

## **Response to selection on mastitis resistance and body reserve mobilization in Holstein and Normande breeds**

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### **Summary**

A divergent selection experiment on mastitis resistance of Holstein and Normande cows was carried out at Le Pin, the INRA experimental farm. Two lines, one resistant to mastitis and one control, were produced by using bulls with contrasted breeding values for a combination of somatic cell score (SCS) and clinical mastitis (CM). In Holstein, the same population was also split into two groups diverging for body condition score (BCS) and obtained by selecting the sires on their BCS breeding values. This paper presents the first results on the observed differences between lines based on data of 268 cows. Overall differences in SCS, CM, infectious status (assessed by bacteriological PCR tests) and BCS were in agreement with genomic predictions. Within-breed differences were similar, but sometimes not significant for udder health traits. These cows support extensive phenotyping for explanatory traits to explain the biological pathways involved in these differences.

*Keywords: bovine, mastitis resistance, body condition, breeding, divergent selection*

### **Introduction**

Mastitis is a major issue in dairy farms with an average incidence of 40% affected cows each year in France (Fourichon *et al.*, 2001). It has negative health and economic impacts on milk production, fertility and welfare.

Selection for milk production has strongly increased body reserve mobilization in Holstein. Mobilization reflects the post-partum adaptation when feed intake does not cover the needs for milk production. However, excessive mobilization increases the risk of impaired health and fertility (Collard *et al.*, 2000). Therefore, improving the capacity to rapidly increase feed intake post-partum would stabilize energy balance earlier and would improve the cow's sustainability.

Therefore, both mastitis resistance and body mobilization are important traits for milk production sustainability, but they have deteriorated in response to selection on milk production. In addition, they are difficult to select: (i) clinical mastitis is essentially environmental and its heritability is low, in spite of a large genetic variability; (ii) we lack indicators of the dynamics of post-partum mobilization available on a large scale for selection.

Nevertheless, different tools are available for selection purposes. Somatic Cell Scores (SCS) are used worldwide. A genomic udder health index, combining SCS and clinical mastitis (CM) information, is available in France since 2012 (Govignon-Gion *et al.*, 2016). Body condition scores (BCS) are recorded once during lactation at the same time as type traits and a genomic estimated breeding value (EBV) is also available.

Little is known however, on the biological mechanisms involved in the genetic differences in mastitis resistance and body mobilization capacity. To address these questions, as well as to demonstrate efficiency of genomic selection, a divergent genetic selection experiment of Holstein and Normande cows on mastitis resistance has been carried out since 2012 at Le Pin, the INRA experimental farm. The divergent lines were selected on a combination of SCS and CM breeding values of their sires. Additionally, the same Holstein cows were also under a divergent selection for BCS. The aim of this paper is to describe the observed differences between the divergent lines, as a response to selection.

## Material and methods

The genetic divergence in mastitis resistance was created in each breed by procreating two cow lines from sires genetically divergent for the SCS and CM EBV (expressed in genetic standard deviation, with a 0 mean): **RES** (for resistant) sires with  $EBV \geq 1$  to improve mastitis resistance and **CTL** (for “control”) sires with negative EBV (from -1.5 to -0.5). Bulls with extreme negative breeding values were excluded because we were mainly interested in resistant animals. The expected difference between lines was 1.5 genetic standard deviations, corresponding to 0.75 point of SCS and about 7% CM incidence. The SCS score being defined in the conventional way as  $SCS = \log_2 (CCS/100\ 000) + 3$ , this SCS difference represents a 40% lower cell count in RES line relative to CTL. The average genetic merit of the two groups of sires was the same for milk yield. In Holstein breed, selection was applied also on BCS with a similar strategy. Holstein bulls were chosen with  $BCS\ EBV \geq 1$  (**BCS+**) or  $\leq -1$  (**BCS-**). Therefore four groups of sires were defined (BCS+/RES, BCS+/CTL, BCS-/RES, and BCS-/CTL). The expected genetic difference between BCS+ and BCS- cow lines was 1 BCS genetic standard deviation, corresponding to 0.5 point BCS, on a 1-5 scale.

In each breed, twenty unselected donor cows were mated with twenty bulls (in Normande) or with ten bulls (in Holstein) of each group. Their embryos were collected and transferred into recipient cows. At the following generation, daughters were mated with bulls of the same group as their sire so that divergence increased in the second generation.

All cows were raised together in one single experimental herd with stable environmental conditions. Milk production and body weight were recorded daily, milk composition (fat, protein, lactose, somatic cell count) twice a week, MIR spectra for comprehensive milk composition (fatty acids, proteins, minerals) weekly. BCS score was recorded monthly by two trained technicians on a 5-point scale with 0.25-point step, whereas the complete conformation was scored once at 90 days in milk. Blood metabolites and progesterone were assayed three times per lactation for energetic status and once a week for post-partum resumption of ovarian cyclicity. In first lactation, bacteriological analyses by RT-PCR (Pathoproof™ test) were performed at 1, 4 and 7 months in milk in a 4-quarter mixed sample to determine the udder infectious status. Each CM event was accurately described and a bacteriological analysis was performed with a sample from the affected quarter to identify the pathogen(s) involved. For each pathogen, the sample was defined as positive if the cycle threshold (CT) was  $< 37$  for major pathogens (*S. Aureus*, *S. Dysgalactiae*, *S. Agalactiae*, *S. Uberis*, *E. Coli*, *Klebsiella*, *Serratia*, *Trueperalla*) and  $< 32$  for minor pathogens (*S. spp*, *Enterococcus*, *C. bovis*). It was defined as contaminated if 3 or more pathogens were detected (Koskinen *et al.*, 2010).

Cows were genotyped with the Illumina 50k or EuroG10k customized chip so that they received individual genomic EBV from the French national evaluation system for about 40 traits including SCS, CM, and BCS. Therefore, two classifications of cows were defined: (i)

based on their sire line; (ii) on their own genomic EBV on mastitis resistance (**gRES** if  $\geq 0.5$  and **gCTL** if  $\leq -0.5$ ) and body condition score (**gBCS+** if  $\geq 0.5$  and **gBCS-** if  $\leq -0.5$ ). Some cows not satisfying these EBV conditions were kept in the sire line comparison analyses but were excluded from the genomic group analyses.

The present study included 268 cows (161 Holstein and 107 Normande) with a complete first lactation in the first four years of the experiment. Out of them, 113 and 39 had a second and third lactation, respectively. In total, 236 of them had lactation bacteriological data. 195 CM events occurred in first lactation before 150 days in milk and at least 7 days after a previous CM case, were considered for CM analysis. Similarly, 100 CM events were retained in second lactation.

Statistical analyses were performed with the GLM procedure of SAS software (version 9.4, Copyright© 2002-2012 by SAS Institute, Cary NC). All comparisons were obtained with a model also including the fixed effects of year, month of calving and age at calving.

## Results

### *Resistance to mastitis*

As expected, selection did not affect 305-day milk yield (table 1). However, it significantly affected protein content in Holstein (+0.4 g/kg,  $p < 0.0001$ ) and fat content in both breeds, with higher values for the RES cows (+1.2 g/kg in Holstein and +0.6 g/kg in Normande,  $p < 0.0001$ ).

RES cows showed a significantly lower SCS mean than CTL cows in both breeds (-0.6 in Holstein and -0.7 in Normande,  $p < 0.0001$ ; figure 1). An even greater effect was observed when considering the female genomic groups with a 1.2 and 1.4 point lower SCS in gRES Holstein and Normande cows, respectively.

In first lactation, 34% cows showed at least one CM in RES line and 39% in CTL line. However selection had opposite effect in the two breeds: whereas Holstein cows displayed the expected response with 32 % of RES cows affected vs 43% in CTL line ( $p = 0.049$ ), RES Normande cows were more affected than CTL ones (42% vs 27%,  $p = 0.013$ ; table 1). The same trend was observed in genomic groups. In second lactation, both breeds showed the expected effect with less cows affected in RES lines.

Number of CM events for cows with at least one CM followed the same pattern: it was lower in the RES line with 0.4 and 0.5 fewer CM event per cow in first and second lactations, respectively. At the breed level, this result was observed only in Holstein in first lactation and in both breeds in second lactation, even though it was not significant in Normande (table 1). It was also lower for gRES cows in first lactation (-0.8 and -0.5 in Holstein and Normande cows, respectively) and for gRES Holstein in second lactation (-0.3).

Proportion of positive bacteriological samples during lactation was significantly lower in Normande RES line with 27% vs 42% (table 1), as well as in gRES cows (-26%,  $p < 0.0001$ ). The same trend, but not significant, was observed in RES Holstein cows (-5%). A greater proportion of samples were positive at 1 month after calving (34.6%) than at 7 months (19.5%). Whatever the line, cows with higher SCC at sampling had more positive samples (15% when  $SCC < 250,000$  cells vs 48% when  $SCC \geq 700,000$ ,  $p < 0.0001$ ). The most frequent infections were due to *S. species* (46%) and *S. uberis* (40%). All samples from CM events were positive to at least one pathogen and mainly to *S. uberis* (86%).

### *Body reserve mobilization*

Body condition selection did not affect milk yield measured as 305-day production (table 2) but significantly affected protein and fat content, with higher values for BCS+ line (+1.1 and +2.1 g/kg respectively,  $p < 0.0001$ ).

Selection had a significant effect on the overall lactation BCS mean with +0.3 point for BCS+ cows (figure 2). An even greater response was observed in the female genomic prediction groups with a difference of +0.5 point for gBCS+ cows. No difference was found between lines and groups for aggregate gain and loss of condition (table 2). Birth and weaning weights were lower in BCS+ veals (-2 kg,  $p < 0.0001$ ) but weights at calving, during lactation and at dry-off were significantly higher (+25, +30, and +15 kg, respectively; table 2).

An effect of BCS line on udder health traits was observed, with less mastitis for BCS+ (1.7 vs 2.1) and less positive samples in lactation (15 vs 26%,  $p = 0.006$ ).

## Discussion

The present study aimed at measuring the efficiency and correlated responses of selection for udder health and body reserve mobilization in an experimental herd. Over the four years and the two breeds, differences between sire lines reached 0.7 for SCS. This value is very close to the 0.75 SCS point predicted by the breeding values. Similarly, the overall observed divergence on CM (2 breeds, 2 lactations) was close to its expectation (5% affected cows), in spite of the limited size of the population studied. In Holstein, the difference in BCS between sire lines was in the expected direction and reached 0.3 BCS point, *i.e.* only a fraction of the 0.5 value predicted by the divergent selection. When partitioning the design per breed and parity, results were more variable, primarily because of the limited size of the different groups. In first lactation, a significant effect was observed on udder health in Holstein with benefit to the resistant line but the opposite effect was observed in Normande. These results could be explained by the low heritability of CM (1.8%) compared to SCS (15%) and, therefore, by higher random variations. In addition, compared with Holstein breed or with the other traits, EBV reliability was lowest in Normande for CM. Nevertheless, in second lactation the observed difference was in the expected direction in both breeds.

These results are still preliminary. Other phenotypes remain to be explored (MIR spectra, blood metabolites, conformation ...) and further investigations will be carried out (molecular characterization, association studies ...) to understand differentiation between lines, especially to understand why cows are genetically more resistant to infection than others or more prone to mobilize reserves. On-going projects intend to explore innate and adaptive immune differences between lines and genetic association between mastitis susceptibility and energy metabolism, as pointed out in this study and revealed in sheep (Bouvier-Muller *et al*, 2016).

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Table 1. Mean 305d production, clinical mastitis (CM) and bacteriological responses to selection for mastitis resistance in the Holstein and Normande divergent lines<sup>1</sup>

	Holstein			Normande		
	RES	CTL	<i>P</i> -value <sup>2</sup>	RES	CTL	<i>P</i> -value <sup>2</sup>
<i>No of first and second lactations</i>	170	79		85	86	
Milk (kg)	7851	7587	NS	5152	5095	NS
Protein content (g/kg)	32.0	31.6	***	34.5	34.6	NS
Fat content (g/kg)	39.5	38.3	***	46.7	46.1	***
<i>No of first lactation CM</i>	54	69		42	30	
% cows with CM	31.8	42.7	*	41.9	26.5	*
Mean CM/cow	1.6	2.2	*	1.5	1.4	NS
% positive lactation sample	18.6	23.9	NS	27.4	41.7	**
<i>No of second lactation CM</i>	32	34		12	22	
% cows with CM	27.3	35.4	NS	19.6	37.6	**
Mean CM/cow	1.4	2.1	*	1.3	1.5	NS

<sup>1</sup> RES = resistant line, CTL = control line

<sup>2</sup> NS : non-significant, \* : p<0.05, \*\* : p<0.01, \*\*\* : p<0.0001

Figure 1. Somatic cell score (SCS) means over lactation in resistant (RES) and control (CTL) lines and in gRES and gCTL genomic groups for udder health in Holstein and Normande breeds

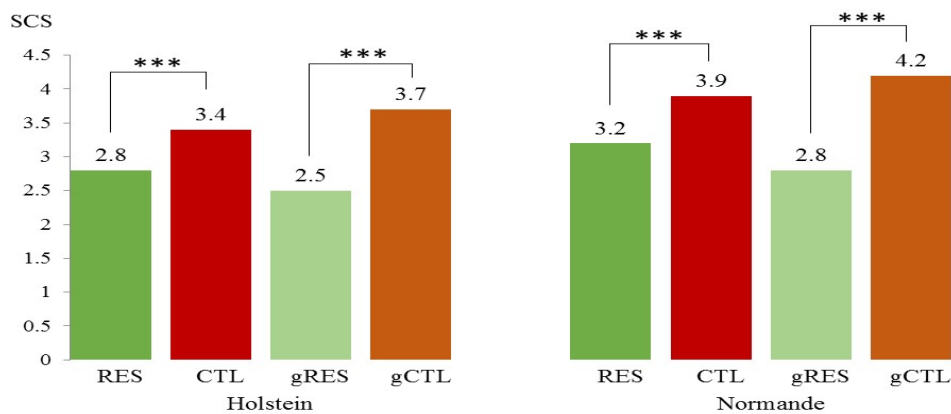


Table 2: Mean 305d production and body condition responses to selection for body reserve mobilization in first and second lactation Holstein cows

Line <sup>2</sup>	n	Milk (kg)	Protein content (g/kg)	Fat content (g/kg)	BCS <sup>1</sup> (point)		Body Weight (kg)				
					Gain	Loss	Birth	Weaning	Calving	Lactation	Dry off
BCS+	73	7884	32.4	40.0	1.3	-1.3	39.8	108	584	603	644
BCS-	76	7802	31.3	37.9	1.2	-1.2	42.3	110	559	573	629
<i>P</i> -value <sup>3</sup>		NS	***	***	NS	NS	***	***	***	***	***

<sup>1</sup> BCS: body condition score

<sup>2</sup> BCS+ = positive line, BCS- = negative line  
<sup>3</sup> NS : non-significant, \* : p<0.05, \*\* : p<0.01, \*\*\* : p<0.0001

Figure 3. Body condition score (BCS) means over lactation in positive (BCS+) and negative (BCS-) divergent lines for body reserve mobilization and in genomic groups in Holstein breed

