be an expression of the local ecosystem and that it can have an impact on the characteristics of the final product (Scintu & Piredda, 2007). Since the typicity is linked to the "terroir", it realizes and expresses the effect of the "terroir" on a product, distinguishing the product linked with this territory from similar ones produced elsewhere. Traditional Feta cheese is produced with raw ewe's locally produced milk, which means that the product is closely linked to the ecosystem of the production zone. The diversity in the microbial flora of raw milk contributes to the special sensory characteristics among traditional cheeses. The aim of this work was to study the autochthonous NSLAB microbiota in respect of species diversity as well as their technological potential, in order to be used as starters for Feta cheese manufacture in each area of production.

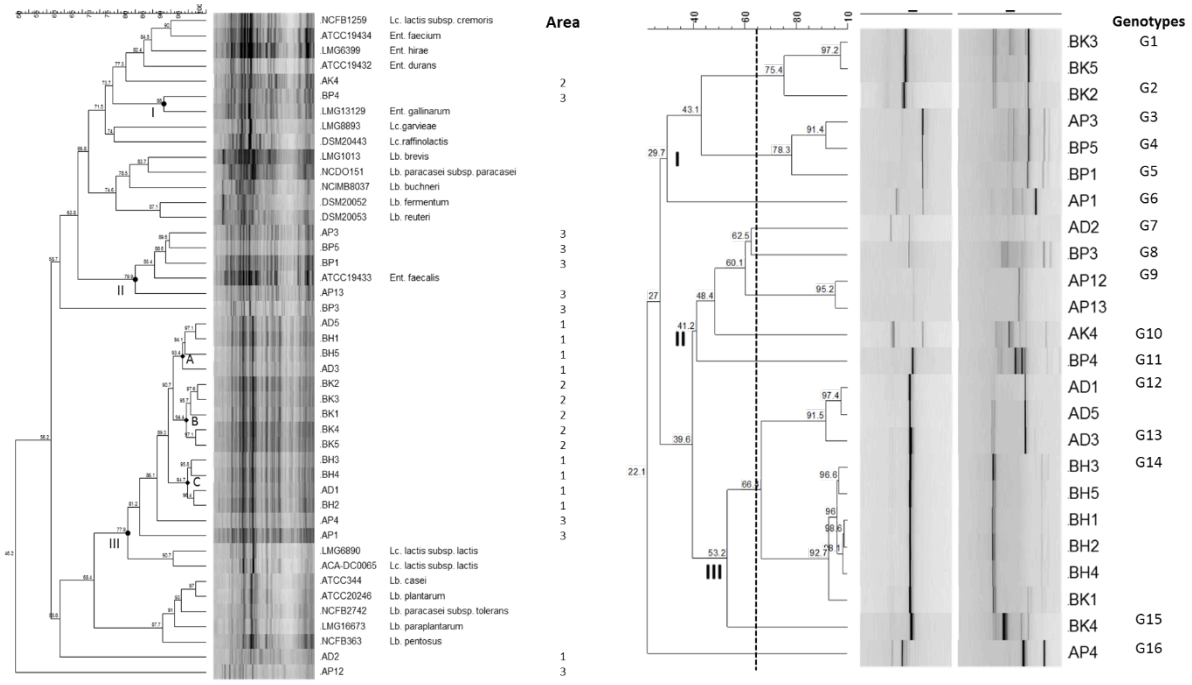
Material and methods
The milk was obtained from 50 sheep, from three different farms, one in Northwest (NW) Greece at ~800m altitude (area 1), one in Southwest (SW) at ~850m (area 2) and one in SW at ~1300m above sea level (area 3), where traditional Feta cheese is produced. The flocks were composed of animals from local herds fed by rough pasture grazed during the day and hand-milked on their return late in the afternoon. Representative 10ml of raw milk samples were

analysed for *Enterobacteriaceae*, Coliforms, *E. coli*, yeasts & moulds, total aerobic counts, *Micrococcaceae*, *Enterococci*, LAB and Gram$^-$ cocci counts. Totally, 24 isolates of LAB were obtained from raw ewes' milk samples and were characterized at species level by SDS-PAGE (Pavlidou *et al*., 2011). Tests applied to the entirety of the strains were inhibitory activity (Kekessy & Piquet, 1970), acid production in milk (Bozoudi *et al*., 2015), proteolytic activity (Andrews, 1983; Church *et al*., 1983), peptidase activity (Piraino *et al*., 2008) and autolysis in buffer (Piraino *et al*., 2008). Statistical analysis was performed through the implementation of the appropriate two-way ANOVA model (SPSS v.15). The genetic heterogeneity of the isolates was assessed by RAPD-PCR (Andrighetto *et al*., 2001).

Results and discussion

The 24 isolates obtained from raw milk, were Gram$^+$, catalase negative cocci and they did not produce CO_2 from glucose. Nineteen out of the 24 were able to grow at 10^{o}C, but not at 45^{o}C, and were characterized as lactococci, while 5 isolates growing at both temperatures were assigned to enterococci (Axelsson, 1993). Results obtained by the SDS-PAGE identification method (Figure 1) indicated that ~83% of the isolates were closely related to *Lc. lactis* subsp. *lactis* (62.5% of the isolates), *Ent. faecalis* (16.7%) and Ent. *gallinarum* (4.2%). Four isolates were found mismatched with all reference strains and delineated separately. The overall similarity of the *Lc. lactis* subsp. *lactis* group of isolates and the reference strains was at 77.9%. Considering the repeatability of the method (≥90%; data not shown), the high degree of similarity (93.4-94.7%) of the various subgroups of strains (A-C), discriminated within the group of *Lc. lactis* subsp. *lactis* strains (group III), suggest very similar enzyme patterns. It is also worthwhile noting, that each subgroup was composed of isolates deriving from the same environment (Bozoudi *et al*., 2015; Pavlidou *et al*., 2011).

Figure 1: Dendrogram of patterns, after SDS-PAGE of cell-free extracts (left) and RAPD-PCR (right) for the isolates from raw ewe's milk produced at three mountainous areas of Greece.



RAPD-PCR analysis was used to assess the genotypic diversity of the NSLAB from raw milk produced at three different mountainous areas. The lowest similarity level obtained by repeated analysis of the same strain was 92.4% and combined patterns with homology above this percentage was considered identical. The dendrogram was able to distinguish 16 different

genotypes (22.1%; Figure 1). The highest number of diverse genotypes was found within the species *Lc. lactis* subsp. *lactis*, which formed 8 different genotypes, followed by 4 of *Ent. faecalis* and 1 of *Ent. gallinarum*. This wide genetic diversity in *Lc. lactis* subsp. *lactis* isolates is possibly related to significant heterogeneity observed, in respect of their biochemical and technological properties. On the other hand, Corroler *et al*., (1998) found a better correlation of the *Lc. lactis* subsp. *lactis* strains with the farm of origin rather than the area of production.

The mean values for several technological properties tested, are summarized in Table 1.

Table 1: Technological properties of NSLAB isolates from raw ewe's milk produced at three different mountainous areas of Greece (n ± SD).

Technological properties tested			Area 1 LL * (G7, G12-G14)	Area 2 LL (G1, G2, G10, G15)	Area 3 EG, EF, LL (G3-G6, G8-G9, G11, G16)
Mean ΔpH after	6h	x±SD	$0.27^{aA} \pm 0.1$	$0.51^{aA} \pm 0.2$	$0.46^{aA} \pm 0.3$
		Range	0.09-0.37	0.27-0.81	0.19-1.23
	24h	x±SD	$2.06^{bA} \pm 0.2$	$2.12^{bA} \pm 0.1$	$1.61^{bB} \pm 0.6$
		Range	1.58-2.23	1.94-2.20	0.88-2.22
Mean % β-casein reduction after	6h	x±SD	$22.02^{aA} \pm 16.5$	$15.53^{aA} \pm 20.8$	$5.88^{aA} \pm 8.8$
		Range	1.20-53.90	0.0-45.70	0.0-22.60
	24h	x±SD	$42.31^{bA} \pm 19.8$	$28.93^{aAB} \pm 16.8$	$22.63^{bB} \pm 14.4$
		Range	7.30-75.30	6.60-46.40	0.40-46.80
Mean % α_s-casein reduction after	6h	x±SD	$32.06^{aA} \pm 23.3$	$16.15^{aA} \pm 25.4$	$10.47^{aA} \pm 11.7$
		Range	5.00-80.40	0.00-57.00	0.00-32.70
	24h	x±SD	$46.53^{aA} \pm 27.2$	$38.74^{aAB} \pm 15.1$	$23.38^{bB} \pm 13.2$
		Range	6.80-85.20	12.80-59.30	1.20-39.60
Mean proterolytic activity (mM L-Glycine) after	6h	x±SD	$0.78^{aA} \pm 0.5$	$0.56^{aA} \pm 0.2$	$0.47^{aA} \pm 0.4$
		Range	0.43-1.87	0.38-0.93	0.0-1.09
	24h	x±SD	$1.74^{bA} \pm 0.6$	$1.68^{bA} \pm 0.3$	$1.40^{bA} \pm 0.5$
		Range	1.01-2.96	1.17-2.02	0.57-2.23
Mean peptidase activity (μKatal.mg^{-1}) after	PepN/C	x±SD	$0.094^{aA} \pm 0.21$	$0.018^{aA} \pm 0.01$	$0.073^{aA} \pm 0.06$
		Range	0.00-0.66	0.00-0.04	0.02-0.16
	PepI	x±SD	$0.031^{aA} \pm 0.03$	$0.038^{aA} \pm 0.03$	$0.042^{aA} \pm 0.04$
		Range	0.00-0.10	0.02-0.08	0.00-0.11
	PepA	x±SD	$0.041^{aA} \pm 0.04$	$0.058^{aA} \pm 0.05$	$0.053^{aA} \pm 0.04$
		Range	0.00-0.11	0.00-0.14	0.02-0.13
Mean autolysis at pH	RA 5.1	x±SD	$0.008^{aA} \pm 0.01$	$0.006^{aAB} \pm 0.01$	$0.002^{aB} \pm 0.00$
		Range	0.00-0.02	0.00-0.01	0.00-0.01
	EA 5.1	x±SD	$0.136^{aA} \pm 0.08$	$0.078^{aB} \pm 0.04$	$0.083^{aB} \pm 0.03$
		Range	0.04-0.29	0.02-0.12	0.04-0.17
	RA 5.5	x±SD	$0.008^{aA} \pm 0.01$	$0.005^{aA} \pm 0.00$	$0.004^{aA} \pm 0.00$
		Range	0.00-0.02	0.00-0.01	0.00-0.01
	EA 5.5	x±SD	$0.120^{aA} \pm 0.10$	$0.121^{aA} \pm 0.10$	$0.095^{aA} \pm 0.08$
		Range	0.04-0.34	0.03-0.27	0.01-0.28
	RA 7.0	x±SD	$0.007^{aA} \pm 0.00$	$0.007^{aA} \pm 0.00$	$0.006^{aA} \pm 0.01$
		Range	0.00-0.01	0.00-0.01	0.00-0.02
	EA 7.0	x±SD	$0.087^{aA} \pm 0.07$	$0.110^{aA} \pm 0.06$	$0.078^{aA} \pm 0.06$
		Range	0.01-0.23	0.05-0.19	0.00-0.19

Rate of autolysis (RA): $OD_{650nm/min}$ and Extend of Autolysis (EA): $\%OD_{0h}$

$^{a, b, c}/^{A, B, C}$: Comparison within the same area / between the three areas, according to the LSD criterion ($P<0.05$)

* LL: *Lactococcus lactis* subsp. *lactis*, EG*: Ent. gallinarum*, EF: *Ent. faecalis*

G: RAPD-PCR genotypes, see Figure 1

Isolates deriving from areas 1 and 2 exhibited higher ($P<0.05$) acidifying ability at 24h but not at 6h. More than 90% of the isolates from area 3 were enterococci, well known to be slow acidifiers (Bozoudi *et al*., 2015). None of the isolates reduced the milk pH to 5.3 in 6h at 30°C and in this respect they were all poor acid producers. However, our strains seemed to establish

a desirable environment in respect of acid production during growth in milk, for Feta cheese production (Abd El-Salam & Alichanidis, 2004). Inspection of the results on the caseinolytic activity suggested that the isolates from area 1 showed stronger ($P<0.05$) degradation of both caseins after 24h of incubation, as reported previously (Bozoudi *et al*., 2015; Pavlidou *et al*., 2011). Mean proteolytic and mean aminopeptidase activity were the same for all strains from all three areas tested. A high ($P<0.05$; data not shown) interstrain heterogeneity was recorded (Pavlidou *et al*., 2011). Piraino *et al*. (2008) reported general aminopeptidase (PepN/C) activity in different groups of LAB as one of the most intense peptidase activities. FAA released by peptidase activities are precursors of several flavour compounds since they are further metabolized by microbial activities.

In addition, the rate and extent of autolysis at pH 5.1 was higher ($P<0.05$) for NSLAB from area 1, which is close to that reached in Feta cheese during coagulation and draining (drop from 6.5 to 5.2 in 6-8h; Abd El-Salam & Alichanidis, 2004). Higher mean caseinolytic as well as mean extend of autolysis activities results, suggest a possible faster degradation of milk constituents and therefore a faster ripening of cheeses made in area 1 by the activity of extracellular and intracellular enzymes. Furthermore, NSLAB from area 1 exhibited a narrow spectrum of antibacterial activity compared to isolates from areas 2 and 3 (data not shown) but inhibited the growth of *Str. thermophilus*, indicating bacteriocin production (Bozoudi *et al*., 2015). The majority of the strains from areas 2 and 3 also inhibited the growth of *L. monocytogenes, St. aureus, E. coli* and *Y. enterocolitica, possibly due to the ability of those strains to create fast (at 6h) acidic conditions (Table 1)*. Variability of technological properties of isolates from natural sources is a common finding (Bozoudi *et al*., 2015; Pavlidou *et al*., 2011; Piraino *et al*., 2008).

Conclusions

The NSLAB microbiota of raw ewe's milk produced at three different mountainous areas of Greece, where Feta cheese is traditionally made, is composed of lactococci, mainly. The strains from each area may exhibit different antibacterial activities and those inhibiting undesirable bacteria could be used as protective cultures in cheese production. In addition, their technological properties in respect of acidifying and proteolytic activities differed according to the production area probably due to the different genotypes retrieved with RAPD-PCR. Future extended studies could possibly determine a link between the area of production and the composition and biochemical activities of NSLAB microbiota. The addition of autochthonous cultures to make cheese of a more constant quality after assessing their safety and flavour formation, while preserving the sensorial peculiarities of cheeses made in each area, deserves to be further investigated.

Acknowledgements

The authors would like to thank the GSRT Greek Ministry of Education for financing the 08SMEs2009 project.

References

Abd El-Salam M.H., Alichanidis E., 2004. Pag. 227-249 in Cheese: Chemistry, Physics and Microbiology, 3rd ed. vol. 2. Elsevier & Academic Press, London.

Andrews A. T. 1983. J. Dairy Res. 50: 45-55.

Andrighetto C., Borney F., Barmaz A., Stefanon B., Lombardi A. 2002. Int. Dairy J. 12: 141-144.

Axelsson L. 1993. Pag. 1-63 in Lactic Acid Bacteria, New York: Marcel Dekker.

Berthier F., Beuvier E., Dasen A., Grappin R. 2001. Int. Dairy J. 11: 293-305.

Bozoudi D., Kotzamanidis C., Hatzikamari M., Tzanetakis N., Menexes G., Litopoulou-

Tzanetaki E. 2015. Int. J. Food Microbiol. 200: 87-96.
Church F.C., Swaisgood H.E., Porter D.H., Catignani G.L. 1983. J. Dairy Sci. 66: 1219-1227.
Corroler D., Mangin N., Desmasures N., Gueguen M. 1998. Appl. Environ. Microbiol. 64: 4729-4735.
Kekessy D.A., Piquet J.D. 1970. Appl. Microbiol. 20: 282-283.
Morandi S., Brasca M., Lodi R. 2011. Dairy Sci. Technol. 91: 341-359.
Pavlidou S., Bozoudi D., Hatzikamari M., Tzanetakis N., Litopoulou-Tzanetaki E. 2011. J. Food Sc. 76: 175-183.
Piraino P., Zotta T., Ricciardi A., McSweeney P.L.H., Parente E. 2008. Int. Dairy J. 18: 81-92.
Scintu M.F., Piredda G. 2007. Small Rumin. Res. 68: 221-231.

Capacities of dairy and suckling herds to valorize grassland resources in mountain areas: use of a bio economic optimization model to study the influence of the structure of livestock systems

Z. Diakité[2, 1]*, C. Mosnier[1, 2], R. Baumont[1, 2], G. Brunschwig[2,1]
[1]INRA, UMR1213, F-63122 Saint-Genès-Champanelle, France;
[2]Université Clermont Auvergne, VetAgro-Sup, UMR1213 BP 10448, F-63000 Clermont-Ferrand, France
*zakary.diakite@vetagro-sup.fr

Abstract
Cattle farming systems with dairy and suckling herds in the Massif Central would improve the valuation of grassland resources and offer better economic performance than specialized herd systems. To verify these hypotheses, we used the bio economic optimization model "Orfée" to represent two Farm-types of the south of Auvergne (ref. BL18 and BL22). The model was enriched with the representation of grassland production from the national typology of permanent grasslands. For each farm, we used the following experimental plan for the simulations: (D for dairy herd LU and S for suckling herd LU) 100% D, 75% D-25% S, 50% D-50% S, 25% D-75% S, 100% S, and a herd distribution equals to the distribution of the considered farm. The result shows a better valuation of grassland resources for intermediate systems with a balanced distribution between dairy and suckling herd, conversely, a lesser one for specialized systems. Balanced LU systems offer the best economic compromise with reasonable loads and provide meat in addition to good quality milk.

Keywords: optimization model, farm-types, grassland resources, valuation, dairy cattle, suckling cattle

Introduction
Research are being done to improve the management of cattle farming systems. In the case of grass-fed cattle farms most of the research concerns forage autonomy and many of them show the essential role of grass valuation by grazing. In the field, the mountainous areas constitute rich territories in grassland resources and are used for cattle farming. But, the geographical characteristics do not play in favor of the valuation of the plots (Fleury et al., 1996). Indeed, mountainous areas present geographical constraints, namely slopes, obstacles in addition to the fragmentation of the plot, which makes it difficult to allocate plots to dairy cows for the pasture (Brunschwig et al., 2006). However, the simultaneous conduct of dairy and suckling herd is likely to optimize the valuation of grassland resources in mountain areas.
In this study, we evaluate the influence of combinations of dairy and suckling herds on the economic performance and grass valuation in the case of mountain cattle farming systems.

Material and methods
We used a bio economic model to realize an in-silico experiment and analyze the influence of the distribution of dairy and suckling cattle herds on the performance of farm system. We chose the model **"Orfée"** (Mosnier, 2009): **Optimization of Ruminant Farm for Economic and Environmental Assessment**. Inspired by Opt INRA (Veysset et al., 2005), this is a bio economic optimization model which maximizes the farm net operating income. Designed from mathematical functions representing biotechnical and economic processes, it represents the exploration system of polyculture farming and helps to making decisions.
This model was developed under the **General Algebraic Modeling System (GAMS).** This mathematical modeling software is useful to describe complex and large models with a

structured language. It also offers the possibility to modify preexisting model, and leaves directly the code of the model "Orfée" to adapt our study.
Our simulations were based on two **Farm-types BL18 and BL22** (Chambre d'Agriculture Cantal, 2015): these operating systems have two flocks, one dairy herd and other suckling herd. Both Farm-types represent two very different farms in terms of herd, structure of the plot, and size and operation of the farms.

Table 1: Summary of main characteristics of Farm-types BL18 and BL22

BL18	BL22
Medium-sized milk-meat systems with a summer mountain pasture, 2 *agricultural workforce* - 44 LU dairy cow 44 LU suckling cow- 70 ha *Agricultural Area* - 30 ha summer mountain pasture - 1.26 LU / ha main fodder area	Large size milk-meat system with an fragmented plot and crops 2 *agricultural workforce* - 72 LU dairy cow 55 LU suckling cow - 112 ha *Agricultural Area* - 5 ha cereals - 1.2 LU / ha main fodder area

Source: Chambre d'Agriculture Cantal, 2015

The farms corresponding to these Farm-types are located in the mountains of southern Auvergne: Cézalier (Monts Cantal) for the BL18 and Planèze of St-Flour (Velay volcanique) for the BL22. Two standard cases describe the cruise operation situations.
We use the bio economic optimization model "Orfée" to find equilibria under optimal operating conditions of the farms. To offer the heterogeneity of plotting to the model, his representation of grassland production has been enriched from the national typology of permanent grasslands (Launay et al., 2011). Geographical constraints are taking into account slopes, obstacles and distances. The result is then parameterized to represent the two Farm-types of the southern Auvergne.

Experimental plan
The experimental plan presents six herds compositions developed to study the influence of herd composition on technical and economic performance of the farming systems using the adapted model.

Table 2: Experimental plan for six situations: (D for dairy herd and S for suckling herd, in LU%) 100% D, 75% D-25% S, 50% D-50% S, 25% D-75% S, 100% S and the last corresponding to the given Farm-type).

Experimental plan	Culture	cutting	Summer Moutain pasture	Repairs dairy LU (D) suckling LU (S)					% ref. Farm-types
				100%D	75%D	50%D	25%D	0%D	
				0%S	25%S	50%S	75%S	100%S	
Farm-type BL18	0	+++	+++	Sc[1].1	Sc.2	Sc.3	Sc.4	Sc.5	Sc.6
Farm-type BL22	+++	+++	0	Sc.A	Sc.B	Sc.C	Sc.D	Sc.E	Sc.F

There are five scenarios designed according to these repairs for each Farm-type and each of them are completed with an additional scenario corresponding to the distribution of the considered farm.
Analyzes of the model outputs permit to evaluate grass valuation by grazing but also the economic advantages (products and gross margin) associated with the combination of dairy and suckling herds. We analyze simultaneously the technical and economic results from the

[1]Scenario

simulations based on four categories of indicators: farm economy, animal's consumption, livestock and plant production.

Results and discussion

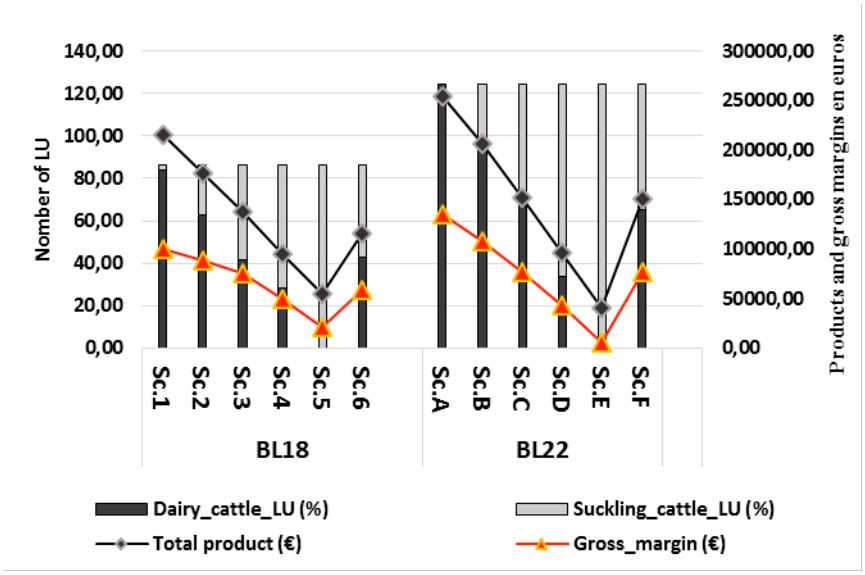


Figure 1: Economic products and gross margins by herd allocations

A better economic product is observed for 100% D systems but heavy loads greatly reduce the gross margin. This gross margin decreases with the increase in the LU of the suckling herd in the farm. Specialized dairy herd are therefore the most economically profitable. Nevertheless, there is a good economic compromise for intermediate distributions (50% D-50% S), which offers a diversity of products (milk and meet), enables to cope with price fluctuations but also to reduce the costs associated with the consumption of concentrates intended for dairy herd.

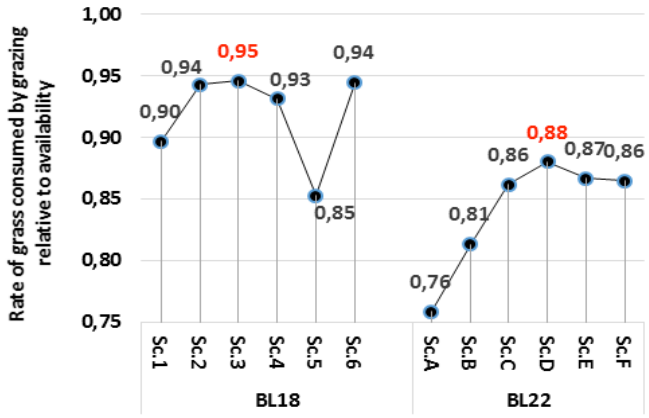


Figure 2: Valuation of available biomass by grazing

Valuation of the grass by grazing is maximum for systems with herd having proportions close to 50% D-50% S. This valuation reaches 95% in the case of BL18 and 88% in the case of BL22. These maximum levels of valuation are different because BL18 operation is more based on grazing than stocks consumption at the opposite of BL22. Valuation of grass by grazing is also good for the quality of dairy products (Farruggia et al., 2008; Martin et al.,

2016). This valuation is lower for specialized systems, except for the 100% S system of the BL22 due to is operation and fragmented plot favorable to grass valuation by suckling herds.

Conclusions

Conducting dairy and suckling herds simultaneously with a balanced distribution between herds is an interesting way to improve the valuation of grassland resources by grazing, as well as the diversity of animal products (milk and meet). These balanced systems provide an operational flexibility to better cope with fluctuating milk prices and a good economic compromise. However, we must continue our analyzes to consider whether the simultaneous conduct of herd with a balanced distribution of dairy and suckling herds permit greater resilience of farm.

References

Brunschwig G., Josien E. Bernhard C. 2006. Pag 83-95 Fourrages 185: 83-95.

Farruggia A., Martin B., Baumont R., Prache S., Doreau M., Hoste H., Durand D. 2008. Productions animales 21: 181-200.

Fleury P., Dubeuf B., Jeannin B. 1996. Pag 199-212 Agric. Sys. 52: 199-212.

Launay F., Baumont R., Plantureux S., Farrie J., Michaud A., Pottier E. 2011. Prairies permanentes-des références pour valoriser leur diversité. éd. Paris: Institut de l'Elevage.

Martin B., Coppa M., Verdier-Metz I., Montel M., Joy M., Casasús I., Blanco M. 2016. Pag 105-115 in proc. 19th Meeting of the FAO-CIHEAM Mountain Pastures Sub-network Options Méditerranéennes A 116. 14-16 June, Zaragoza, Spain.

Mosnier C., Duclos A., Lherm M., Lelyon B., Agabriel J., Gac A. 2015. Renc. Rech. Ruminants 22: 89.

Veysset P., Bébin D., Lherm M. 2005. Pag Agric. Sys. 83: 179-202.

Chambre d'Agriculture Cantal, 2015. Cas types laitiers d'INOSYS Réseaux d'Elevage d'Auvergne-Lozère. http://www.cantal.chambagri.fr/pages-hors-menu-internet/ menu-horizontal/les-etudes-et-references/systemes-laitiers-et-mixtes-cas-types. html

Study of starter culture persistence and performance in pasture production of Fromadzo PDO cheese

S. Zenato[1], R. Pramotton[1], L. Vernetti[1], P. Dolci[2]*, A. Barmaz[1]
[1]Institut Agricole Régional, La Rochère 1/A - 11100 AOSTA (AO)
[2]DISAFA, University of Turin, Largo P. Braccini 2, 10095, Grugliasco (TO)
*paola.dolci@unito.it

Abstract
The aim of the present research was to evaluate the performance of two autochthonous *Lactococcus lactis* starter cultures, in pasture productions of Fromadzo PDO cheese, in order to establish their potentiality in terms of cheese colonization, persistence and quality of the final product. Three Fromadzo productions were set up, starting from pasture milk, and two autochthonous starter mixtures (AS1 and AS2) plus a commercial starter were used, separately, in each production. The fate of the starters inoculated was followed by Repetitive element palindromic-PCR and their influence on the quality of the final product was evaluated by a taste panel. The results obtained in this study highlight the potential of AS2 mixture and, in particular, of the strain *L. lactis* subsp. *lactis* 60ME1MRS, in terms of colonization and persistence in Fromadzo PDO cheese. The performance of this strain, which seems to positively affect the flavour richness of the final product, deserves to be more extensively studied for possible future applications.

Keywords: Fromadzo PDO cheese; starter culture; *Lactococcus lactis*; starter performance

Introduction
Fromadzo is the second Protected Designation of Origin (PDO) cheese in Aosta Valley, in terms of commercial importance. It is a semi-hard, low- or semi-fat cheese obtained from cow milk coming from at least two milkings, with the possible addition of small percentages of goat milk, derived from farms located in the region and coagulated with natural rennet.
The attribution of PDO from European Community (European Regulation 1263/96) emphasized the interest in the link of the product with Aosta Valley territory. Thus, in a previous research (Bal, 2010), lactic acid bacteria ecology in Fromadzo cheese was studied starting from traditional spontaneous fermentations, without the additions of starter cultures. On the basis of the results obtained relatively to the dominant species and the physiological characterization of the strains isolated, two mixtures of autochthonous starter cultures were formulated.
The aim of the present research was to evaluate the performance of the two selected autochthonous starter cultures, in pasture Fromadzo productions, in order to establish their potentiality in terms of cheese colonization, persistence and quality of the final product.

Material and methods
Three Fromadzo PDO productions (I, II, III) were set up (totally, nine cheeses), starting from pasture milk, in the period from June to July 2016, and two autochthonous starter mixtures were used, separately, in each production. The composition of the starter cultures was the following: starter culture 1 (AS1), *Lactococcus lactis* subsp. *lactis* 34PISM17/7 and *Lactococcus lactis* subsp. *cremoris* 47MRSF6/15; starter culture 2 (AS2), *L. lactis* subsp. *lactis* 60ME1MRS and *L. lactis* subsp. *cremoris* 47MRSF6/15. Moreover, a commercial starter (CS) was also used in cheesemaking in order to compare autochthonous and commercial starter performance in terms of overall quality of the final product.
Presumptive lactococcal populations were analysed in samples of milk (M), curd (Cu) and

cheese at 7 (Ch 7d), 30 (Ch 30d) and 60 (Ch 60d) days of ripening by traditional plating on M17 agar incubated at 22 °C for 48 h. Moreover, cheese samples at 7 and 30 days of ripening were undergone analysis in order to evaluate the presence and persistence of the added starter cultures. In particular, 240 colonies of presumptive lactococci, isolated on M17 agar, were submitted to DNA extraction by using the kit Microlysis (Microzone, UK), and to molecular characterization by Repetitive element palindromic-PCR (rep-PCR) using $(GTG)_5$ primer in order to compare their genetic profiles with those of the added *L. lactis* starter strains.
Finally, a taste panel session was planned for the sensory evaluation of the products at 60 days of ripening, in order to compare starter performance and evaluate their influence on the quality of the final product.

Results and discussion
Presumptive lactococcal populations were found in Fromadzo cheeses with load ranging from about 10^7 to 10^8 cfu/g in curd samples and from about 10^8 to 10^9 cfu/g in cheeses at 7, 30 and 60 days of ripening (Figure 1). The trend was similar in cheeses manufactured with AS1 and AS2 while cheeses produced with the addition of CS showed loads of about one logarithmic unit less. Lactococcal counts reached values which were comparable to those reported for other Italian pasture ripened cheeses (Morandi et al., 2011; Dolci et al., 2008).
Rep-PCR technique allowed to obtain clear genetic profiles of the three strains composing the two autochthonous starter cultures (Figure 2a). Thus, their fingerprinting was efficiently compared with the rep-PCR profiles of the colonies isolated on M17 plates (Figure 2b) in order to study the effective colonization and persistence of the inoculated starter strains in the products. Rep-PCR belongs to the group of molecular characterization techniques which are considered fundamental, in dairy microbiology, to follow the fate of the starters once inoculated in raw milk (Ndoye et al., 2011).

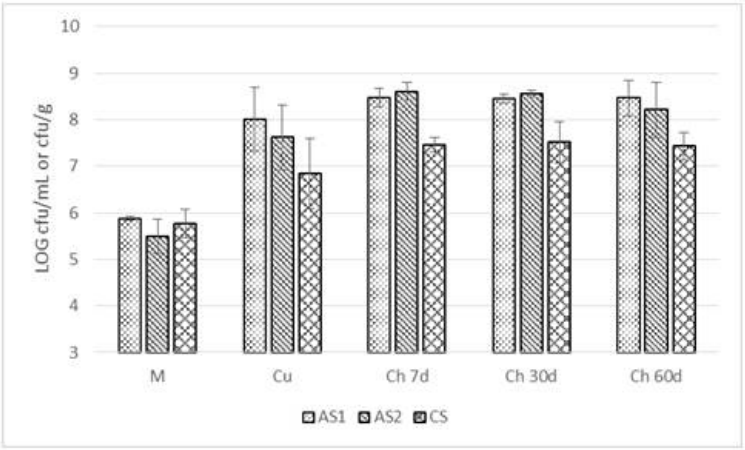


Figure 1. Presumptive lactococcal loads obtained from milk (M), curd (Cu) and cheese samples at 7 (Ch 7d), 30 (Ch 30d), 60 (Ch 60d) days of ripening added of autochthonous (AS1 and AS2) and commercial (CS) starters. Microbial load values are calculated as average of the three values obtained from the three different productions.

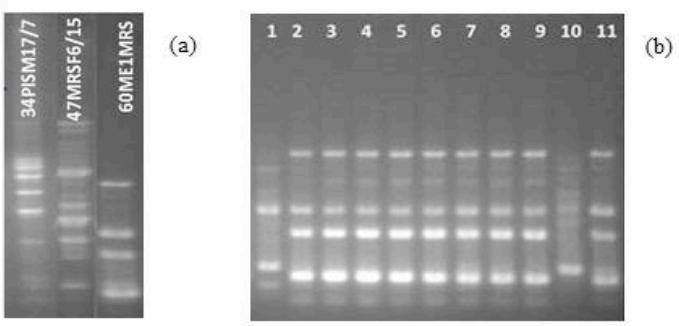


Figure 2. (a) Rep-PCR profiles of the three L. lactis autochthonous starter strains used in the study. (b) Rep-PCR profiles of 10 colonies (lines 1-10) obtained from a cheese analysed at 7 days of ripening (Ch 7d_III) compared with the rep-PCR profiles of the autochthonous starter strain 60ME1MRS (line 11).

The results obtained from the molecular characterization by rep-PCR showed that AS2 mixture had the best performance with regard to the colonization of cheeses (Table 1). In particular, in pasture production I, AS2 represented the 90% of lactococcal microbiota in cheese samples at both 7 and 30 days of ripening (Table 1). Most of the colonies showed rep-PCR profiles referable to the genetic fingerprinting of the strain *L. lactis* subsp. *lactis* 60ME1MRS which prevailed in all the three manufacturing. On the contrary, the strain *L. lactis* subsp. *cremoris* 47MRSF6/15, common to the two starters, was found with frequency not higher than 30%. Similarly, the colonization of the strain *L. lactis* subsp. *lactis* 34PISM17/7 was occasional in the cheese analyzed. These results highlight, once again, the technological differences existing among strains belonging to the same species and subspecies and the importance of a selection at strain level (Franciosi et al., 2009; Carafa et al., 2016; Hickey et al., 2007).
Finally, the cheeses manufactured with the mixture AS2 received the best scores from the taste panel which, in general, penalized cheeses made with CS because considered "plain" in terms of flavour. The possibilities of using autochthonous strains as potential starters in order to obtain products characterized from typical aroma is a topic widely discussed (Wouters et al., 2002).

Table 1. Frequency (%) of isolation of autochthonous starter culture (AS1 and AS2) strains (34PISM17/7, 47MRSF6/15, 60ME1MRS) obtained from cheeses analysed at 7 (Ch 7d) and 30 (Ch 30d) days of ripening and produced in three different cheesemaking (I, II, III) in the period from June to July 2016. NM: microbial populations naturally contaminating cheese samples.

		AS1		NM	AS2		NM
		34PISM17/7	47MRSF6/15		60ME1MRS	47MRSF6/15	
I	Ch 7d	0%	0%	100%	90%	0%	10%
	Ch 30d	0%	20%	80%	90%	0%	10%
II	Ch 7d	0%	0%	100%	90%	0%	10%
	Ch 30d	40%	0%	60%	30%	30%	40%
III	Ch 7d	0%	0%	100%	90%	10%	0%
	Ch 30d	60%	0%	40%	50%	10%	40%

Conclusions

The results obtained in this study highlight the potential of AS2 mixture, and in particular of the strain *L. lactis* subsp. *lactis* 60ME1MRS, in terms of colonization and persistence in Fromadzo PDO cheese. The performance of this strain, which seems to positively affect the flavour richness of the final product, especially when compared with the commercial starter, deserves to be more extensively studied for possible future applications.

References

Bal M.C. 2010. Caratteristiche igienico-sanitarie di Valle d'Aosta Fromadzo DOP d'alpeggio. Tesi di Laurea, Università di Torino, 117 pp.
Carafa I., Clementi F., Tuohy K., Franciosi E. 2016. Food Microb. 53: 94-103
Dolci P., Alessandria V., Rantsiou K., Rolle L., Zeppa G., Cocolin L. 2008. Int. J. Food Microbiol. 122: 302–311.
Franciosi E., Settanni L., Cavazza A., Poznanski E. 2009. Int. Dairy J. 19:3–11.
Hickey D.K., Kieran N., Kilcawley L., Beresford T.P., Sheehan E.M., Wilkinson M.G. 2007. J. Dairy Res. 74:9–17.
Morandi S., Brasca M., Lodi R. 2011. Dairy Sci. Technol. 91:341–359.
Ndoye B., Rasolofo E.A., LaPointe G., Roy D. 2011. Dairy Sci Technol 91:495–524.
Wouters J.T.M., Ayad E.H.E., Hugenholtz J., Smit G. 2002. Int. Dairy J. 12:91–109.

Genetic and non-genetic factors contribute to differences in relative proportion of α_s-casein phosphorylation isoforms among Montbéliarde cows

Z.H. Fang[1-2], H. Bovenhuis[2], M.H.P.W. Visker[2], A. Delacroix-Buchet[1]*, G. Miranda[1], and P. Martin[1]

[1]GABI, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[2]Animal Breeding and Genomics Centre, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, the Netherlands
*agnes.delacroix-buchet@inra.fr

Abstract
Post-translational phosphorylation of casein (CN) is one of the key factors responsible for constructing and stabilizing casein micelles. Variation in phosphorylation degree of α_s-CN is suggested to affect milk cheese-making properties. This study aimed to investigate the variation in phosphorylation degree of α_s-CN (α_s-CN PD) and to explore to what extent genetic and other factors contribute to the variation in relative concentrations of α_s-CN phosphorylation isoforms and the α_s-CN PD. Morning milk samples from 529 French Montbéliarde cows were analyzed using LC/ESI-MS. Additional α_{s2}-CN phosphorylation isoforms were detected and we found considerable variation in the relative concentrations of α_s-CN phosphorylation isoforms. We observed that the α_s-CN PD and relative concentrations of α_s-CN phosphorylation isoforms changed during and between lactations and that genetics also impacts the phosphorylation profile and α_s-CN PD. Therefore, changing α_s-CN PD in milk is possible by selective breeding.

Keywords: casein, phosphorylation isoform, lactation stage, heritability, LC/ESI-MS

Introduction
Detailed milk protein composition exhibits high heterogeneity due to quantitative variation in the content of individual milk proteins, numerous genetic variants, and isoforms with different level of post-translational modifications such as glycosylation in κ-CN and phosphorylation in all caseins. Phosphorylation of caseins is one of the key factors responsible for constructing and stabilizing casein micelles (De Kruif and Holt, 2003). Although all 4 caseins (α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN) are phosphoproteins, α_{s1}-CN and α_{s2}-CN are the most phosphorylated, and their phosphorylation profiles are more heterogeneous than those of β-CN and κ-CN. The phosphorylation degree of α_s-CN (α_s-CN PD) is one of the factors affecting cheese-making properties of milk (Bijl *et al*., 2014b, Jensen *et al*., 2012). Therefore, it is of interest to explore variation in α_s-CN PD and analyze to what extent genetic and other factors contribute to the variation in α_s-CN phosphorylation profile. Genetic parameters for relative concentrations of α_{s1}-CN-8P and α_{s1}-CN-9P have been reported (Bijl *et al*., 2014a, Buitenhuis *et al.*, 2016). However, no information is available regarding α_{s2}-CN phosphorylation profile as well as detailed milk protein composition in the Montbéliarde breed. The objective of this study was to investigate the variation in α_s-CN PD among milk of individual cows and explore the relationships among different phosphorylation isoforms of α_s-CN. We also examine the genetic and non-genetic sources of variation in α_s-CN PD, and in relative concentrations of α_s-CN phosphorylation isoforms and other major milk proteins in French Montbéliarde cattle from Franche-Comté cheese production area.

Material and methods
Test-day morning milk samples from Montbéliarde cows were collected from commercial herds across 3 French departments (Doubs, Jura and Haute-Saône) located in the production area of protected designation of origin (PDO) cheeses: Comté, Morbier, Mont D'Or and Bleu

de Gex, and the protected geographical indication (IGP) cheese French Gruyère. The sampling periods were during Oct-Dec 2014 and Apr-Jul 2015. The sampling was developed to maximize genetic and milk content diversity, to obtain an optimal representation of the variation in milk protein composition from the current French Montbéliarde cattle population in Franche-Comté. For this purpose, we sampled cows across different parities (1-5) and lactation stages (7-652 days), and based on paternal pedigree and on protein and calcium content in milk from previous lactation records. Finally, milk samples from 529 cows descending from 191 sires and 68 paternal grandsires, 52 of which being also maternal grandsires, located in 430 herds, were collected. Milk (25 mL) was preserved with Bronopol after collection, transported on ice to the laboratory, then frozen at -20°C until analyzed by Liquid chromatography/electrospray ionization-mass spectrometry LC/ESI-MS. We implemented the LC/ESI-MS method developed at INRA to simultaneously measure the relative concentrations of the major milk proteins and their isoforms, notably their phosphorylation isoforms (Miranda et al., 2013). Protein variants and isoforms of the six major milk proteins (αs1-, αs2-, β-, and κ-CN, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG)) were identified by matching measured molecular masses with an in-house calculated mass database on bovine milk proteins. The phosphorylation degrees of αs1-CN and αs2-CN were defined as the proportion of isoforms with higher degrees of phosphorylation, which were calculated as αs1-CN PD = (αs1-CN-9P/ total αs1-CN) x100 and αs2-CN PD = [(αs2-CN-12P+ αs2-CN-13P+ αs2-CN-14P)/ total αs2-CN] x100.

To estimate variance components and genetic parameters, the following animal model was used: $y_{ijklm} = \mu + region_i + parity_j + lstage_k + season_l + animal_m + e_{ijklm}$, where y is the observation of the trait of interest; μ is the overall mean; $region_i$ is the fixed effect of the i^{th} region; $parity_j$ is the fixed effect of the j^{th} parity class; $lstage_k$ is the fixed effect of the k^{th} lactation stage class; $season_l$ is the fixed effect of the l^{th} season class; $animal_m$ is the random additive genetic effect of animal m and is assumed to be distributed as $N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, where **A** is the additive genetic relationships matrix consisting of 5,546 animals with pedigree traced back for 5 generations, and σ_a^2 is the additive genetic variance; e_{ijklm} is the random residual effect and is assumed to be distributed as $N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where **I** is the identity matrix, and σ_e^2 is the residual variance. The heritability was defined as $h^2 = \frac{\sigma_a^2}{\sigma_p^2}$, where the phenotypic variance $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$. The effect of the herd x date of sampling was not included in the model because it was not estimable due to the data structure (as it was generally collected only one cow per herd). Statistical analyses were performed using ASReml (Gilmour et al., 2009).

Results and discussion

Variation in major milk proteins

α-LA was the least abundant protein, and α_{s1}-CN the most abundant protein among milk samples. The CV values ranged between 7 and 20%, implying substantial variation in protein composition among milk of individual Montbéliarde cows. The average relative protein concentrations for the 6 main milk proteins are in the range reported in previous studies in other breeds (Bobe et al., 1998; Heck et al., 2008). Two protein variants were found for α_{s1}-CN (B and C) and α_{s2}-CN (A and D), three for κ-CN (A, B, and C) and β-LG (A, B, and D), and four for β-CN (A1, A2, B, and I). Protein variants κ-CN E and β-CN I have not been reported before in the French Montbéliarde population.

Variation in α_{s1}-CN and α_{s2}-CN phosphorylation isoforms

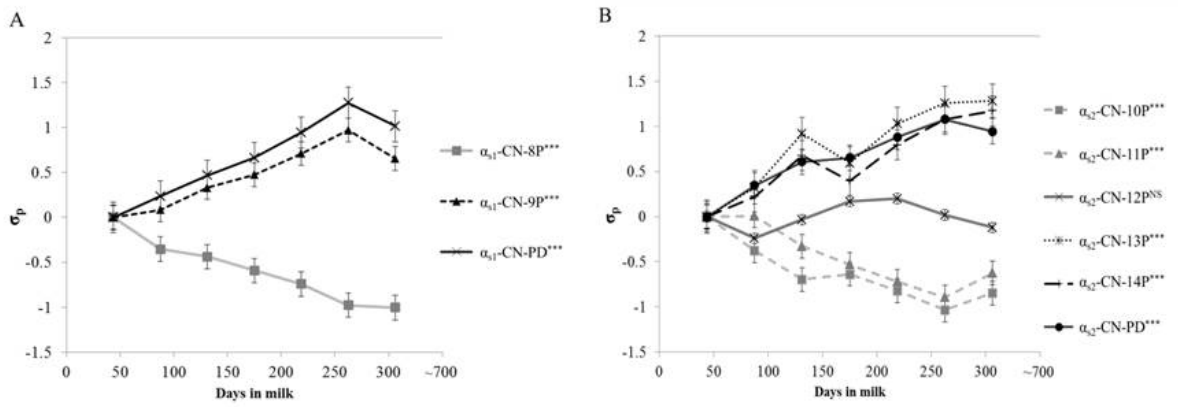
Among milk samples, we found 77% of α_{s1}-CN carrying 8P and 23% carrying 9P, on average. Thus, α_{s1}-CN-8P was the abundant isoform whose mean concentration accounted for 25.3% of total protein in milk. For α_{s2}-CN phosphorylation isoforms, we estimated only the relative concentrations of α_{s2}-CN-10P to -14P from the A variant because only one milk sample

contained the D variant, which is present with isoforms from 7P to 11P. We detected 3 new phosphorylation isoforms: α_{s2}-CN-9P, -14P, and -15P in bovine milk, in addition to the known isoforms α_{s1}-CN-8P and -9P, and α_{s2}-CN-10P, -11P, -12P, and -13P. The relative concentrations of each α_s-CN phosphorylation isoform varied considerably among individual cows. α_{s2}-CN-9P and -15P occurred in trace amounts, and they were not observed in all milk samples. Out of 529 milk samples, α_{s2}-CN-9P was detected in 21 samples, and α_{s2}-CN-15P was detected in 4 different samples. We found 8% of α_{s2}-CN having 10P, 36.5% having 11P, 32% having 12P, 19% having 13P, and 4.5% having 14P, on average. Thus, α_{s2}-CN-11P and -12P were the abundant isoforms, whose mean concentrations accounted for 3.1% and 2.7% of the total protein in milk, while α_{s2}-CN-13P had a mean concentration of 1.6%. The CV values of α_s-CN phosphorylation isoforms ranged between 8 and 42%, suggesting large variation in relative concentrations of different α_s-CN phosphorylation isoforms. The estimated mean concentrations of α_{s1}-CN-8P and -9P and of α_{s2}-CN-10P to -12P, as well as their CV values, were comparable to the results reported by Heck et al (2008).

Factors affecting phosphorylation of α_{s1}-CN and α_{s2}-CN

Parity significantly affected relative concentrations of β-CN and α-LA (all $P<0.001$). For α_s-CN phosphorylation isoforms, parity significantly affected α_{s1}-CN PD, α_{s2}-CN PD, and relative concentrations of all α_s-CN phosphorylation isoforms except α_{s2}-CN-11P and α_{s2}-CN-12P. Lactation stage significantly affected α_{s1}-CN PD, α_{s2}-CN PD and relative concentrations of all α_s-CN phosphorylation isoforms except α_{s2}-CN-12P (all $P < 0.001$). The magnitude of the effects varied from 0.01 to 1.5 phenotypic standard deviation (Figure 1). As lactation progressed, we observed a significant decrease in relative concentrations for the group of isoforms with lower degrees of phosphorylation (α_{s1}-CN-8P, α_{s2}-CN-10P, and α_{s2}-CN-11P) and a significant increase in relative concentrations for the group of isoforms with higher degrees of phosphorylation (α_{s1}-CN-9P, α_{s2}-CN-13P, and α_{s2}-CN-14P) as well as a significant increase in both α_{s1}-CN PD and α_{s2}-CN PD.

Figure 1: Effects of lactation stage on α_{s1}-CN (A) and α_{s2}-CN (B) phosphorylation profile and their phosphorylation degree (PD) throughout lactation.



X-axis shows days in milk. Y-axis shows the effect of lactation stage, expressed as a fold change in phenotypic standard deviation (σ_p) of the relative concentration of each phosphorylation isoform. α_{s1}-CN PD = (α_{s1}-CN-9P/total α_{s1}-CN) x 100; α_{s2}-CN PD = [(α_{s2}-CN-12P + α_{s2}-CN-13P+α_{s2}-CN-14P)/total α_{s2}-CN] x 100. NS: not significant, *** $P < 0.001$.

Heritability

Heritability estimates for relative concentrations of the 6 major milk proteins were moderate to high and ranged from 0.22 (α-LA) to 1.00 (α_{s1}-CN) (not shown). The standard error of the heritability estimate for α_{s1}-CN concentration could not be approximated accurately as the estimate was at the boundary of the parameter space. Likelihood ratio test suggested that the

95 % confidence interval of the heritability estimate for α_{s1}-CN concentration ranged from 0.75 to 1.00. For α_s-CN phosphorylation isoforms (Table 1), heritability estimates for relative concentrations of α_{s1}-CN-8P (0.84) and α_{s1}-CN-9P (0.56) were high, for α_{s1}-CN PD (0.37) was moderate and for relative concentrations of α_{s2}-CN phosphorylation isoforms and for α_{s2}-CN PD were low to moderate (0.07 to 0.32). In summary, we can say that, even if both the 'region' and 'season' effects could explain a part of the herd variation, the model we used was not optimal because it can lead to confusions between genetic and herd variations and thus, overestimate genetic variance.

Table 1: Mean, standard deviation (SD), phenotypic variance (after adjusting for the systematic effects: sampling region, parity, and lactation stage) (σ^2_p), and heritability (h^2) for the individual phosphorylation isoforms of α_{s1}-CN and α_{s2}-CN, and the phosphorylation degrees (PD) of α_{s1}-CN and α_{s2}-CN measured on test-day morning milk samples from 529 Montbéliarde cows (SE in brackets).

Phosphorylation isoform (% wt/wt)	Mean	SD	σ^2_p	h^2
α_{s1}-CN-8P	25.27	2.08	3.60	0.84 (0.18)
α_{s1}-CN-9P	7.65	0.96	0.84	0.56 (0.18)
α_{s1}-CN PD	23.27	2.80	6.19	0.37 (0.15)
α_{s2}-CN-10P	0.72	0.30	0.08	0.11 (0.09)
α_{s2}-CN-11P	3.04	0.55	0.28	0.32 (0.14)
α_{s2}-CN-12P	2.68	0.34	0.11	0.09 (0.12)
α_{s2}-CN-13P	1.57	0.31	0.07	0.07 (0.11)
α_{s2}-CN-14P	0.40	0.14	0.02	0.14 (0.13)
α_{s2}-CN PD	57.05	8.36	55.94	0.23 (0.12)

Conclusions

We report for the first time the difference in relative concentrations of α_s-CN phosphorylation isoforms and the α_s-CN PD due to systematic environmental effects (parity and lactation stage) and genetic variation between cows. From the heritability estimates for α_s-CN phosphorylation isoforms, exploitable genetic variation for the phosphorylation degrees of α_{s1}-CN and α_{s2}-CN exists in the French Montbéliarde cattle. Further investigations were conducted and published (Fang *et al.*, 2016, 2017).

Acknowledgements

This study was funded by the French Ministry of Agriculture, Agro-food and Forest, the French Dairy Interbranch Organization (CNIEL), the Regional Union of Protected Designation cheeses of Franche-Comté (URFAC) and the Regional Council of Bourgogne Franche-Comté, under the project FROM'MIR. The first author benefited from an Erasmus-Mundus fellowship and a grant by APIS-GENE (Paris, France), within the framework of the European Graduate School in Animal Breeding and Genetics (EGS-ABG, Paris, France).

References

Bijl E., van Valenberg H.J.F., Huppertz T., van Hooijdonk A.C.M., Bovenhuis H. 2014a. J. Dairy Sci. 97: 7240–7246.

Bijl E., van Valenberg H.J.F., Sikkes S., Jumelet S., Sala G., Olieman K., van Hooijdonk T., Huppertz T. 2014b. Int. Dairy J. 39: 215–221.

Bobe G., Beitz D.C., Freeman A.E., Lindberg G.L. 1998. J. Agric. Food Chem. 46: 458–463.

Buitenhuis B., Poulsen N.A., Gebreyesus G., Larsen L.B. 2016. BMC Genet. 17: 114.

Fang Z., Bovenhuis L.B., Visker M.H.P.W., Delacroix-Buchet A., Miranda G., Boichard D., Martin P. 2017. J. Dairy Sci. *in press*.
Fang Z.H., Visker M.H.P.W., Miranda G., Delacroix-Buchet A., Bovenhuis H., Martin P. 2016. J. Dairy Sci. 99: 8168–8177.
Gilmour A.R., Gogel B.J., Cullis B.R., Thompson R., Butler D. 2009. ASReml user guide release 3.0. VSN Int. Ltd, Hemel Hempstead, UK.
Heck J.M.L., Olieman C., Schennink A., van Valenberg H.J.F., Visker M.H.P.W., Meuldijk R.C.R., van Hooijdonk A.C.M. 2008. Int. Dairy J. 18: 548–555.
Jensen H.B., Poulsen N.A., Andersen K.K., Hammershøj M., Poulsen H.D., Larsen L.B. 2012. J. Dairy Sci. 95: 6905–6917.
De Kruif C.G., Holt C. 2003. Pag. 233–276 in Advanced Dairy Chemistry-1 Proteins. Fox P.F. & McSweeney P.L.H., editors. Springer US.
Miranda G., Krupova Z., Bianchi L., Martin P. 2013. In 10th annual International Milk Genomics Consortium symposium. 1-3 October, Davis, California USA.

Characterization of milk and estimation of cheese yield for dairy ewes in Tunisian mountains

W. Nasri[1, 2*], S. Smeti[1], I. Mekki[1], N. Atti[1]
[1]University of Carthage, INRA Tunisia, Laboratory of Fodder and Animal Productions, 2080 Ariana, Tunisia.
[2]University of Carthage, National Agronomic Institute of Tunisia, 1082 Tunis, Tunisia.
*waad.nasri@gmail.com

Abstract
This study aims to characterize Sicilo-Sarde ewes' milk and cheese yield in the region of Beja in north-western Tunisia. The experiment was carried out on a private farm with 82 multiparous lactating ewes grazing mountain grassland. Throughout the lactation, five controls had taken place and individual milk samples were collected. The physicochemical composition of the milk (pH, density, freezing point, fat, protein, lactose and total solids) and the individual cheese yield were measured at each control. The total dairy production and the daily milk production ranged from 65 and 130 l/ewe and from 0.87 to 1.73 l/day. The pH, density and freezing point mean values were respectively of 6.63, 30.09 and -0.69 %. The mean percentages of fat, protein, lactose and total solids were respectively 6.48, 6.21, 5.35 and 9.35. The mean value of the cheese yield was 23.65 %. The pH values were almost stable throughout the lactation, while the freezing point values were varying over time. The highest density value was recorded at the first dairy control. The fat content and the cheese yield increased over lactation. The lactation stage affects the physicochemical composition of the Sicilo-Sarde ewes' milk as it affected three of the most important parameters and the cheeseability.

Keywords: dairy ewes, mountain grassland, milk composition, cheese yield

Introduction
Sheep farming plays an important socioeconomic role in rural areas. It has always been anchored in Tunisian pastoral traditions. The Sicilo-Sarde breed is the nucleus of dairy sheep farming in Tunisia. During the last years, in Tunisia, interest in the production and the processing of sheep's milk has increased (Atti *et al.*, 2006). Sheep milk is mainly intended for cheese production. The milk and cheese's production and characteristics can vary according to several factors. The lactation stage and diet significantly influence the composition of sheep's milk (Atti *et al.*, 2006, Buccioni *et al.*, 2006). According to Bousselmi and Othmane (2015), the individual laboratory cheese yield can be affected by several factors like the days in milk, parity, lambing type and the lactation stage. In this context, this experiment aims to study the characteristics of the Sicilo-Sarde ewe's milk and its cheese yield.

Material and methods
Animals and diets
The experiment was carried out in a private farm in the mountains of Beja. The studied flock, was composed of 82 multiparous ewes and 5 rams of the Sicilo-Sarde breed. The mating was performed during autumn. The weaning was applied 15 days after the lambing. The ewe's feeding was based on the mountain grassland and green barley grazing. The number of grazing hours depended on the weather. The feeding system also included an amount of 300-700 g/ewe/day of oat or barley hay which amount varied according to the climatic conditions; it increased during the rainy days.

Milking controls

Five bi-monthly controls were carried out during the experimental period. The dairy controls were carried out about 6 weeks after the milking beginning. The first control involved the morning and the afternoon milking and the results were used to estimate the daily milk production. The following ones only concerned the afternoon milking. They consisted in determining the quantity of milk produced by each ewe and collecting 25 ml individual samples. The latter were transported to the laboratory in a cooler and then stored at -20 °C to be used for further analyzes. Indeed, 15 ml were reserved to realize the physicochemical analyzes and 10 ml to determine the individual laboratory cheese yield (ILCY). Each milk sample was well homogenized before analysis. The physicochemical composition was determined using a Milkcoscan 4000.

Individual laboratory cheese yield

The ILCY was determined based on the method described by Bousselmi and Othmane (2015). Samples of 10 ml milk were warmed and mixed by shaking until reaching 30 °C. The samples were then transferred into weighed empty tubes (W_{ET}) and 10 µl of rennet (1/5000) were added. The mix was, afterwards, rapidly stirred using a vortex. The renneted milk tubes were placed in a water bath for an hour at 37 °C to ensure the coagulation of the milk. The coagulum obtained was cut vertically (in the form of a cross) and was centrifuged for 15 min at 36 °C at 1750 g. The whey was expulsed manually and the tubes were put upside-down in the open air in order to ensure the total expulsion of the remaining whey. Finally, the tubes contacting the curd were weighed (W_{CT}). The ILCY is calculated as the weight of the curd (W_{CT} - W_{ET}) * 100 / 10 (%).

Statistical analyzes

Based on the data collected from the morning and afternoon milking on the first control, the daily milk production (MP_d) was estimated from the afternoon milking controls for the next controls (MP_a) using the SAS System regression procedure according to the following model: $MP_d = a\ MP_a + b$ (1). Data of the quantitative and qualitative milk production was submitted to an analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS in order to test the effect of the number of the milking control according to the following model: $Y_i = \mu + NC_i + e_i$ (2); Y_i: studied parameter (MP, physicochemical parameter or cheese yield); µ: mean value; NC_i: number of the control's effect; e: standard error. A mixed procedure was also used in order to test the effect of the animal as a random effect, and the birth mode (simple or double) and the lactation stage as fixed effects on the studied parameters.

Results and discussion

The performances of total and daily milk production of the studied flock, during the experimental period, are shown in Table 1.

Table 1: Dairy production performances of the Sicilo-Sarde ewes' milk

	Mean	Minimum	Maximum
Total milk production (l/ewe)	94	65	130
Milk production (l/day)	1.26	0.87	1.73
Milk production on 1st control (l/day)	1.73[a]	1.05	2.70
Milk production on 2nd control (l/day)	1.44[b]	1.00	2.04
Milk production on 3rd control (l/day)	1.10[c]	0.72	1.66
Milk production on 4th control (l/day)	1.08[c]	0.53	1.47
Milk production on 5th control (l/day)	1.00[c]	0.53	1.47

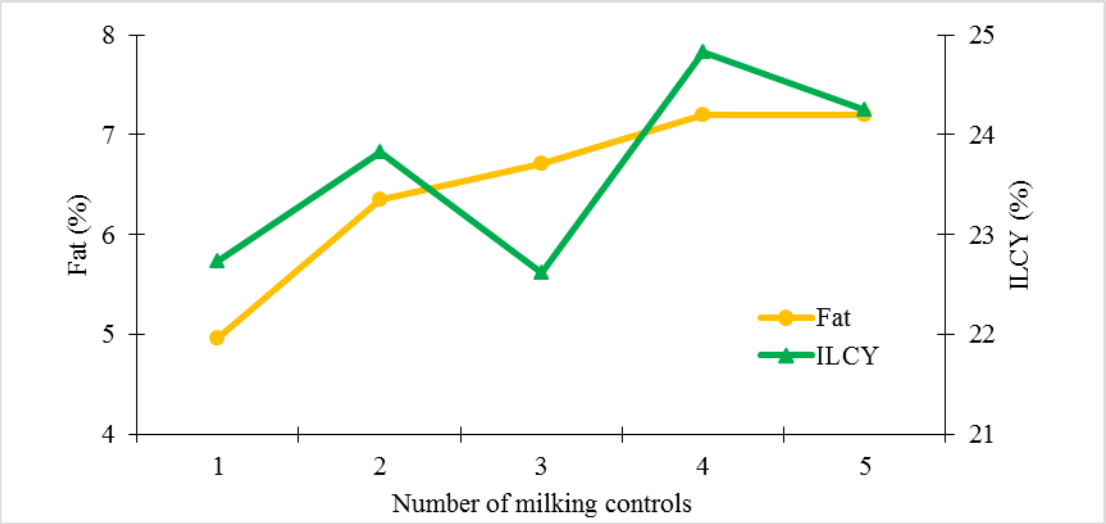
The mean value of the herd's total MP was 94 l. The daily MP ranged from 0.87 l to 1.73 l with a mean of 1.26 l. The mean value of the daily MP (1.26 L/day) was relatively high for the Sicilo-Sarde breed; which traduced the benefic feeding system of the experimental herd based on mountain grassland. Indeed, this value is much higher than 0.46 l/day on concentrate and barley grazing and 0.62 l/day on grazing barley reported by Atti and Rouissi (2003) and Atti *et al.* (2006), respectively. A significant difference was recorded between the daily MP means throughout the progress of the lactation stage. In fact, this production decreased progressively, during time, from 1.73 L/day on the first control to reach 1.00 L/day during the fifth; this last mean value is still high confirming that the ewes of this flock are good milk producers. The evolution of the composition of the milk throughout the lactation is reported in Table 2. The milk's pH mean value was 6.63. Indeed, Assenat (1985) reported that the average pH value of sheep milk is around 6.65.This parameter was almost stable during the different controls. The mean value of the density was 30.09; the only significant difference was recorded between the 1st milking control (31.97) and the next ones. Contrariwise, the freezing point mean values showed significant differences during the controls. Its mean value was -0.687. This is in agreement with the values indicated by Hilali *et al.* (2011) varying between -0.86 and -0.56. The mean percentages of the chemical parameters of ewes' milk were 10.23, 6.48, 6.21 and 5.35 respectively for total solids, fat, proteins and lactose. The last did not significantly fluctuate throughout the controls. For the proteins content, the only significant difference was recorded between the 1st milking control (6.56 %) and the rest. Its mean value (6.21 %) is almost similar to the value indicated for the same breed (Atti and Rouissi, 2003; Atti *et al.,* 2006). The total solids and fat contents both significantly varied over time from the first to the fifth control. The mean value of fat (6.48 %) is lower than those recorded by Atti and Rouissi (2003) and Atti *et al.* (2006) respectively of 7.10 % and 7.72 %. This difference can be explained by the differences in feeding and also the late lactation stage. The lactation stage significantly affected the milk's production and chemical composition as well as the density; contrarily, it did not affect the pH and the cheese yield. The animal factor significantly affected all the parameters except the pH. The birth mode did not have any effect on the studied parameters.

Table 2: Physicochemical composition of the Sicilo-Sarde ewes' milk

	1st control (day 42)	2nd control (day 56)	3rd control (day 70)	4th control (day 84)	5th control (day 98)	Pr > F
pH	6.69	6.67	6.57	6.60	6.62	0.0022
Density	31.97^{a}	29.15^{b}	29.55^{b}	29.97^{b}	29.80^{b}	< 0.0001
Freezing point (°C)	-0.688^{bc}	-0.669^{a}	-0.677^{ba}	-0.703^{d}	-0.698^{dc}	< 0.0001
Total Solids (%)	10.52^{a}	10.05^{c}	10.04^{c}	10.32^{b}	10.22^{cb}	< 0.0001
Fat (%)	4.96^{d}	6.35^{c}	6.71^{b}	7.20^{a}	7.19^{a}	< 0.0001
Proteins (%)	6.56^{a}	6.09^{b}	6.09^{b}	6.18^{b}	6.15^{b}	< 0.0001
Lactose (%)	5.40	5.24	5.28	5.42	5.39	0.17

The fat contents values of the milk increased progressively from 4.96 % on the beginning of the experiment to reach 7.19 % at the fifth (last) control (Figure 1). The ILCY mean value was 23.65 %. It is lower than the value of 34.9 % indicated for the same breed by Maamouri *et al.* (2009) and Bousselmi and Othmane (2015) respectively of 34.9 % and 33.87 %; but this value is within the range of variation indicated by these authors which is from 10.17 % to 49.95 %. In fact, the ILCY mean values increased from 22.73 to 23.82 % then decreased to, almost, the initial value (22.61 %) and later it increased again to reach its maximum at the fourth control (24.83 %). No significant difference was recorded between the ILCY mean values at the different milking controls. The slight observed differences can be explained by the difference of the days and the stage of the lactation.

Figure 1: Evolution of the fat content and ILCY throughout the controls; ILCY: Individual Cheese Yield



Conclusion

The results indicate that the studied ewes' flock reared on mountain grassland had a high production even at the end of the lactation. The birth mode did not have any effect on the milk's production and quality. The lactation stage significantly affected the quantitative and qualitative characteristics of the milk except the pH and the cheese yield. It is important to study the effects of the lactation stage since it strongly affects the milk's chemical composition.

Acknowledgments

The authors would like to thank Mr. Zied Ben Youssef breeder and owner of the farm BorjLella and the staff of the laboratory of Fodder and Animal Productions especially the technician, Mrs. Zina Taghouti, and the driver, Mr. Lassaâd Touati.

References

Assenat L. 1985. Le lait de brebis. Composition et propriétés; in: Lait et produits laitiers. 1. Les laits de la mamelle à la laiterie. Ed. Tec & Doc, Lavoisier Paris.
Atti N., Rouissi H. 2003. Ann. De l'INRAT. 76: 209-224.
Atti N., Rouissi H., OthmaneM.H. 2006. Livest. Sci. 104: 121-127.
Bousselmi H., Othmane M.H. 2015. Greener J. Biol. Sci. 5: 001-009.
Hilali M., El-Mayda E., Rischkowsky B. 2011. Small Rumin. Res. 10: 92-101.
Maamouri O., Othmane M.H. & Atti N. 2009Ann. de l'INRAT. 82: 122-137.

Technological and biochemical characterization of Drâa goat milk and cheese

Y. Noutfia[1]*, S. Zantar[2], M.E. Hilali[3], C. Alem[4] and Y. Filali Zegzouti[5]
[1] National Institute of Agronomic Research (Errachidia – Morocco)
[2] National Institute of Agronomic Research (Tangiers – Morocco)
[3] International Center for Agricultural Research in the Dry Areas (Amman – Jordan)
[4] Research Team in Biochemistry & Natural Substances, FSTE-UMI (Errachidia – Morocco)
[5] Research Team in Biology Environment and Health, FSTE-UMI (Errachidia – Morocco)
* noutfiaa@yahoo.fr

Abstract
Drâa goat is the main indigenous goat breed raised in oases of South-East of Morocco. The objective of this study was to characterize some technological quality parameters of milk and cheese of this goat breed. Milk samples from a herd of Drâa goats raised at the Experimental Station of Errachidia were characterized and used for cheese production at laboratory scale. The results show that the biochemical composition of milk is about 6.64 for pH, 1.77 g/l for lactic acid, 13.3% for dry matters, 4.16% for fat, 3.60 for total nitrogenous matters (TNM) and 0.73% for ash. The percentage of long, medium and short chain fatty acids is respectively 33.5%, 46.1% and 20.4% with a predominance of Palmitic acid. Cheese produced from Drâa goat milk can be classified into the category of mid dry cheeses because of its high content of dry matters (41.9%). The concentration of fat, TNM and ash was respectively 24.2%, 15.3% and 2.26%. No significant effect ($P>0.05$) of coagulation duration on cheese yield, water content and fat ratio was observed.

Keywords: goat, Morocco, Drâa, fatty acids, cheese, coagulation

Introduction
At the oasis area of southern Morocco, the sector of goat farming, in general and Drâa specie in particular, has been selected among the priority for research in this areas. In addition, goat cheese production can be considered as a mean of sustainable rural development in small farms of oases and remote areas where goat cheese is made, usually in small women cooperative and dairies. Furthermore, the valorization of goat milk cheese seems to ensure better profitability of milk production and thus, is an important force behind the development of the goat population in this area which has a very fragile economy.
In this context, this study was conducted on Drâa goat milk in order to (i) characterize the biochemical quality and the fatty acids profile of this milk and to (ii) study the effect of coagulation duration on some quality parameters of produced cheese.

Material and methods
Seven milk samples (250 ml/sample) were obtained periodically from a milk mixture of evening and morning milking from a herd of Drâa goats raised at the Experimental Station of Errachidia. The characterization of the main biochemical parameters of the Drâa goat milk and cheese was conducted in concordance with AFNOR standards. Analyses were performed with two replicates for each sample. The profile of fatty acids was determined by a gas chromatography.
Seven Cheese making trials (in each trial, 3 liter of milk was used) were performed in the laboratory. Pasteurization is done in a water bath at 72°C for 20 seconds. After that, milk is cooled and inoculated with a lyophilized culture at a rate of 32.5mg/liter, and then it is kept for thirty minutes at room temperature. Rennet (0.3 ml/liter) and $CaCl_2$ are incorporated into

the mixture to accelerate coagulation and strengthen the matrix Casein-Calcium. Three coagulation durations of 45min, 90min and 240min were tested for the soft cheese produced from the same sample of milk. After coagulation, the curd was drained. All data of this study was analyzed by using Excel-2007 software.

Results and discussion

Characterization of the biochemical composition of Drâa goat milk

The physicochemical of Drâa goat milk is given in table 1.

Table 1. Biochemical characteristics of Drâa goat milk.

Parameter	pH	Acidity (°D)	Dry matters (%)	Fat (%)	Total Nitrogenous Matter (%)	Ash (%)	Density (kg/l)
Mean ± SD	6.64 ± 0.15	17.75 ± 2.27	13.3 ± 1.63	4.16 ± 1.57	3.60 ± 0.41	0.728 ± 0.078	1.0313 ± 0.003

The mean values for the various biochemical parameters of Drâa goat milk are comparable to those reported for North Moroccan goat milk except for acidity which is much lower (18°D Vs 27°D for the North) (Zantar et al., 2009). In general, the obtained values are largely situated in the upper level of the range reported in other studies by several Moroccan and foreign authors (Le Jaouen., 2004), (El Alamy., 1992) and (Chilliard et al., 2003).

The fatty acids profile of analyzed milk is reported in the table 2.

Table 2. Fatty acids profile of Drâa goat milk.

Short chain fatty acids		Medium chain fatty acids		Long-chain fatty acids	
Fatty acid	Percentage (%)	Fatty acid	Percentage (%)	Major Fatty acid	Percentage (%)
C4:0	0.30	C14:0	4.95	C18 :0	11.2
C6:0	1.23	C14:1	0.95	C18:1	16.6
C8:0	1.76	C15:0	1.66	C18:2n6c	1.11
C10:0	10.56	C15:1	0.48	C20:3n3	1.43
C11:0	0.15	C16:0	37.6	C20:4n6	0.81
C12:0	6.37	C16:1	0.53		

The major fourth fatty acids found in Drâa goat milk are respectively: Palmitic Acid (C16**:** 37.6%), Oleic Acid (C18:1n9c/1n9t with 16.6%), Stearic Acid (C18:0 with 11.2%) and Capric Acid (C10**:** 10.56%). In general, these findings are in concordance with the studies of (Tudisco et al., 2014), (Kompan and Komprej., 2012) and (Ayadi et al., 2009) for goat milk's of some Moroccan and foreign breeds.

Characterization of cheese quality

Table 3 reports the mean values and standard deviation of physical and chemical parameters of the produced cheese.

Table 3.Physical and chemical characteristics of produced cheese.

Parameter	pH	Acidity (°D)	Humidity (%)	Fat (%)	FAT/DM	DNF (%)	TNM (%)	CY (%)	Ash (%)
Mean ± SD	4.58 ± 0.15	211 ± 17.3	58.1 ± 2.65	24.2 ± 3.07	0.51 ± 0.05	20.8 ± 2.34	15.3 ± 2.60	19.3 ± 1.55	2.26 ± 0.54

The pH and acidity of the Drâa cheese is 4.58 and 211°D respectively. These values are higher than the average reported by Kouniba et al. (2007) and Zantar et al. (2009) for cheese produced from Alpine and local races of Morocco milk**.**
The water content is about 58.1%, indicating an average dry matter of 41.9%, which is higher than that found by Kouniba et al. (2007) for cheese of Alpine and local goat breeds of Morocco and Benkerroum and Tamime. (2004) for Northern Morocco goat cheese. This is due to the richness of Drâa goat milk in DM, manufacturing practices and also to the ambient humidity at laboratory scale which is low (25%).
Fat content varies between 19 and 25% with an average value of about 24.2%. This average is higher than that found for other traditional goat cheeses, whose fat fraction does not exceed 20% (Zantar et al., 2009) and (Kouniba et al. 2007). The second major component of cheese DM is the TNM whose content is 15.3±2.60. This average value is lower than that reported by Elmarakechi and Hamama. (1995) (16.4%), but superior to that reported in the work conducted by Kouniba et al. (2007) for the Alpine (13.3%) and indigenous goat breed (14.3%).
These results can be attributed to several parameters; the most important are the processing method, the composition of milk and goat feeding.
Cheese yield (CY) is about 19.3 ± 1.55. This yield is slightly higher than that obtained (17.7%) by (Zantar et al., 2009) for the goat population in northern Morocco**.**

Effect of coagulation duration on some quality parameters of produced cheese
The effect of coagulation duration on CY, water content, fat and whey losses is reported in table 4.

Table 4. Effect of coagulation duration on some quality parameters of goat cheese.

	45 min	90 min	240 min	P value
Cheese yield (%)	19.1	19.9	18.9	0.33
Water content (%)	57.8	58.5	57.9	0.89
Fat (%)	22.5	25.7	24.4	0.35
Whey losses (%)	39.7	32.6	37.7	0.20

Statistically, no significant effect of coagulation duration (on parameters cited in the above table) was observed ($p > 0.05$). However, it seems that the best coagulation duration is 90min, as the cheese yield and the recovery rate of fat are in a higher level.

Conclusion
Drâa goat milk composition is situated in the upper level of the range reported by studies conducted on Moroccan and foreign goat species.
Valorization of this milk on cheese can be qualitatively (chemical composition rich in nutrients like Fat and proteins) and quantitatively (high cheese yield) interesting. These findings can be exploited for the development of other dairy goat products typical to the oases southern Morocco regions in order to improve incomes of rural communities.

References
Zantar S, Chentouf M, Ibnelbachyr M, Chikhi A and L Iniguez (2009). Improvement of the goat keeper's income through the valorization of milk in cheese production in North and South of Morocco. In: El gharous M, Dahan R and El Mourid M (Ed.). Annual Report 2007-2008 (MCGP). Rabat. 2009: 68 – 88.
Le Jaouen J C (2004). Composition chimique et structure physicochimique du lait. In : Institut d'élevage (Ed.). La fabrication du fromage de chèvre fermier. Paris. 2004: 18 – 25.

El Alamy H.A., Senaity A., Kholif A.M. 1992. J. Agric. Sci. 17: 3875-3880.
Chilliard Y., Ferlay A., Rouel J., Lamberet G. 2003. J. Dairy Sci. 86: 1751–1770.
Tudisco R., Grossi M., Addi L., Musco N., Cutrignelli M.I., Calabrò S., Infascelli F. 2014. Fatty Acid Profile and CLA Content of Goat Milk: Influence of Feeding System. J. Food Res. 3.4: 93.
Kompan D., Komprej A. 2012. The Effect of Fatty Acids in Goat Milk on Health, Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health, Prof. Narongsak Chaiyabutr (Ed.), In Tech, DOI: 10.5772/50769. Available from: http://www.intechopen.com/books/milk-production-an-up-to-date-overview-of-animal-nutrition-management-and-health/the-effect-of-fatty-acids-in-goat-milk-on-health.
Ayadi M., Keli A., Chentouf M. 2009. Effect of ensiled olive cake with molasses on production, chemistry composition and fatty acid milk of goats in north Morocco. Renc. Rech. Ruminants 16.
Kouniba A., Berrada A., El Marakchi A. 2007. Revue Méd. Vét. 158 (03): 152-160.
Benkerroum N., Tamime A.Y., 2004. Food Microbiol 21: 399-413.
El Marrakchi A., Hamama A. 1995. Pag 24-33 in ''Journées sur Perspectives de développement de la filière lait de chèvre dans le bassin méditerranéen'', Etude FAO, Production et Santé Animale, Rome, 131: 24-33.

Identification of bacterial biodiversity and volatile fraction of Bitto Storico cheese in different Alpine pasture areas

F. Turri[1]*, P. Cremonesi[1], G. Gandini[2], G. Battelli[3], M. Severgnini[4], F. Pizzi[1]

[1]Institute of Agricultural Biology and Biotechnology, Lodi Unit, National Research Council, via Einstein, 26900 Lodi, Italy;

[2]Department of Veterinary Medicine, University of Milan, via Celoria 10, 20133 Milano, Italy.

[3]Institute of Sciences of Food Production, National Research Council, via G. Celoria 2, 20133 Milan, Italy.

[4]Institute of Biomedical Technologies, National Research Council, via Fratelli Cervi 93, 20090 Segrate, Italy

*turri@ibba.cnr.it

Abstract

Bitto Storico cheese is a raw milk seasonal cheese produced in the Orobic Alps, whose production is closely associated to local traditional activities and alpine pastures.

The aim of this work was to characterize the microbiota diversity and the volatile fractions of cheese produced in different alpine pasture areas.

Fifty-four Bitto Storico cheeses samples, produced in six different Alpine pastures, were collected and processed. Bacterial DNA was extracted using an optimized protocol and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina). The volatile fraction was characterized by means of Solid Phase Extraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS).

The microbial community of ripened cheese was mainly dominated by *Firmicutes* phylum with a high abundance of *Streptococcaceae* and *Lactobacillaceae*. Each Alpine area was characterized by peculiar bacterial communities, due, probably, to the pasture composition and the cheesemaking process. A total of 24 volatile compounds resulting from microbial activity and diet were found, the formers being mostly alcohols and esters, the latter being terpenes derived directly from the pasture.

Keywords: alpine area, cheese, bacteria, next generation sequencing, lipophilic fraction

Introduction

The Bitto Storico is an alpine heritage cheese made with raw milk and produced at an altitude of at least 1,500 m only in a specific period of the year (between June 1 and September 30), in the Orobic Alps (Central Alps, Northern Italy), where the rotational pasture grazing is contemplated. The cheesemaking process depends on local traditions that enhance the alpine biodiversity of the production areas, such as the use of raw cow milk processed within one hour from the end of the milking, thus making use of the natural warmth of the milk, with a supplement of fresh raw goat milk (10-20%). For this, the cheesemaking process starts immediately after milking in the *calècc*, a stone structure where cheese is produced in itinerant dairies. Since the use of feeds and silage for animal feeding and probiotics during curdling is prohibited, the organoleptic variability of the cheese depends on local bacteria that change according to pastures and environment. These conditions, in combination with an acidification due to indigenous microflora, guarantee a variability of organoleptic characteristics, probably associated to different microbial communities, highly appreciated by consumers.

Here, we present an evaluation of these aspects, in a study aiming to: (i) the characterization of the microbial variability by Next Generation Sequencing, and (ii) the analysis of the

volatile fraction of the Bitto Storico cheese, produced in different alpine pasture areas.

Material and methods

Sample collection

Cheese samples were collected from 54 Bitto Storico cheeses with 95÷110 days of ripening, manufactured in six different Alpine areas identified as TV, FP, OS, OV, CZ, BS , in three Lombardy geographical zones (Val Gerola, Valli del Bitto, Valle Brembana), in three different cheesemaking periods of the summer season (July, August and September). For each Alpine area and for each cheesemaking period, three cheese replicates from independent wheels were collected in the same week of cheesemaking.

Metagenomic and volatile fraction analysis

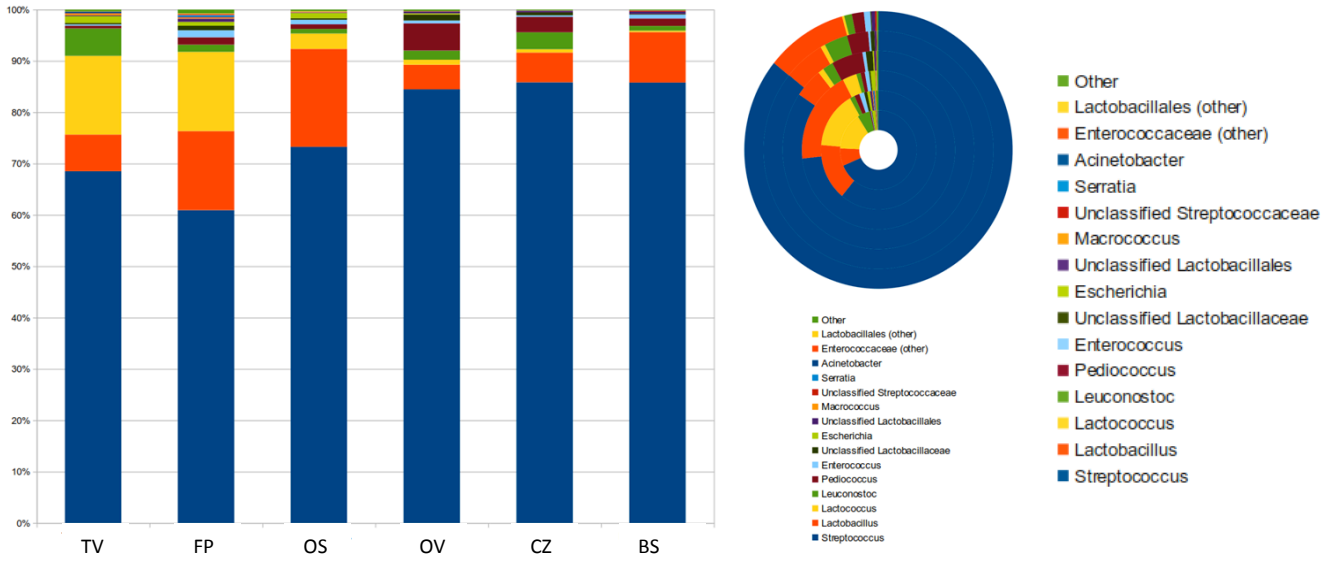
The bacterial DNA was extracted using an optimized protocol (Cremonesi et al., 2006) and 16S rRNA gene amplicons on V3-V4 region sequenced on a paired 2x300 bp run on a Miseq platform (Illumina, San Diego, CA, USA). Raw sequences were processed rebuilding full amplicon fragments via pair overlapping and quality-filtered using the "split_libraries_fastq.py" utility of the QIIME suite (Caporaso et al., 2010), which discards from further analyses any sequence having more than 25% low-quality nucleotides. Quality filtered reads were, then, analysed with the standard QIIME pipeline. A random subset of 120000 reads for each sample was used. Sequences were grouped into OTUs (Operational Taxonomic Units) by clustering together reads at 97% identity or higher and taxonomically classified against the Greengenes bacterial 16S rRNA database (release 13_8, http://greengenes.lbl.gov) by using the RDP classifier (Wang et al., 2007) at 50% confidence.
The volatile fraction was determined by Solid Phase Extraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS) on 2 g of cheese sample, with the conditions described elsewhere (Masotti et al., 2012).

Results and discussion

Metagenomic analyses

Cheese microbiota composition was dominated by bacteria belonging to *Firmicutes* phylum which made up to about 99% of the total bacteria presence. The Bitto Storico microbial composition results very semplified, with the major 5 genus, again, accounting for >95% of the total relative abundance, on average. *Streptococcus* and *Lactobacillus* sum up to about 85% on average relative abundance, whereas subdominant generawere *Lactococcus*, *Leuconostoc* and *Pediococcus*.
As reported in Figure 1, showing the average relative abundance at genus level, each Alpine area was characterized by a peculiar microbial diversity, due, probably, to the cheesemaking process and the local vegetation of each area.
For example, Alpine area OV showed a consistent presence of *Pediococcus* spp. (avg. rel ab 5.2%), while *Leuconostoc* (5.3%), *Lactococcus* (15.4%) and *Lactobacillus* (19.1%) characterized respectively the alpine areas identified as TV, FP, and OS.

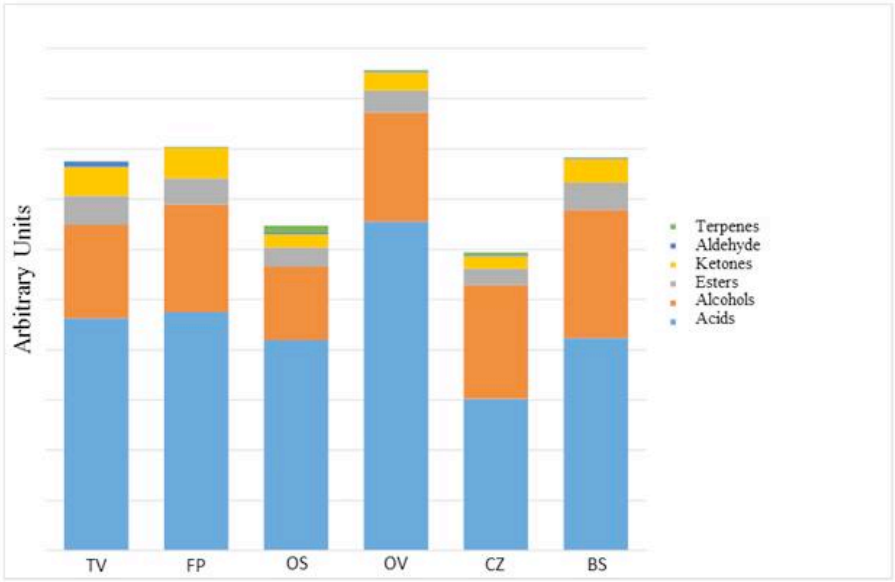
Figure 1: Relative abundance of bacteria for the six different Alpine areas of the study



Volatile fraction

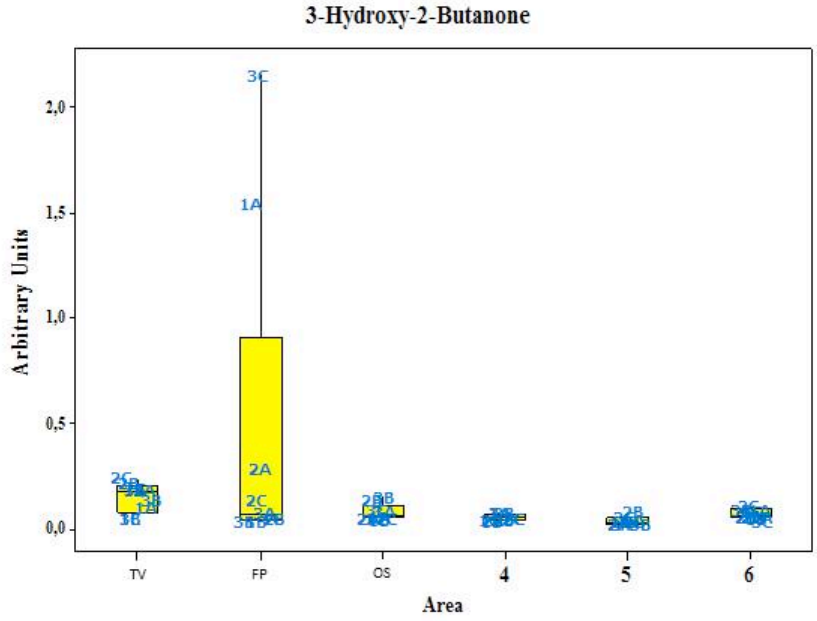
Volatile fraction included 7 acids, 8 alcohols, 4 ketones, 3 esters, 1 aldehyde and 2 terpenes, all of them, excluding the latter, deriving from the catabolism of the principal constituents of milk (proteins, lipids and sugars) by microbiota activity. Different areas were characterized by different abundance of volatiles, as shown in Figure 2, being cheeses from area OV richer than areas OS and CZ.

Figure 2: Relative abundance of Volatiles for the six different Alpine areas studied



During the production season, the microbiota activity can change dramatically especially in some areas, as shown in Figure 3, where the relative abundance of 3-hydroxy-2-butanone (from citrate metabolism) practically absent in areas OV, CZ and BS, is very high in area FP, but not in all periods (1-2-3), nor in all samplings (A-B-C).

Figure 3: Relative abundance of 3-hydroxy-2-butanone for the six different Alpine areas in the 3 samplings (A, B, C) of the 3 periods (1, 2, 3) during the season of production



Conclusions

Preliminary results showed that the bacteria present in all samples correspond to three main phyla: *Firmicutes*, *Streptococcaceae* and *Lactobacillaceae*. Moreover each alpine area shows a peculiar microbial diversity, probably indicating a possible scarce standardization of niche products, a sign of local production in a traditional area.

Volatiles also showed a huge diversity that cannot be related to the different periods of sampling.

Further correlation analysis will be performed between the microbiome and the volatile cheese fraction, in order to verify if the volatile fraction composition was influenced by bacteria and the different production zone.

Acknowledgements

This work was funded by the Agreement Regione Lombardia/CNR, Project FilAgro "Strategie Innovative e sostenibili per la filiera agroalimentare".

References

Cremonesi P., Castiglioni B., Malferrari G., Biunno I., Vimercati C., Moroni P., Morandi S., Luzzana M. 2006. J. Dairy Sci., 89(1):163-169.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., and Knight R 2010. Nature Methods., 7(5): 335-336.

Masotti F., Battelli G., De Noni I. 2012. J. Dairy Sci. 95 (9): 4760-4767.

Wang Q., Garrity G.M., Tiedje J.M., and Cole J.R. 2007. Appl. Environ. Microbiol. 73: 5261-5267.

Performance of different native starter cultures for Fontina PDO production

L. Vernetti-Prot[1]*, S. Zenato[1], R. Pramotton[1], Andrea Barmaz[1]
[1]Institut Agricole Régional, Rég. La Rochère 1/A, 11100 Aosta
*l.vernetti@iaraosta.it

Abstract
Fontina PDO is a cheese traditionally produced in Valle d'Aosta from the Valdostana cattle breed. It comes from the single milking of raw whole cow's milk. Fontina PDO Production Guidelines provide for the addition to vat milk of lactic acid bacteria cultures selected within the production area in order to guide correct Fontina cheese making procedures. The aim of this research was to evaluate the ability of three different indigenous starter cultures to positively influence the microbial composition of the vat milk and the product quality in three different lactation periods.
The experimental protocol provided for monitoring the comparative processing of the same batch of milk and the subsequent cheese using three different lactic acid bacteria formulations, compared to a fourth devoid of starter culture which functioned as control reference. Monitoring was carried out by both microbial and chemical composition analysis. Results showed that all the starter cultures acted effectively right from the initial milk processing stages, preventing the growth of total coliforms, *Escherichia coli* and coagulase-positive staphylococci during cheese ripening. At tasting, the cheese produced with the three starter cultures presented similar characteristics and complied with the Production Regulations, unlike the cheese devoid of starter culture inoculation.

Keywords: cheese, native, starter, Fontina PDO

Introduction
Fontina PDO Production Regulations provide for the addition of starter cultures containing indigenous lactic acid bacteria selected in the Fontina production area to the vat milk. The Institut Agricole Régional and Regione Valle d'Aosta Department of Agriculture and Natural Resources isolated and characterized the lactic microbial flora taken from some traditional farms "alpeggi" during the period of highland summer pasture in Valle d'Aosta [1] and developed three different starter cultures composed with different strains of the genera: *Streptococcus* [2], *Lactococcus* and *Lactobacillus* as an effective guidance tool for correct cheese-making. This would not only improve the cheese quality but significantly reduce its microbial-related organoleptic defects. [3].
The aim of the study was to assess the ability of the three lactic formulations to integrate the microbial composition of the raw vat milk intended for Fontina cheese production, favourably influencing the cheese making properties in three different lactation stages. This was carried out by studying the evolution of the cheese microbiota during ripening, assessing the hygienic quality of the products and evaluating its compliance with Fontina PDO Production Regulations.

Materials and method
The experimental protocol provided for monitoring the comparative processing of the same batch of milk and the subsequent cheese in three different lactation periods when the cows are hay fed indoors and grazing outdoors: post partum (P1), oestrus (P2) and early gestation (P3), using three different lactic acid bacteria formulations, compared to a fourth devoid of starter culture functioning as control reference. Monitoring was carried out by analysing both the

microbial and chemical composition of both the vat milk before and after starter inoculation and the relative cheese during its various ripening stages (24 hours, 15-30-84 days).
The starters comprising the lactic formulations composed with different strains were follows

- Culture 1: *Streptococcus thermophilus, Lactococcus lactis Lactobacillus delbrueckii lactis*
- Culture 2: *Streptococcus thermophilus, Lactococcus lactis Lactobacillus rhamnosus*
- Culture 3: *Streptococcus thermophilus, Lactococcus lactis Lactobacillus paracasei*

The following microbial analysis were performed :

- Thermophilic lactococci on M17 agar at 45°C for 24-48 hours;
- Mesophilic lactococci on M17 agar at 22°C for 24 hours;
- *Lactobacillus* on MRS agar in anaerobiosis at 45°C for 48-72 hours;
- Coliform and *E. coli* on Petrifilm 3M at 37°C for 24-28 hours;
- Obligate heterofermentative bacteria on MSE agar at 21°C for 4 days;
- Moulds and yeast on Petrifilm 3M at 30°C for 24-48 hours;
- Total count on Petrifilm 3M at 30°C for 3 days;
- Proteolytic bacteria on Skim Milk Agar at 30°C for 48 hours;
- Coagulase- positive staphylococci on Baird-Parker + RPF (Rabbit Plasma Fibrinogen)/Agar at 37°C per 48 hours.

Results and discussion

The milk was found to have excellent hygienic and health conditions, despite presenting a higher bacterial count during period P3 when increased ambient temperatures most likely furthered the proliferation of microbes.
Prior to inoculation of the starter cultures, there was seen to be a depletion of lactic flora in the lactic microflora naturally present in the freshly-milked milk. After adding the three starter cultures to the vat milk, the lactic microflora reached a count of between 10^3 and 10^5 UFC/g in all three tested periods. Hence, the three starter cultures worked effectively right from the very first milk processing stages despite differing in composition and acidification activity (Figure 1).

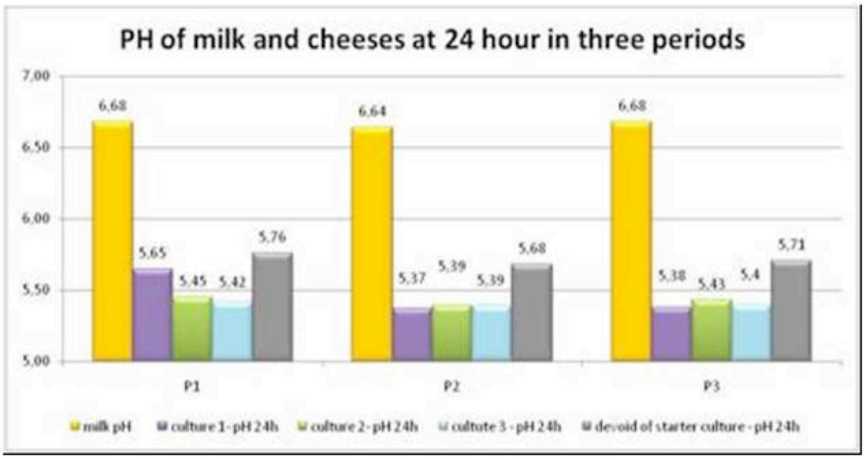


Figure 1. pH of the milk and the cheese 24 hours prior to the three starter blends in three periods

In all the tested periods, the lactic bacteria and the three cultures demonstrated a tendency to increase during cheese ripening, reaching a peak between 24 hours and 15 days followed by a slow decrease during the course of ripening. On the whole, the lactic bacteria count resulted slightly higher in cheese produced using cultures 2 and 3, with average counts exceeding 10^8 UFC/g. an increase in the mesophilic lactococci bacteria count in all the cultures in period P3 while, on the contrary, the count was lower in periods P1 and P2, especially in culture 3 (Figure 2).

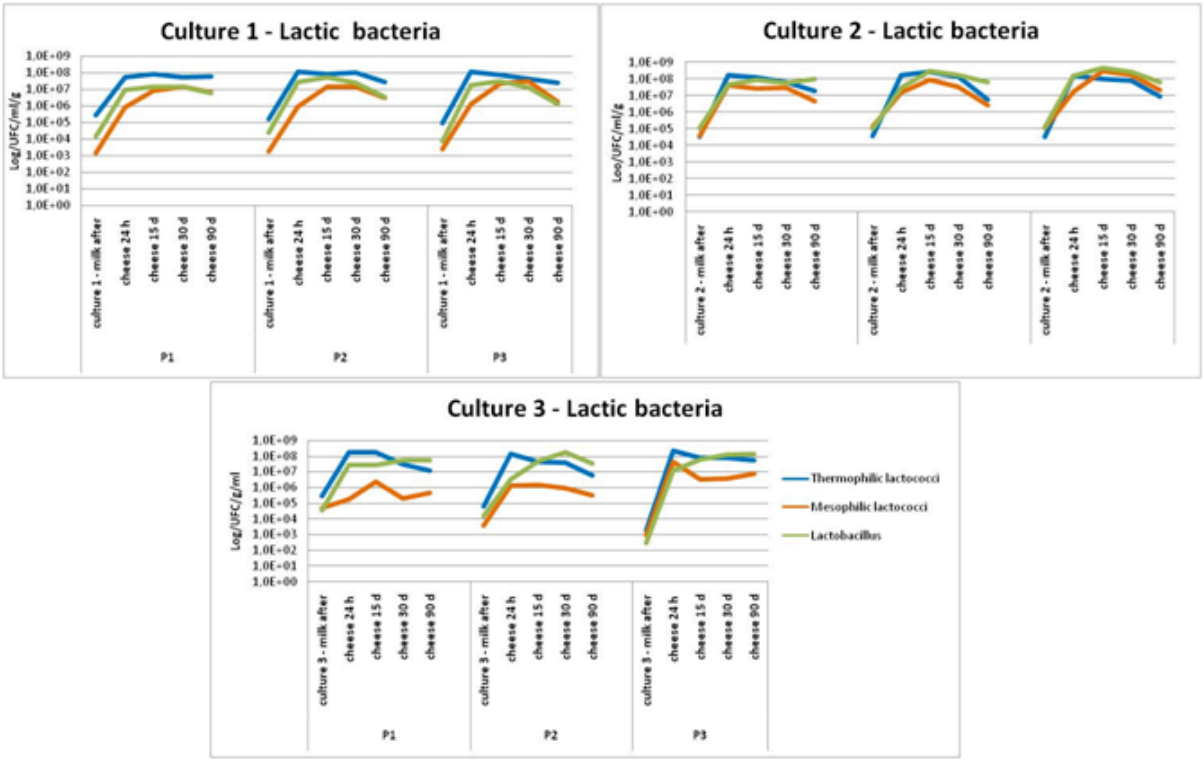


Figure 2. acid lactic bacteria development while cheese ripening achieved through starter blends

During cheese ripening, the three starters performed correctly in all the periods, working well to impede the growth of total coliforms and *E. coli.* They were however only partially capable of impeding coagulase-positive staphylococci that exceeded regulation thresholds at the 24 hour step of period P3 in the cheese making procedure that employed cultures 1 and 2. None of the three cultures was sufficiently capable of preventing the development of obligate heterofermentative bacteria in period P2. Furthermore, in this period the bacteria count remained alarmingly high throughout the entire cheese ripening stage, akin to cheese devoid of starter inoculation, while during the other periods, culture 3 (and to a lesser degree culture 2) were seen to thwart the growth of this bacterial group. Likewise, yeast counts showed an upward trend during ripening in all the cheese making processes without any indication of being particularly influenced by the presence of the starter cultures. At tasting, the cheese produced with the three starter cultures presented similar characteristics, such as typicity, organoleptic characteristics, hygienic quality and complied with the Production Regulations, unlike the cheese devoid of starter culture inoculation (Figure 3).

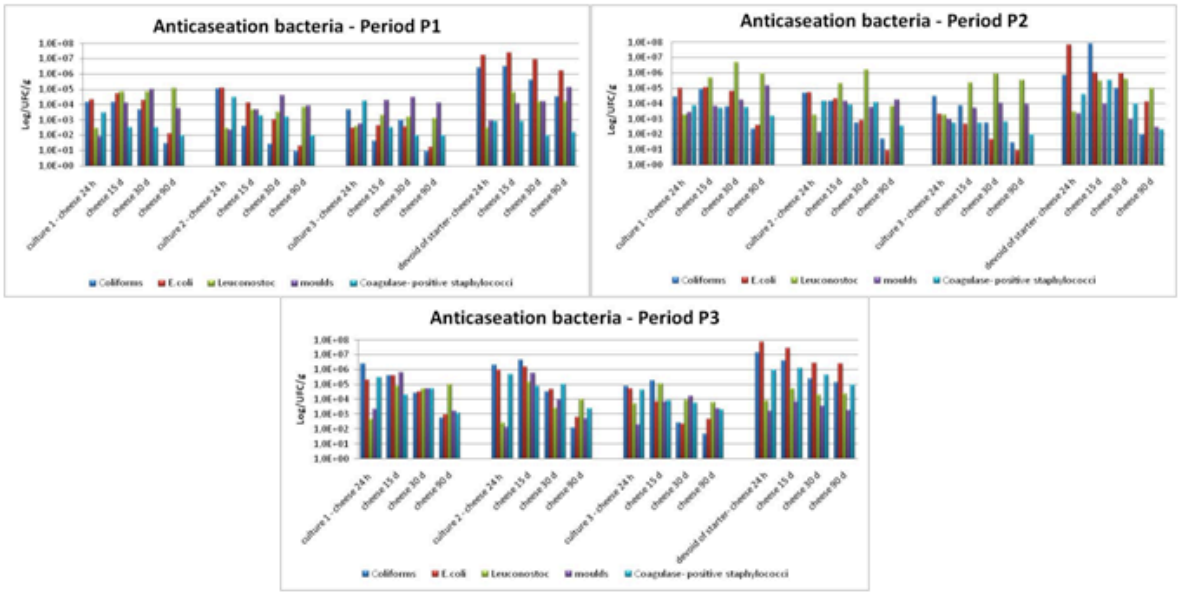


Figure 3: anticaseation bacteria while cheese ripening achieved through starter blends

The cheeses produced with starter cultures showed no differences in the examined periods; the culture 1 showed a greater linearity with the production regulations while the culture 3 has a better behavior in critical situations. The culture 2 has an intermediate behavior to the first two, while without starter forms were found for the visual appearance of non-compliant and in addition to this lack of sanitary requirements, especially with regard to the charge of coagulase-positive staphylococci.

Conclusions

The cheese produced using indigenous cultures offered a similar profile in all the periods under examination and were, on the whole, compliant with Production Guidelines even though, at tasting the wheels of cheese presented striking differences that were linked to the three starter cultures. However, the wheels devoid of inoculation were not visually compliant and above all lacked the hygiene and health requirements as regards their coagulase-positive staphylococci count. Therefore, the results of this study demonstrate that even if the composition of the three lactic cultures differ, the end product presents similar aspects overall.

Acknowledgements

12th INTERNATIONAL Meeting on Mountain cheese 20-22 June 2017 Padua, Italy

References

Andrighetto C., Borney F., Barmaz A., Stefanon B., Lombardi A. 2001. Genetic diversity of *Streptococcus themophilus* strains isolated from Italian traditional cheeses. Short communication alla Conferenza Nizo sui microrganismi lattiero-caseari. Ede 13-15 luglio.

Treu L., Vendramin V., Bovo B., Campanaro S., Corich V., Giacomini A. 2014. Genome Announc. 2(1): e00067-14.

Battistotti B., Bottazzi V., Vola G.1977. Impiego di *Streptococcus faecium, Streptococcus thermophilus* e bacilli lattici nella caseificazione del formaggio Fontina: scienza e tecnica lattiero-casearia, vol. XXVIII: 331-341.

DAFN

Department of Agronomy
Natural resources Anima

ISBN 978-88-6938-059-4