Breeding for resistance to gastrointestinal nematodes in French dairy sheep: towards an increase in resilience and sustainability of sheep dairying

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Abstract

Anthelmintic resistance is increasingly present in French sheep flocks. The issue is particularly acute in dairy sheep, as the only anthelmintic drug still available during the lactating period without milk withdrawal time is eprinomectin, a macrocyclic lactone. In the Pyrenean dairy sheep breeds, for which the breeding system is based on pasture grazing under an Atlantic climate, some flocks are in dire straits with no more efficiency of the treatments against the gastrointestinal nematodes (GIN) during the lactating period. Management of GIN in France aims at an integrated control, based on a combination of solutions. It is advocated that treatment should be targeted and selective, grazing should be managed to limit the sources of contamination and resistance of sheep should be increased through genetic selection.

This paper focuses on the genetic lever and describes the process undertaken by the Pyrenean dairy sheep organisations over the last decade to implement a selection for resistance to parasites in the Blond-Faced Manech and Basco-Béarnaise breeds. Phenotyping of resistance to GIN has been achieved since 2008 by experimental infection of the rams entering the insemination centre. The protocol is based on two successive infections with L3 larvae of *Haemonchus contortus*, separated by a 15-day recovery period. Faecal egg count (FEC) one month after each infection and the variation of packed cell volume (ΔPCV) between the time of infection and one month afterwards were measured. FEC and ΔPCV may be considered as indicators of, respectively, the resistance to parasites and the resilience of the animal. 1,826 Blond-Faced Manech rams and 520 Basco-Béarnaise rams have been phenotyped for this trait since 2008. All of them are genotyped. The estimation of genetic parameters of FEC and ΔPCV at each infection shows that FEC is moderately heritable while ΔPCV isplays a lower heritability. The genetic correlations between FEC and ΔPCV are quite high, meaning that the more resistant rams are also the more resilient. The genetic correlations between FEC and ΔPCV on the one side, and the traits currently under selection (milk, fat and protein, somatic cell count, udder morphology) on the other side are low, close to zero. Consequently, the inclusion of resistance to parasites in the breeding objective would not hamper too strongly the efficiency of selection on current traits. A genomic evaluation of FEC and ΔPCV was performed in 2022 and the breeding organisation of the Blond-Faced Manech and the Basco-Béarnaise breeds decided to include resistance and resilience to parasites in the breeding criteria of these breeds. FEC and ΔPCV have been combined in a composite sub-index related to parasite resistance and resilience. This sub-index was then combined with the current selection

index to produce a new Total Merit Index that is now used to select the rams in the breeding program.

Keywords: dairy sheep, resistance to parasites, breeding objective, genetic parameters
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Introduction

Gastrointestinal nematodes (GIN) are ingested by sheep while grazing. They cause significant economic losses (mortality, milk production losses, direct costs of anthelmintics pharmaceuticals). The ecotoxicity of some anthelmintics (e.g. macrocyclic lactones) can provoke undesired effects on non-targeted fauna, coprophagous insects of the pastures mainly (Verdú *et al*, 2018). Moreover, the important adaptation capacity of GIN has made them develop anthelmintic resistances (including multidrug resistance) which has been growingly evidenced over the last few years, leading to a true risk of therapeutic impasse (Jacquiet *et al*, 2024). For all these reasons, breeding for resistance to GIN in sheep is a relevant selection objective.

However, we must have in mind that the genetic selection to increase the resistance/resilience of the host is only one of the pillars of the integrated control of GIN, along with the elimination of the GIN (use of targeted selective treatments, discovery of new molecules) and the drying up of the sources of contamination (e.g. through a better management of pastures and grazing). Use of multiple tools among the integrated parasite management toolbox is the right way to efficiently fight GIN infections (Jacquiet *et al*, 2024).

In France, the Pyrenean dairy sheep breeds (Manech and Basco-Béarnaise breeds), raised mainly outdoors in the western part of the Pyrenean mountains, close to the Atlantic Ocean are subject to severe natural GIN infections due to production systems based on grazing associated to a mild and wet climate. Therefore, the breed organisation of these breeds pioneered in the implementation of genetic selection for resistance and resilience against parasitism in France.

Phenotyping resistance and resilience to gastrointestinal parasites

While many countries and breeds, especially in Australia, New Zealand, UK, Uruguay measure resistance to parasites in situation of natural infections on pastures (Morris *et al*, 2010; Woolaston and Windon, 2001; Ciappesoni *et al*, 2023, Cunha *et al*, 2024), an original design has been set up in France, based on a standardised protocol with controlled infections with *Haemonchus contortus* (Gruner *et al*, 2004a, Jacquiet *et al*, 2015). This protocol is applied to young rams, never exposed to parasites, gathered in breeding/AI centres, which are genetically important because they are the future sires in the selection process. The objective of the protocol is to display differences between rams without hampering their fitness.

The figure 1 summarizes the principles of the standardised protocol and the different measures that are collected. It is based on two successive infections of a given and known dose of L3 of *Haemonchus contortus* (a hematophagous GIN). At the first infection, 3,500 doses of L3 are administered, and 5,000 at the second infection. At each infection, feces are collected 30 days after infection to measure Fecal Egg Count (FEC) through a coprological analysis (Jacquiet *et al*, 2011). A blood sample is also collected the day of the infection and 30 days after to measure packed cell volume (PCV), the purpose being to measure blood loss during infection. Rams are drenched between both infections. When the protocol is applied to meat sheep breeds, L3 doses may

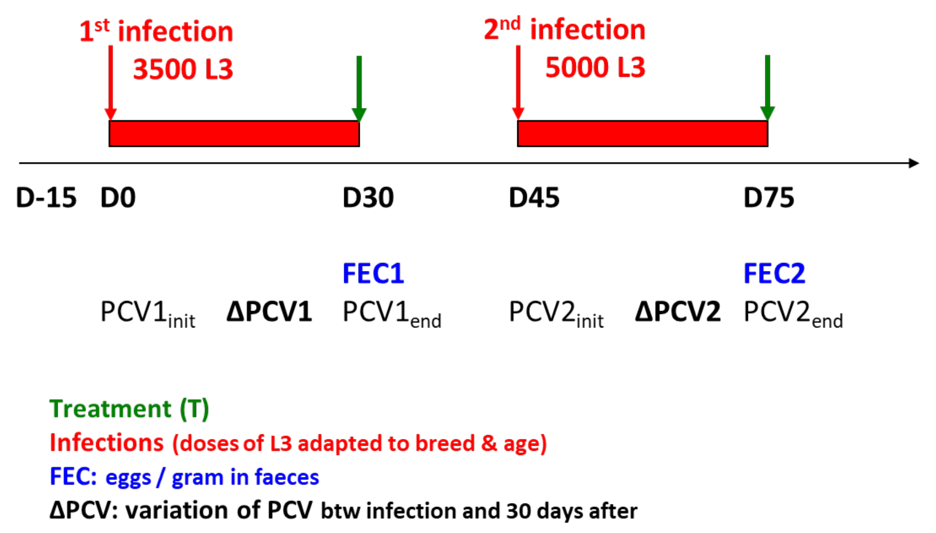

Figure 1. Protocol of experimental infestation (from Jacquiet et al, 2015).

vary depending on age of rams and how they respond to infection. In the case of the Pyrenean dairy sheep breeds (Red-Faced Manech – RFM and Basco-Béarnaise - BB), the protocol occurs when the rams are one-year-old, before their first use as AI rams. When the phenotyping protocol is applied, they have never known the pasture and are therefore naïve regarding parasites.

FEC are an indicator of the resistance to GIN, by assessing the capacity of the sheep to decrease the establishment, the development, the fecundity and the fitness of the worms. The variation of PCV between start and end of infection (ΔPCV) is an indicator of the resilience of the animal regarding the parasitic challenge. Besides, kinetics of PCV also allows to verify the fitness of the sheep after each infection and thus monitor the well-being of the animals.

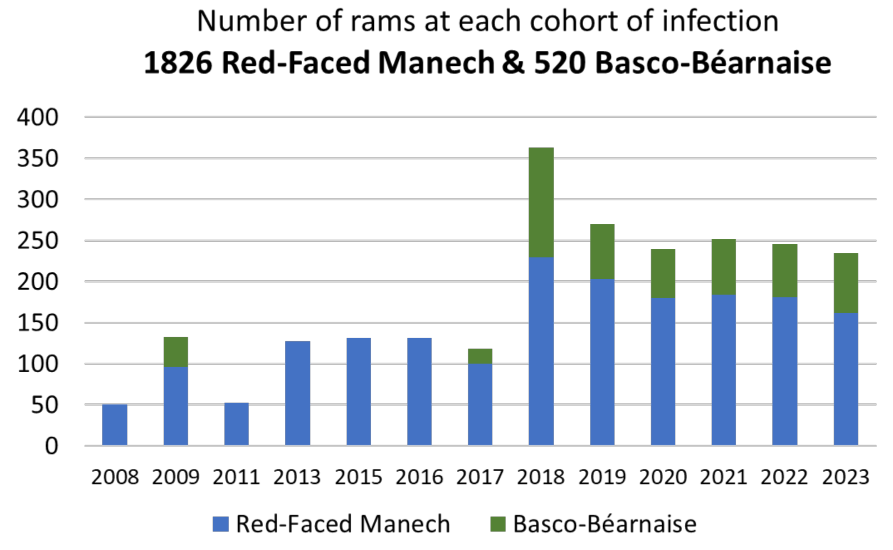

Figure 2. Number of rams submitted to the protocol of phenotyping over the years.

FEC is the commonly used criterion worldwide to measure host resistance to parasites. As it is time-consuming and costly to measure, targeting rams gathered in collective centres makes the selection process more cost-effective, money and time wise. The use of *Haemonchus contortus* is relevant for the following reasons: (i) it is a pathogenic and thermophile GIN; (ii) it is the most prevalent species in south-western France; (iii) most of the time, this species is concerned in case of resistance to drugs. In any case, literature has shown a very high genetic correlation ($\approx$ 1) between resistance to different species of GIN (Gruner *et al*, 2004a). Finally, a very high genetic correlation ($\approx$ 0,9) has been found between natural infections and experimental infections (Gruner *et al*, 2004b).

The first protocol of phenotyping in the Pyrenean breeds was implemented in 2008. Since then, new rams have been phenotyped almost every year. Finally, phenotypes are available for 1,826 RFM and 520 BB rams in 2024. This dataset was used for genetic analyses (Figure 2).

Genetic parameters and genetic evaluation of resistance to gastrointestinal nematodes and resilience traits

Computation of traits

The traits used in genetic analyses are the following:

- The square root of FEC at first and second infection (both infections being considered as a different trait): FEC1 and FEC2. Such a transformation resulted in more symmetrical distributions.
- The variation of PCV between start and end of infections (both infections being considered as a different trait): ΔPCV1 and ΔPCV2 (ΔPCVi=PCVi[init] – PCVi[end]).

Genetic parameters

Genetic parameters of resistance and resilience traits, as well as their standard errors, were estimated using the restricted maximum likelihood estimation with the VCE package. The model used is the following:

$$Y_{ijk} = \mu + P_i + A_j + R_k + e_{ijk}$$

Where Y_{ijk} is the dependent variable (FEC1, FEC2, ΔPCV1 and ΔPCV2), μ is the population mean, P_i is the fixed effect of protocol i (representing the contemporary group, one per year), A_j is the fixed effect of age j of the ram, R_k is the additive genetic random value of the ram k and e_{ijk} is the random residual effect. As the rams were submitted to only one protocol in their lifetime, there were no repetition and therefore no permanent environmental random effect.

The table 1 presents the genetic parameters obtained in Red-Faced Manech.

All heritabilities are significantly different from zero. FEC are moderately heritable, with a higher heritability of FEC at the second infection (0.35 vs 0.20). ΔPCV have a lower heritability (0.12 and 0.14). Genetic correlations across both infections are high (more for FEC than for ΔPCV), but without being the same trait. Genetic correlations are high between resistance and resilience, especially within infections: the most resistant animals are also the most resilient. These parameters show that selection is feasible. The parameters in BB (not shown here) follow the same pattern, yet with some differences (there is no higher heritability of FEC in infection 2 in BB). These results are consistent with those estimated previously (Aguerre *et al*, 2022).

Table 1. Genetic parameters and standard errors of estimation of Fecal Egg Count (FEC) and variation of packed cell volume (ΔPVC) in Red-Faced Manech at infection 1 and 2. Heritabilities are on the diagonal (bold characters), genetic correlations above the diagonal and phenotypic correlation below the diagonal.

	FEC1	FEC2	ΔPCV1	ΔPCV2
FEC1	**0.20 ± 0.04**	+0.82 ± 0.05	+0.96 ± 0.05	+0.47 ± 0.10
FEC2	+0.37	**0.35 ± 0.05**	+0.70 ± 0.07	+0.79 ± 0.10
ΔPCV1	+0.30	+0.14	**0.12 ± 0.03**	+0.40 ± 0.14
ΔPCV2	+0.15	+0.43	+0.06	**0.14 ± 0.01**

Genetic evaluation

The genetic evaluation is performed using the same model as for genetic parameters. Until 2022, the evaluation was polygenic. From 2023 onwards, we have run a genomic evaluation (all the rams phenotyped are also genotyped), enabling the inclusion of resistance and resilience to parasites in the genomic pre-selection step of young rams, simultaneously with other traits.

EBV's of elementary traits (FEC1, FEC2, ΔPCV1 and ΔPCV2) are provided to the breed organisation. In addition, composite indexes are also calculated and provided:

- -a FEC index combining both infections: FECindex = ¼ FEC1 + ¾ FEC2, weights of ¼ and ¾ reflecting the difference of heritability across infections.
- -a ΔPCV index combining both infections: ΔPCVindex = ½ (ΔPCV1 + ΔPCV2), weights of ½ reflecting the same heritability across infections.

-a so-called parasitism index combining resistance and resilience: PARASITISMindex = ¾ FECindex + ¼ ΔPCVindex. The weights were chosen by the breed organisation, with the intention of giving a significant weight to resilience, while keeping a higher (conservative) weight for resistance, arguing that FEC is the most universal indicator worldwide. However, which weights should be given to resistance and resilience is a key question. The answer could be updated in the future, according to new scientific results or following new decisions from the breeding organisation.

Defining a selection objective that accounts for resistance and resilience to parasites and building a selection criterium accordingly in the Pyrenean dairy sheep breeds

To have a quick glance at the background on the selection in the Pyrenean dairy sheep breeds, we must have in mind the following statement:

- the breeding programs of the RFM and BB breeds have been shifted towards genomic selection since 2017, with a reference population reaching 3,300 rams in RFM and 1,100 rams in BB. They are efficient programs, generating in a genetic gain (expressed in genetic standard deviation of the selection index) reaching 0.16 in RFM and 0.15 in BB (Astruc *et al*, 2022).
- the selection criteria include in both breeds fat and protein yield, fat and protein content, somatic cell count, udder morphology traits.
- high level of anthelmintics resistance in the area has made the breeders fully aware of the importance of increasing resistance/resilience to GIN. Yet there is a strong inclination to find the right compromise between currently selected traits and novel

parasite-related traits. Hence, the genetic correlations between all the traits were a key indicator to consider.

Genetic correlations between traits included in the current selection index and traits of the parasite index

Genetic correlations between traits as well as their standard errors, were estimated using the restricted maximum likelihood estimation with the VCE package. The model used is the bivariate model, without repetitions, the fixed effects being those used in the genetic evaluation (described above for FEC and ΔPCV, described in Aguerre *et al*, 2022 for currently selected traits). We present in the table 2 the genetic correlations between FEC1, FEC2, ΔPCV1, ΔPCV2 on the one hand, milk yield (MY), fat and protein content (FC and PC), somatic cell score (SCS), teat angle (TA), udder depth (UD) on the other hand.

Most correlations are close to zero. As the size of the dataset is quite small, half of the correlations are not significantly different from zero. Bearing in mind this limit and looking at the general patterns, whether for resistance or resilience traits, favourable correlations are somewhat balanced with unfavourable correlations. Aguerre *et al* (2022) found correlations slightly different, mainly due to a smaller dataset and the high standard error of estimation. We can state that the genetic correlations are globally low in RFM. Therefore, selecting for resistance and resilience to GIN challenges would not jeopardise the efficacy of the selection on other traits. The smaller size of the dataset in BB does not allow to give consistent results.

In a complementary approach, we calculated the Pearson correlation coefficient between current total merit index (TMI) and the parasitism index combining resistance and resilience, for 663 young rams born in late 2023 and genotyped for the genomic pre-selection. The correlation was at the very low level of 0.04, suggesting neither favourable nor unfavourable relationship, confirming what we observed on elementary traits.

Defining a new Total Merit Index

To define a new TMI including resistance and resilience to GIN, we built a composite index as follows:

TMInew = TMIcurrent + k * PARASITISM, with:

Table 2. Genetic correlations and standard errors of estimation in Red-Faced Manech between Fecal Egg Count (FEC) and variation of packed cell volume (ΔPVC) at infection 1 and 2, and 6 major traits included in the current total merit index.

	FEC1	FEC2	ΔPCV1	ΔPCV2
Milk yield	-0.20 ± 0.11 *	-0.03 ± 0.07	-0.06 ± 0.17	-0.07 ± 0.13
Fat content	0.12 ± 0.13	0.10 ± 0.09 *	0.06 ± 0.17	0.10 ± 0.13
Protein content	-0.26 ± 0.12 *	-0.19 ± 0.08 *	-0.21 ± 0.17 *	-0.02 ± 0.13
Somatic Cell Score	-0.17 ± 0.12 *	-0.12 ± 0.09 *	-0.48 ± 0.23 *	-0.09 ± 0.14
Teat angle	-0.17 ± 0.11 *	0.04 ± 0.08	-0.08 ± 0.19	-0.22 ± 0.12 *
Udder depth	0.12 ± 0.11 *	0.04 ± 0.08	0.41 ± 0.19 *	0.11 ± 0.13

Sign * spots correlations that are significantly different from zero. Correlations are coloured in green when favourable, in orange when unfavourable.

- TMIcurrent is the current TMI including dairy traits (60% in RFM; 65% in BB), SCS (20% in RFM; 17.5% in BB) and udder morphology (20% in RFM; 17.5% in BB)
- PARASITISM defined as above: PARASITISM = ¾ FECindex + ¼ ∆PCVindex

We made k vary from 0 to 1000 and we applied a selection of 25% on a set of young rams submitted to genomic preselection, based on TMInew. We then plot the evolution of TMIcurrent and PARASITISM of the selected rams against k. The results were discussed with the breeding organisation that choose the k value corresponding to their desired compromise between not losing too much on TMIcurrent while sufficiently improving PARASITISM. The re-ranking of the rams was also accounted for. The k chosen resulted in a loss of genetic progress on TMIcurrent of 5% in RFM and 8% in BB, which is weak. The gain on PARASITISM is equivalent to a decrease of 200 eggs per gram in the selected rams of each cohort. In terms of percentage in the TMInew, the weight of PARASITISM is 24% in RFM and 35% in BB.

This new TMI was used for the first time at the genomic preselection step of the young rams born in late 2023. These rams will be used at AI in 2025.

Conclusion and perspectives regarding ICAR guidelines

Breeding for resistance to parasites became necessary in the Western Pyrenean breeds with regards to the situation on anthelmintic resistance in the area. This is in line with the strong willingness of the breeding organisation to select for resilience and generate animals more adapted to agroecological transition (towards less chemical treatments) and to global warming (*Haemonchus contortus* is a thermophile species). Yet the selection will be effective in the mid to long term. For this reason, management of GIN should integrate a combination of solutions. Besides genetic selection to increase resistance of sheep, it is advocated that treatment should be targeted and selective, grazing should be managed to limit the sources of contamination. Beyond the case of Pyrenean breeds, the Lacaune breed, which has also been confronted to anthelmintic resistance these last years, will start phenotyping resistance and resilience to GIN in late 2024, allowing to include these traits in the selection objective within 4 years.

The SMARTER project (Horizon 2020 research and innovation program No. 772787) produced in 2023 a deliverable on recommendations to phenotype resilience. It included resistance/resilience to parasites, with the different ways of phenotyping (traits, proxies, experimental vs natural infection). This deliverable was intended to be translated into ICAR guidelines. To include these recommendations into a new section of ICAR guidelines constitutes a major objective of the Sheep-Goat-Camelid WG.

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