GENETIC VARIATIONS AND ASSOCIATIONS FOR IMPROVING MILK PRODUCTION AND MILK PRODUCT QUALITIES IN SHEEP AND GOATS.

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SUMMARY

The genetic variation of dairy traits for small dairy ruminants is revised. Examples are given on the exploitation of this genetic variation in large populations using a classical polygenic approach (sheep) or taking profit of major genes (goat). The genetic determinism of secondary traits, including meat and functional traits, is also described. Finally, perspectives for new profitable technologies, as molecular genetic and reproductive technology, are discussed.

Keywords: **Dairy sheep, dairy goat, cheese, breeding scheme, genetic determinism, dairy traits, functional traits, reproductive technology, molecular genetics.**

INTRODUCTION

This presentation will focus on European populations to introduce genetics of milk production. In Europe, dairy sheep are distributed mainly in Southern and Eastern regions, and usual husbandry systems include a suckling period of at least one month, before the milking only period, which reflects a double purpose dairy-meat. In Mediterranean countries, local goats are generally managed as dairy sheep, while in continental Europe the system of milking directly after kidding is the most frequent as for cattle. As a general trend for both species, the income from milk is the most important, the milk being usually processed into cheese. Furthermore in the Europe Union countries, there is often a complete association between a region of production, a breed and the brand name of a high quality cheese.

A great diversity of production systems, from extensive to intensive management (Boyazoglu and Flamant, 1990; El Aich *et al.,* 1995) is combined to a great diversity of genetic material. Most sheep or goat milk has been traditionally produced by local breeds with low or medium milk yields. However some European populations have evolved into dairy types (*i.e* in France, Greece, Italy, Norway, Portugal, Spain, Switzerland) with a simultaneous adaptation of the breeding system to modern husbandry methods and sometimes the implementation of sound breeding programmes (Barillet, 1997). While crossing is used in somes cases for creation of synthetic breeds (*i.e.* Assaf in Israel; Epstein, 1985) or for absorption of low producing local breeds, the most frequent situation is purebreed selection.

I) GENETIC VARIATION OF DAIRY TRAITS

When using the standard A_4 milk recording test (monthly recording) and genetically connected data via AI, genetic parameters on a total lactation basis for dairy traits of sheep (Barillet and Boichard, 1987; Sanna *et al.,* 1997) and goat (Boichard *et al.,* 1989) follow the

same patterns as in cattle: heritabilities for milk (M), fat (F) and protein yields (P) are moderate (~ 0.30) and smaller than for fat (F%) and protein (P%) contents (~ 0.50 to 0.60), whereas the genetic coefficients of variation,which best represent the possible genetic gain are higher for yields. Furthermore fat is more variable than protein, and the fat/protein ratio of the genetic standard deviations is in the range of 1.1 to 1.4 for yields and 1.5 to 1.9 for contents wathever the species. Milk and matter yields are highly related (~ 0.8 to 0.95), but their genetic correlations with contents are very different: while M is negatively related to contents, and generally more strongly to P% (- 0.4) than with F% (- 0.3), the genetic correlations between matter yields (F,P) and contents (F%, P%) are close to zero or slighty negative, except for the correlation between F and F% which is clearly positive (~ 0.2 to 0.3).

Exceptions to this pattern have been reported on different breeds and varying environmental conditions, but in recent studies, based on test-day data for sheep and goat, heritabilies for yields are about 0.3, with important variations according to lactation stage (Barillet and Boichard, 1994 ; Schaeffer and Sullivan, 1994 ; Barbieri, 1995 ; Baro et al., 1994). These parameters describe genetic variation under polygenic assumptions but interest in candidate genes affecting dairy traits is growing, specially for milk proteins.

In goats, studies on biochemical genetics indicated a highly variable αs1-casein polymorphism, variants being associated to different rates of synthesis (Grosclaude et al., 1994). Molecular studies on the αs1-cas locus reviewed by Martin (1993) revealed a complex genetic polymorphism and throwed light into the alternative splicing process underlying the relationship observed between protein structures and expression levels. So far, 7 variants corresponding to 14 alleles have been identified in many breeds in different countries (Grosclaude et al., 1994) including the nihil allele O (Leroux et al., 1990), with important genomic deletions and no synthesis of αs1-cas. PCR-allele specific techniques are today available for genotyping young animals of any sex. The quantitative effects of the goat αs1-cas on dairy traits were studied by independant on-station (Barbieri et al., 1995) and on-farm experiments (Mahé et al., 1994) indicating that the difference between the extreme genotypes AA (favourable) and FF, in terms of P%, was 4.5 g/kg, for an overall mean of 29 g/kg and a polygenic standard deviation of 1 g/kg. Also, the favourable allele A outperformed the F allele in P and F%. Milk from AA genotypes had a higher ratio casein/total protein than FF milk with a corresponding advantage of 15 % in terms of cheese yield (Vassal et al., 1994). At present, research is oriented to clarify the relationship between fat and protein syntheses in the mammary gland which could explain the differences in goaty flavour associated to the αs1-cas polymorphism reported by Delacroix et al (1996).

Major genes may explain global genetic variation. Barbieri et al (1995) compared estimates of genetic parameters for Alpine goats under station conditions using an animal model including or not a αs1-cas fixed effect. The heritability of P% changed from 0.66 to 0.34 when the αs1-cas effect was included in the model. Corresponding values for the correlation between P and P% were 0.09 and -0.22, thus suggesting that a pleïtropic effect of the αs1-cas gene is hiding a polygenic opposition between both traits. It is likely that estimated genetic parameters under polygenic models concerning P% and, at a lesser extent P and F%, will be influenced by αs1-cas allelic frequencies which vary widely among goat populations.

While the quantitative effects of the αs1-cas locus has been thoroughly studied, loci of other caseins may affect goat performances and milk chemical composition, in particular β-cas where nihil alleles were found in Italian breeds (Chianese *et al.*, 1993) and in Corsica (Mahé and Grosclaude, 1993).

For sheep, research on the genetic polymorphisms of milk proteins is not yet as extensive as for goat or cattle and it is less conclusive. Two αs1-cas variants « Normal » and « Welsh » had been detected (King, 1967) until Chianese *et al* (1996) identified 5 variants (A-E), but only the Welsh (D) variant has been related to modification of casein content and rennet coagulation (Pireda *et al.*, 1993). Also, the allelic frequency of the Welsh variant is generally very weak, in the range of 0.0 to 0.06 for investigated breeds (Corsica, Lacaune, Manech, Sarda) so that its potential use for sheep selection appears often limited. Variants A and B are the most common for β-lactoglobulin, and so far the relations with dairy traits or technological properties of milk are too controversial (Lopez-Galvez *et al.*, 1994; Pellegrini *et al.*, 1997; Recio *et al.*, 1997) to consider presently this polymorphism as a potential breeding tool.

Other genes are being studied as candidates for explaining genetic variation of dairy traits: GH and GH receptor (Gootwine *et al.*, 1993), or prolactin receptor gene (Le Provost *et al.*, 1994). The RFLP studies at the GH locus have demonstrated the existence of two alleles for sheep due to variation in the number of copies of the gene (Gootwine *et al.*, 1993).

II) EXPLOITATION OF GENETIC VARIATION OF DAIRY TRAITS

In a context of increasing comsumption of sheep and goat cheese, the market demand is to improve the milk yield, via P+F, with an adequate chemical composition, via F%+P%. Two examples, the first using polygenic inheritance and the second taking profit of major genes, will illustrate how genetic variation can be exploited in large populations to contribute to satisfy market demands.

1) The dairy sheep Lacaune breeding scheme (France)

In France, the first region of sheep milk production is the Roquefort area in the Massif Central plateau with 750,000 Lacaune ewes on 2600 farms in 1996. In the 1960s, the dairy plants worried about the stagnation of milk production due to the low milk yield level despite the increase of the Roquefort cheese market. In order to improve milk yield, a cooperative projet was established between INRA, breeders and milk companies to organize an open nucleus selection scheme based on AI and on-farm milk recording.

In 1996 the open nucleus totalized 169,000 ewes on 392 farms (22 % of the population), and the rate of IA reached 80 % which allowed to progeny-test 472 AI young rams, a figure comparable to the number of bulls progeny tested yearly in the most efficient European dairy cattle programmes. To maximize both the annual genetic gain and the discounted benefits, 50 % of the inseminated ewes in the nucleus flocks were mated with sampling rams being progeny and 50 % with AI proven rams, especially the 120 top ranked rams.

The published genetic gain for milk yield (expressed as mature equivalent lactation), validated according to the methods recommended by Interbull in cattle (Barillet *et al.*, 1996), was close

to 6 liters per year between 1980 and 1994, *i.e.* about 2.4 % year[-1] of the nucleus mean .

According to the evolution of the market demand, the selection objective was periodically revised in order to improve both the dry matter yield and the dry matter content. This new goal has actually been reached, since from 1990 the annual genetic gain for F% and P% is in the range of 0.2 to 0.3 g l^{-1}, whereas the genetic trend for milk yield is about 5 liters per year during the same time (figure 1). About 15 years after the beginning of the breeding scheme, a constant annual genetic progress for milk yield was obtained in the open nucleus. At the same time, the diffusion rate (AI plus natural mating rams) from the nucleus to the base population reached about 100 % for commercial breeders using a simplified milk recording (554,000 ewes on 1533 farms in 1996 with an AI rate of 40 %). Thus, since 1980 the phenotypic trend is the same in the nucleus and the base population (phenotypic gain of 100 liters between 1980 and 1995), with a gap of about 40 l corresponding to 5-7 years of gain (figure 2). This result is in agreement with the gene-flow studies carried out in the 1970s to design and optimize the breeding scheme (Barillet and Elsen, 1979).
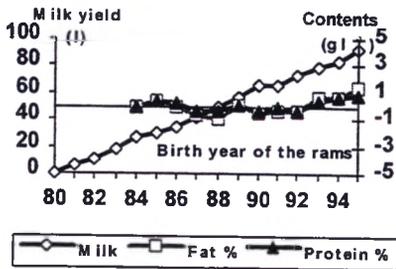

Figure 1. Genetic trend for Lacaune rams in the nucleus flocks (animal model evaluation 1997).
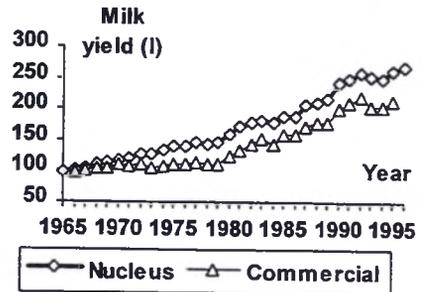
Figure 2. Phenotypic trend for milk yield in the Lacaune nucleus flocks and commercial

Finally the sheep milk production from the Roquefort area quadrupled from 38.8 millions liters in 1965 to 171.7 millions liters in 1996.

2) The αs1 casein polymorphism in the French goat selection scheme

In a context where goat milk is marketed almost exclusively as cheese, caseins are essential to guarantee cheese yield. However, P% in goat milk has been often reported as low when compared to sheep or even cow milk.

The use of the αs1-cas polymorphism as a selection tool was studied in order to preserve the polygenic background (Barbieri, 1995). In France, the selection schemes for the predominant Alpine and Saanen breeds rely on 2 open nuclei totalizing 120,000 goats for both breeds in 1000 herds genetically connected via 60,000 AI practised yearly which allow to progeny-test each year 80 AI young bucks. The selection scheme follows the usual two step selection of AI males: ancestor selection by planned matings between sires and dams of bucks and progeny selection. Reproducers are evaluated using an univariate BLUP animal model

(Boichard *et al.,* 1992) and combining P and P% in an index. The selection practised, ignoring the molecular information, has yielded a positive global genetic trend for M, P and P%, and a positive evolution of the frequencies of favourable alleles (Manfredi *et al.,* 1995a). Nevertheless, a retrospective study of Manfredi *et al* (1995b) indicated that sons of unfavourable genotype FF born from matings between AF parents had very weak chances of being selected after progeny testing. Thus, putting into progeny test FF young bucks is economically inefficient and risky for the farmers participating to the sampling programme. Since 1996, the αs1-cas genotypes of sires and dams are used in the planned matings and bucks are within family selected at birth in order to control mendelian sampling without altering the polygenic background. Costs of this program are alleviated by genotyping females with electrophoretical techniques and by including male blood sampling for PCR analyses in the routine paternity test protocole.

III) SECONDARY TRAITS

As for dairy cattle, the dairy breeding objectives for small ruminants suppose that an increase of individual milk yield will be followed by a progress in the economic margin per animal. This usual simplification is acceptable only if the dairy selection keeps or increases the milk feed efficiency. To verify this point, a divergent selection experiment on dry matter yield (F+P), using AI Lacaune rams from the nucleus, has been in progress since 1989, the ewes being fed with *ad libitum* good quality roughage and about 15 % of concentrate whatever the milk yield level. After 4 years of experiment (during the second and third month of lactation), the High line's ewes (HL) produced, as expected, 20 % more milk compared to the Low line (LL) (*i.e.* 2 standard genetic deviations or 60 liters per lactation) without increase of the body weight, ingested 8% more feed and targeted more adequately their body reserves towards milk production. Thus Gross Efficiency is strongly improved for HL *vs.* LL (0.36 *vs.* 0.31) and the smaller residual feed intake of HL tends to show a biological increase of efficiency for this line (Marie *et al.,* 1996). Therefore the simplification of dairy breeding goal based only on the increase in milk income appears fully acceptable. Furthermore, it must be kept in mind that such a favourable indirect response (specially the absence of indirect response on body weight and the increase of feed intake capacity of roughage) is due to the fact that the Lacaune breed has been on-farm efficiently selected in the framework of feeding systems based on the use of high quantities of roughage to increase the self-sufficient production of the farms in the Roquefort area.

Meat income for dairy sheep is based usually on the milk suckled lamb production, which depends basically on prolificacy. In most cases, the balance between milk and meat income is such that a genetic increase in prolificacy is not desirable: the prolificacy has a very small heritability (0.03 to 0.05) and its genetic correlation with milk yield is slightly positive (0.2 to 0.3), so that a very slow genetic increase in prolificacy is expected with or without including this trait in the global selection criteria (Barillet *et al.,* 1988). Thus, prolificacy may usually only be monitored to avoid any possible genetic drift. However in some particular situations where meat income is comparable or over milk income, prolificacy may become a main selection criteria so that the breeding strategy may be dramatically modified as in Israel

where the Booroola FecB gene is being introgressed in the Awassi and Assaf breeds (Gootwine, 1995).

Functional traits leading to decrease production costs via reduction of unvolontary culling or reduction of input costs can be split into the following major categories: fertility, milkability and udder health, length of productive life and resistance to disease.

Heritability of fertility is classically reported as low and these traits are not usually included in the selection objectives. One exception in goat is the selection against polled reproducers (Ricordeau, 1981), since this trait is associated to intersexuality via an autosomal unknown gene (Syrstad, 1991). The availability of molecular markers for horns (Vaiman et al., 1996) represents a first step to understand the relationship between both traits.

Until now no dairy sheep or goat breed has been directly selected on milkability and udder health but an indirect genetic response on milkability can be expected in the case of an efficient breeding programme managed by breeders who have simplified machine milking routine. In goats, the existence of a major gene influencing the milk flow rate has been demonstrated using segregation analysis methods (Le Roy et al., 1995): the difference between the means of $hdhd$ high milk flow and ++ normal goats was about 3 phenotypic standard deviations, heterozygotes being intermediate and the residual heritability being estimated to 0.25. In sheep, milking speed, using an automatic milk meter (Guillouet et al., 1990) exhibits an important phenotypic variation. Heritabilities for linear scored udder traits ranged from 0.16 to 0.24 in sheep (de la Fuente et al., 1996 ; Fernandez et al., 1997) and from 0.19 and 0.38 in goat (Luo et al., 1997). Heritabilities for somatic cell counts are small to moderate (Baro et al., 1994). So genetic variation exists for these traits but more information is needed on the genetic associations among udder traits and on the definition of the traits to be kept for large scale recording, keeping in mind that any extra on-farm records are expensive to implement in small ruminants compared to dairy cattle.

The length of productive life may be considered using linear scored type traits as practised for dairy cattle. Again, considering the difficulties to implement extra measurements in small ruminants, it is more likely that a global approach will be profitable for sheep and goat as proposed now for cattle using the theory for the analysis of survival data (Ducrocq, 1994).

Disease genetic resistance is not systematically consider in any sheep or goat breeding scheme. Here, we can expect progress by using major genes as the PrP gene for scrapie (Hunter et al., 1992). More research is needed on candidate genes (i.e, Ruff and Lazary (1989) for the CAE virus) or markers for mastitis as suggested in cattle (Georges et al., 1996).

CONCLUSION

Present knowledge on genetic variation in dairy sheep and goats allows genetic gains comparable to the best dairy cattle situation when breeding programmes are based on on-farm milk recording, accurate genetic evaluation, and open nuclei selection schemes using AI. This approach is in progress today in several European countries (Barillet, 1997) and it is recommended for dairy sheep or goat populations starting modern genetic improving. New genetical and reproductive technologies can make substantial contributions if they are inserted into this general approach.

Concerning the reproductive techniques, an extended use of AI is the first priority for most small dairy ruminant populations. The theoretical contributions of closed nuclei using MOET (Rossi *et al.*, 1995) should be carefully revised regarding the cost and the number of reproducers born per donor from MOET (Cognié *et al.*, 1995), and, more critical, the pertinence of selecting animals in a single environment.

Candidate genes or markers are already used in small dairy ruminants genetic programmes and perspectives are promising for improving accuracy, selection intensity and early selection of reproducers. Molecular information will be specially useful when adding secondary traits to the selection objective, in particular for antagonistic traits. Genetic linkage maps are now available for goat (Vaiman *et al.*, 1996) and sheep (Crawford *et al.*, 1995). Projects for QTL detection for dairy sheep started in Europe using purebreed and crossbreeding designs and in Australia by crossing Merino and Awassi sheep (Raadsma, personal communication).

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