



Use of genomic data in French dairy sheep breeding programs: results and prospects

J.M. Astruc¹, G. Lagriffoul¹, H. Larroque², A. Legarra², C. Moreno², R. Rupp², F. Barillet²

¹Institut de l'Élevage – BP 42118 – F-31321 Castanet-Tolosan Cédex, France

²INRA-UR631, Station d'amélioration génétique des animaux, F-31326 Castanet-Tolosan, France

Abstract

The selection of the 5 French dairy sheep breeds is presently based on the usual quantitative genetics approach, both for milk production and udder functional traits, using pedigree and recorded phenotypes in nucleus flocks. Moreover since 2002 a gene-assisted selection (GAS) has also been implemented to select for scrapie resistance against classical scrapie and BSE strain, based on a large scale PrP gene genotypings. This national scrapie plan was included in the existing breeding schemes : between 2002 and 2009, a total of 160,000 PrP genotypings have been funded and designed, mainly in the nucleus flocks, in the aim to be efficient at the whole population level. As a result in 2009, 95 % of the rams used in the nucleus flocks were homozygous ARR sires, illustrating the efficiency of PrP GAS in French dairy sheep populations. Now a high-density SNP array (the Illumina Ovine SNP50 BeadChip) available in sheep since January 2009 makes feasible in the future either marker-assisted selection (MAS)/GAS, or genomic selection (GS), at least in the Lacaune and Manech red faced breeds, thanks to the size of the nucleus of the 5 French dairy sheep breeds using extensively AI and to the storage of the DNA/blood of the AI rams since the middle of the 90's. Therefore we present an overview of different genomic on-going projects involving the French dairy sheep breeds.

Keywords: dairy sheep, genomics, selection, SNP, breeding schemes.

1.0 Introduction

The selection of the 5 French dairy sheep breeds is presently based on the usual quantitative approach, both for milk production and udder functional traits, using pedigree and recorded phenotypes in nucleus flocks. This selection has given sound results, with differences from one breed to another, according to the size of the selected population in nucleus flocks, the starting year of each breeding program, and the more or less extensive use of animal insemination (AI).

Since 2002 a gene-assisted selection (GAS) has also been implemented to select for scrapie resistance against classical scrapie and BSE strain, based on a large scale PrP gene genotypings. This national scrapie plan was included in the existing breeding schemes and has allowed a successfully increase of the frequency of the ARR allele, firstly in the nucleus flocks, then in the commercial flocks. Such a GAS has been made possible through the development of both strategies and tools, which would be useful to any other gene selection.

The Illumina Ovine SNP50 BeadChip, available in sheep since January 2009, opens the door to a more systematic utilization of genomic tools in the future, either by applying marker-assisted selection (MAS)/GAS, or genomic selection (GS) in the French dairy sheep breeds.

In this paper, we first intend to present the breeding programs of the French dairy sheep breeds, in order to bring to light the assets of these programs for a possible use of genomic tools. We describe the main features of the GAS implemented for ovine PrP gene, underlining the organization and tools that have been set up to succeed in spreading the favorable ARR allele as a genomic way to increase scrapie resistance. Finally, we present the different on-going genomics projects in French dairy sheep breeds, whose common applied purpose is to test MAS/GAS, or GS.

2.0 The dairy sheep breeding programs in France

Dairy sheep breeding in France is mainly located in 3 mountainous areas of Southern France, in which one or more local breeds are milked to produce high added-value cheese: the Lacaune breed in the Roquefort area (Roquefort cheese), the Manech (red-faced and black-faced) and the Basco-Béarnaise breed in the Western Pyrenean hills and mountains (Ossau-Iraty cheese), the Corsican breed in the Corsica Island (various kinds of cheese, bruciu cheese).

Since the early seventies, breeding programs have been implemented for each breed, based on extensive milk recording, heat synchronization and animal insemination (AI), progeny-test and assortative matings. They are also based on a pyramidal organization of the population with, at the top, breeders of the nucleus flocks, where breeding programs are carried out, and from where the genetic gain is transferred afterwards to the commercial flocks by using natural mating or AI rams born in the nucleus flocks (Barillet, 1997). The table 1 highlights main features of the selection breeding programs, showing differences between breeds.

Table 1. Size of the selection programs in the French dairy sheep breeds (year 2009)

Breed	Total population	Population recorded in nucleus flocks (%)	Number of AI (AI rate within the nucleus flocks)	Progeny-tested AI rams
Lacaune	900,000	173,568 (19%)	395,800 (85%)	445
Red-faced Manech	270,000	70,712 (26%)	56,800 (50%)	151
Black-faced Manech	100,000	14,509 (15%)	7,900 (45%)	36
Basco-Béarnaise	80,000	21,984 (27%)	14,100 (50%)	52
Corsican	95,000	21,050 (23%)	6,300 (30%)	31

For each breed, the selection criteria and the efficiency of the breeding schemes depend on the size of the entire population and the part of population included in nucleus flocks (submitted to official AC milk recording), the impact of AI, the number of AI rams progeny-tested, the history and age of each breeding scheme, according to the organization of the breeders and industry interest. The table 2 summarizes selection criteria as well as indicators illustrating the efficiency of the breeding programs. Among the main features:

The program of the Lacaune breed has included functional traits at a 50% level since 2005 (Barillet *et al.*, 2006; Barillet, 2007). The program of the Pyrenean breeds has included fat and protein, steadily since 2001, up to a 2009 new criterion whose objective is to increase fat and protein yields and contents.

The relationship between the impact of AI and progeny-test and the efficiency of the selection program is high, as shown when looking at tables 1 and 2.

Table 2. Selection criteria and efficiency of the breeding schemes in the French dairy sheep breeds.

Breed	Selection criteria ¹	Annual Genetic gain in MY for rams (1995-2007) (liters)	Average lactation in 2009 in liters (length in days)
Lacaune	1/2 FY+PY+F%+P%) + 1/2 (SCC+U)	6.4 272	(164)
Red-faced Manech	FY+PY+F%+P%	3.8	180 (155)
Black-faced Manech	FY+PY+F%+P%	1.2	134 (139)
Basco-Béarnaise	FY+PY+F%+P%	2.9	164 (146)
Corsican	MY	0.7	133 (182)

MY = milk yield, FY = fat yield, PY = protein yield, F% = fat content, P% = protein content, SCC = somatic cell count, U = udder morphology.

¹Economic values for each trait included in the subindex for milk production (MY, or FY, PY, F%, P%) and in the subindex for functional traits (SCC, U) are not described in the selection criteria of the table 2.

3.0 A large scale gene assisted selection: selecting for scrapie resistance

In addition to the phenotypic selection on quantitative traits, the BSE crisis context has been the starting point, from 2000, of the implementation of a GAS for scrapie resistance, based on PrP gene genotyping. The objective of the program was to eradicate the unfavorable VRQ allele and to select for the favorable ARR allele (Leymarie *et al.*, 2009). Since the breeding programs were efficient and the diffusion AI-oriented, the efforts of genotypings focused first in the nucleus flocks, in the aim to combine selection on the PrP gene and selection on production and functional traits, while maintaining genetic variability of each breed. Regarding initial allelic frequencies, the situation was heterogeneous from one breed to another, from only 17% of favorable ARR allele in the Red-Faced Manech to 55% in the Lacaune breed.

To tackle the issue, from 2000 to 2009, 160,000 genotypings have been carried out. The genotyping strategies were different from one breed to another, depending on the initial ARR allelic frequency of the PrP gene and on the relative part of AI. In the Lacaune breed, the genotypings concerned the young rams candidates to enter the breeding centers, whereas in the other breeds, both candidate rams and elite dams were genotyped, to take into account a more unfavorable initial ARR frequency as well as a less extensive use of AI in the nucleus flocks. The organization of the genotypings were made to allow the young rams be PrP genotyped very early, before entering the breeding center at 1 month-old. To face this constraint a blood sampling is taken at about 10 days-old, sent to an agreed lab, which sends back the result as quickly as possible to allow the program manager to select young rams for the breeding center.

A national molecular information system has been set up to manage and valorize PrP genotypes. The molecular database, when exchanging with the dairy sheep genetic information system (named SIEOL), enables passing on of checked genotypes for selection purposes and computation of genotype predictions on relatives (Astruc *et al.*, 2008 ; Morin *et al.*, 2008).

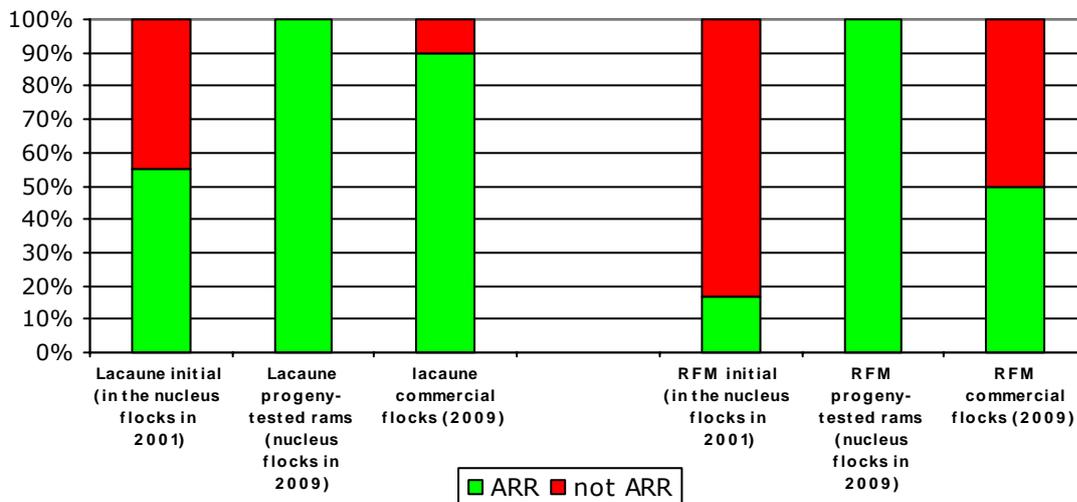


Figure 1. Evolution of the PrP allelic frequencies in the Lacaune and Red-Faced Manech (RFM) breeds between 2001 and 2009.

The increasing number of genotypes and predictions, combined with the use of AI and diffusion of natural mating rams, has allowed a dramatic change in the allelic frequencies. The figure 1 illustrates the results of the PrP breeding plan, within the breeding program (nucleus flocks) and in the whole population (commercial flocks) for the Lacaune and Red-Faced Manech breeds.

4.0 Genomics in the French dairy sheep breeds : on-going projects and strategies for using genomic data

Using the opportunity of the PrP scrapie plan as well as research programs for QTL detection in the French dairy sheep breeds, a DNA-bank was constituted, from the middle of the nineties. Blood samples of all the AI rams progeny-tested in the 5 French dairy sheep breeds were systematically collected and sent to the molecular laboratory Labogéna in Jouy-en-Josas. In 2009, the collection of DNA-samplings of AI progeny-tested rams reached 9,100 rams, as shown in the table 3.

Since January 2009, a high-density SNP array (the Illumina Ovine SNP50 BeadChip) has been available in sheep, opening the door in the future to an either marker-assisted selection (MAS)/GAS, or genomic selection (GS).

Table 3. DNA-collection on the French dairy sheep breeds (year 2009).

Breeds	Number of AI progeny-tested rams with storage of DNA	Year of beginning of the DNA-storage
Lacaune	5961	1995
Red-Faced Manech	1940	1995
Black-Faced Manech	436	1995
Basco-Béarnaise	544	1995
Corse	219	2003

Furthermore, the recent developments on genomic selection and their growing applications in bovine have stimulated the breeding organizations involved in dairy sheep in France, alongside with the organizations of research and development (INRA and Institut de l'Élevage) to set up a strategy of utilization of the genomic tools. In this purpose, different projects have been built and are on-going. Thus, several programs have been launched, which are complementary and serve the adopted strategy.

According to the size of the selection programs, which is different from one breed to another (Table 1), the proposed strategy consists in studying and testing the utilization of genomics based either on MAS/GAS, or genomic selection (GS) with the idea to test different methods or procedures to run genomic evaluations (Aguilar *et al.*, 2010). Indeed, the implementation of GS seems feasible at least in the Lacaune and Red-Faced Manech breeds, whose potential training populations, higher than a minimum of 1,000 animals (table 3), could allow genomic estimated breeding value (GEBV) predictions to be accurate enough for further utilization to compute GEBVs for young rams at birth. In the other breeds with smaller training populations, an across-breed genomic selection approach will be tested, particularly for the Manech breeds, whose racial and environmental proximity to the Latxa breeds from Spain might allow to mix all the progeny-tested rams with true proofs in a single training population, which could be sufficient to estimate accurate enough genomic equations. We will check if this across-breed approach, using Illumina ovine SNP50 BeadChip, is relevant.

The strategy lays on the following programs, mainly based on genotyping but also on engineering inputs:

The SheepSNPQTL project (2009-2011) (Moreno *et al.*, 2009), partially founded by research and breeder organizations, besides fine detection of QTL, aims at determining a first set of procedures to calculate genomic breeding value estimates (GEBV) for selected traits in the Lacaune breed, using a thousand of well-known progeny-tested rams.

The European 3SR project (2009-2011) is devoted to major gene and QTL detection in sheep and goats. The final aims are, on the one hand, to provide enhanced insight into mechanisms controlling resistance to mastitis, resistance to gastrointestinal parasites and ovulation rate, and, on the other hand, to provide lists of SNP associated to those traits and that can be used for selection.

One of the goal of the Roquefort'in project (2010-2013) is to test a genomic selection approach in the dairy Lacaune breed with the following tasks : increasing the accuracy of the GS procedures previously estimated in the SheepSNPQTL project, using 2,000 rams more in order to reach a training population of 3,000 rams; experimenting GS, by computing GEBV for a part of two batches of young rams to be progeny-tested in 2011 and 2012, two-third of each batch being chosen classically on EBV ancestor selection, and one-third of each batch being chosen with a

GEBV selection pressure, in the aim to compare EBV or GEBV efficiency with official proofs got in 2013. On the whole, 4,000 rams will be SNP genotyped.

The across-border project Genomia (2010-2012) involves the French Manech and Spanish Latxa breeds. Regarding genomic selection, the purpose is to test GS in Manech red faced breed alone (training population of 1,000 rams); then to test an across-breed GEBV approach, by genotyping 2,600 AI rams, corresponding to the mixing of Red-Faced and Black-Faced Manech, Blond-Faced and Black-Faced Latxa training populations.

The on-going project PhenoFinLait (2008-2013) (Faucon *et al.*, 2009) (www.phenofinlait.fr), also partially founded by research and breeder organizations, concerns cattle, goats and sheep and aims mainly at searching QTL for milk fatty acid profiles and milk proteins, but will be also useful for fine QTL detection on all the selected traits recorded in the nucleus flocks. In dairy sheep, 2,000 SNP genotypings will be performed in this project, corresponding to a daughter design in Lacaune and Manech red faced breeds.

The Genovicap project (2010-2012), jointly led by INRA and Institut de l'Élevage associated in a partnership unit named UMT GENEPR is devoted to the engineering issues: how to handle genomic data, how to run the breeding schemes using genomic breeding values, how to adapt them, how to organise the genotypings (founding, chip purchase, genotypings).

The challenge and hopes in GS for dairy sheep in France, associated or not with fine QTL detection or knowledge of causal mutations, would be to increase the selection efficiency, by speeding up selection on routinely recorded traits, and/or by implementing selection of new traits expensive or difficult to record. Thus the first step will be to test the accuracy of GEBV prediction for selected milk and functional traits. Assuming that accuracies of GEBV will be sufficient to select elite sires very early, the way(s) towards which the increasing efficiency will have to be used and oriented will be a major concern for the breeding organizations and those who manage the breeding programs:

Reducing costs (removing the progeny test of AI rams and reducing the total number of AI rams). The selection tools are expensive in sheep regarding the gross margin per animal (milk recording, AI). Cutting costs while maintaining the genetic relevance/efficiency has always been a key concern in dairy sheep programs in France.

Increasing genetic gain on actual selected traits. This is of most importance since all breeds have a long generation interval for the sire pathways in present phenotype selection regarding progeny-test of AI rams; this could be dramatically reduced by GS.

Adding new traits in the selection criteria of dairy sheep breeds (Barillet, 2007). The demand of both industry, breeders and consumers is still growing regarding healthy and functional animals and foods; thus encouraging the breed organizations to introduce new traits in the breeding goals: production traits (milk fatty acid profiles, milk persistency and once a day milking ability), functional traits (as milking ability or disease resistance, such as mastitis, nematodes or scrapie resistance) ...

These different ways are all relevant and will probably mix to an expected improvement of the selection efficiency.

At the same time, the breeding organizations will have to think and face evolutions of the breeding schemes:

What new management of the AI livestock without progeny-testing ?

Which size of the open nucleus population, with official animal recording ?

How to re-organize engineering ?

How to take into account genomic data in the information system, as it has been successfully completed for the integration of PrP gene ?

5.0 Conclusion

The breeding programs of the French dairy sheep breeds are known to be efficient on a classical phenotypic selection basis. The management of the PrP gene to reinforce scrapie resistance of the entire population, which is the first application of using genomics data on a large scale, was a success story.

Regarding the availability of the Illumina Ovine SNP50 Bead Chips since January 2009 and the recent developments on genomic selection, the French dairy sheep breeding schemes are in a favourable situation to successfully introduce genomic data in their programs: the Lacauine and Red-Faced Manech programs are based on a high number of progeny-tested rams due to an extensive use of AI in their nucleus flocks. Furthermore, the storage of DNA has been implemented for more than a decade. Thus the breeders of the French dairy sheep breeds have decided to test and implement a genomic selection or a marker/gene-assisted selection (both are not antagonistic). Therefore, several genomic projects are ongoing and will output results and answers in the next years, basically on the accuracies of GEBV for the selected traits and then on the cost-effectiveness of such a selection, compared to the actual phenotypic selection. The structuring power of these on-going projects on the breeders, research and development, and the co-operation between all these organizations involved in provide a favourable situation to face this exciting challenge in dairy sheep. Nevertheless unexpected issues arise, among them the legal aspects around the status of the GEBV: shall an individual breeder be authorized to use genomic tools and consequently GEBV from its own within-flock rams, given that the computation of such GEBV has required a huge collective investment? This kind of questions is far from being clearly answered.

References

- Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., Lawlor, T.J., 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. *J. Dairy Sci.* 93: 743-752.
- Astruc, J.M., Lagriffoul, G., Morin, E., Barillet, F., Bonaiti, B., Rehben, E., 2008. SIEOL : implementing a global information system for genetic and techno-economic support in dairy sheep in France. Proc. 36th Session of ICAR, Niagara Falls, USA, 18-20 June 2008, ICAR Technical Series, N° 13, 123-127.
- Barillet, F., 1997. Genetics of milk production, Chap 20. In: Piper, I., Ruvinsky, A. (Eds.), *The Genetics of Sheep*. CAB International, pp. 539-564.
- Barillet, F., Astruc J.M., Lagriffoul, G. 2006. Taking into account functional traits in dairy sheep breeding programs through the French example. 35th ICAR Session, Kuopio, Finland, EAAP publication N° 121, 57-64.
- Barillet, F., 2007. Genetic improvement for dairy production in sheep and goats, chapter of the special issue entitled «The outlook of quantitative and molecular genetics. Application in improving Sheep and Goats», edited by D. Notter, R. L. Baker and N. Cokett. *Small Ruminant Research* 70 (2007) 60–75
- Faucon F., Brochard M., Barillet F., Bollard M., Brunshwig P., Duhem K., Eggen A., Esvan S., Ferrand M., Fritz S., Gastinel P.L., Guerin J.L., Journaux L., Krychowski T., Lagriffoul G., Larroque H., Lecomte C., Leray O., Leve rrier S., Martin P., Mattalia S., Miranda G., Palh iere I., Pe yraud J.L., Boichard D. PhenoFinLait (LactoScan) : French national program for high scale phenotyping and genotyping to detect QTL linked with fine composition of ruminant milk. International Milk Genomics Consortium, Paris, France, 28-30 Septembre 2009.
- Leymarie C, Bouffartigue B, Astruc JM, Bael den M, Barillet F, Bibé B, Bonnot A, Boscher MY, Bouix J, Brochard M, Dion F, François D, Jouhet E, Jullien E, Orlianges M, Moreno C, Palhière I, Perret G, Raoul J, Sidani C, Tiphine L, Raynal A, Bouchel D, Catrou O, Chibon J, Tribon. The national breeding program for scrapie resistance in the French sheep population. *Renc.Rech.Ruminants* 2009, 16. 411-414.
- Moreno C., Servin B., Faraut T., Klopp C., Rupp R., Mulsant P., Robert-Granié C., Barillet F., Delmas C., Bouix J., François D., Allain D., Manfredi E., Bodin L., Elsen J.M., Robelin D., Mangin B., Aurel M.R., Bouvier F., Calavas D., San Cristobal M., Jacquiet P., Foucras G., Boissy A., Legarra A.. SheepSNPQTL : a 60,000 SNP chip to fine map QTL affecting production traits, resistance to diseases and behavior in sheep. *Renc.Rech.Ruminants*, 2009, 16. 420.
- Morin, E., Astruc, J.M., Lagriffoul, G., SIEOL, a new global information system for genetic and techno-economic support for dairy sheep breeders in France. Proceedings of the International Mediterranean Symposium of EAAP in Corte, France, 6-8 November 2008.

6.0 Acknowledgements

Financial support of the different projects described in this paper was provided by Apis-Gène, ANR, Casdar, Conseils Généraux de l'Aveyron et du Tarn, Communauté de Communes du Grand Rodez, France Agri-Mer, Feder, FGE, FUI, Ministère de l'Agriculture, Région Aquitaine, Région Midi-Pyrénées.