

# Meta-Analysis for Heat Tolerance Traits in Holstein in France, the Netherlands and Spain

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## Abstract

In the context of climate change, livestock production systems face the challenge of ensuring that, although more and more exposed to heat-stress conditions, animals will be able to remain healthy while maintaining satisfactory production, responding to consumer's demand. As part of the European project RUMIGEN, France (INRAE, Idele), Spain (INIA, IRIAF), and the Netherlands (WUR) studied the response of different dairy traits to heat-stress conditions for Holstein cattle breed. Production (milk, fat and protein yields) and udder health (somatic cell score) traits were investigated under different meteorological conditions, represented by the temperature humidity index (THI) averaged over three days on the day of recording and the two days before. A THI=50 was considered as neutral condition (i.e. no heat/cold-stress) and used as reference for level comparisons. Specific rates of changes in production/health traits at a given THI were measured as the slopes (first derivative) of the estimated reaction-norm curves for each trait. Genetic evaluations were performed by each country using test-day records of cows in their first lactation between 2010 and 2020, to estimate both levels and slopes for each trait. Estimated breeding values (EBVs) and reliabilities were obtained for sires with at least 20 daughters with test-day records. A meta-analysis was performed to estimate the genetic correlations between the three countries, using the Multiple Across Country Evaluation approach. For each country, de-regressed proofs (DRPs) and effective record contributions (ERCs) were computed using a single trait model from the EBVs, reliabilities, and variance components estimated at a national level for specific THI values. The estimated genetic correlations obtained with slopes were weak and not credible, which could be explained by the low heritability of the slopes and by the small proportion of performances recorded under heat-stress conditions. The DRPs on slopes, as they were calculated, were not able to capture the genetic (co)variability associated to these traits. Conversely, estimated genetic correlations for all level traits were high (between 0.81 and 0.97) even if they were slightly lower than under thermo-neutral conditions. This shows a very good consistency of the three national genetic evaluations under heat-stress conditions. In conclusion, valuable predictions under heat-stress conditions could be obtained through international evaluations, that would result in Northern countries benefiting from the information that already exists in the Southern countries. However, the approach for low heritable traits such as slopes should be improved if there are not enough data at high THI.

**Key words:** heat tolerance, dairy cattle, reaction norm model, meta-analysis

## Introduction

In the context of climate change, cattle will be exposed to more frequent and more intense heat waves, inducing acute and chronic heat-stress. Their welfare, health and production will be negatively affected by this stress (West,

2003, Becker et al., 2020). Genetic selection could be a useful tool to improve heat tolerance and help dairy cattle facing future weather conditions (Carabaño et al., 2019).

As part of the European Horizon 2020 project RUMIGEN, the impact of heat-stress conditions on performances and the genetic

variability for heat tolerance in dairy cattle were studied at the national level in France, Spain and the Netherlands.

The aim of the meta-analysis was to estimate genetic correlations between countries for the Holstein breed for different traits related to heat tolerance. These correlations will be helpful to determine the potential interest of international genetic evaluations for heat tolerance, in a context where phenotypic data recorded under heat-stress conditions are more widely accessible in some countries.

## Material and methods

### *Phenotypes*

Test-day records for milk yield (MY), fat yield (FY), protein yield (PY), and somatic cell score (SCS; defined as  $SCS = 3 + \log_2(SCC/100,000)$ , with SCC being somatic cell counts in cells/ml) of 5,753,268 French, 1,016,403 Spanish and 474,273 Dutch Holstein cows in their first lactation between 2010 and 2020, were extracted from the respective national genetic evaluation databases (BDNZ, France; CONAFE, Spain; and CRV, the Netherlands).

For each trait, different traits indicators of heat tolerance were defined as levels of production at 150 days-in-milk (DIM) under thermo-neutral and heat-stress conditions, and slopes of production decline under heat-stress conditions. In accordance with the results of previous analyses performed at the population level (Mattalia et al., 2022), thermo-neutral conditions were defined as being equal to a THI of 50 for the three countries, while heat-stress conditions were defined specifically for each country (THI equal to 65, 68, and 77, for France, the Netherlands, and Spain respectively). The slopes were defined as the response curve to increasing heat loads of each trait at the heat-stress THIs.

### *Weather data*

Weather data were provided by the national meteorological agencies (Météo-France for

France, the National Meteorological Agency (AEMET) for Spain, and the Koninklijk Nederlands Meteorologisch Instituut (KNMI) website for the Netherlands. Meteorological records were available from 1,993 Spanish and 34 Dutch weather stations distributed throughout each national territory. In France, grid weather data included various meteorological variables for each of the 9,892 8 x 8 km squares covering the whole country (Safran database). Each herd was associated to the closest weather station or square through its ZIP code.

Daily temperature-humidity indices were computed using the formula proposed by the National Research Council (1971):

$$THI = (1.8 * T + 32) - (0.55 - 0.0055 * RH) * (1.8 * T - 26)$$

where T is the average daily temperature (in degrees Celsius) and RH is the average daily relative humidity (in percent).

The heat load measure was defined as the THI averaged over three days, including two days before test day and the test day.

### *Model*

A genetic evaluation was performed to estimate breeding values (EBVs) and associated reliabilities at the national level using the following random regression model:

$$y = Xb + Za + Wp + e$$

where **b** is fixed effects, **a** and **p** contain additive genetic and permanent environmental random regression coefficients on THI and DIM for each animal in the pedigree and each cow with records, respectively. **Z** and **W** are matrices containing the Legendre polynomial covariates appropriate for each THI and DIM corresponding to a record. Cubic and quadratic Legendre polynomials were fit for THI and DIM, respectively, providing five EBVs for each animal in the pedigree. The fixed effects were as follows:

- France: herd-test-day of record, DIM, gestation stage and age at calving;
- Spain: THI, herd-year-season of record, age at calving, and DIM.
- The Netherlands: herd-test-day, DIM, gestation stage, age at calving-year of calving-season of calving, age at calving-year of calving-season of calving-lactation stage;

Then, the DRPs and ERCs were derived from the national EBVs and reliabilities, using a deregression approach (VanRaden et al., 2014) implemented in an INRAE program. This approach assumes that EBVs were obtained with a single-trait model. For the levels, the heritabilities used for the deregression were estimated using the afore mentioned random regression model. For the slopes, which are obtained from the estimated random regression coefficients, no proper estimates of heritability can be obtained and therefore, heritabilities were assumed to be equal to 0.10 for MY, FY and PY, and 0.03 for SCS in order to obtain DRPs and ERCs, assuming a heritability similar to that of functional traits relative to heat tolerance (e.g. heritability of rectal temperatures).

For each country and for each trait, the analyses included DRPs and ERCs of all Holstein bulls with at least 20 daughters with performances and a reliability of at least 0.25. However, no selection was performed regarding the number of daughters with performances in heat-stress conditions. For milk production levels, 7,932 French sires, 3,624 Spanish sires and 2,281 Dutch sires met these criteria, with a total of 328 common sires to the three countries. For SCS levels, 7,932 French sires, 3,607 Spanish sires and 2,257 Dutch sires met the requirements, with a total of 325 common sires. The pedigree of each bull was traced back for 3 generations.

Genetic correlations between countries were estimated using the Multiple Across Country Evaluation (MACE) approach (Schaeffer, 1994). In this study, it consisted in a pedigree-based animal model, considering each country as a separate trait. The following multiple trait model was implemented:

$$y = Xc + Za + e$$

where  $y$  is the vector of DRPs from each country,  $c$  is the vector of country of evaluation fixed effects,  $a$  is the vector of random additive genetic effects in all participating countries, and  $e$  is the vector of residual effects. It is assumed that  $\text{Var}(a) = G_o \otimes A$  and  $\text{Var}(e) = R_o \otimes D$ , where  $G_o$  and  $R_o$  are the genetic and residual matrices of (co)variances between countries,  $A$  is the pedigree-based relationship matrix and  $D$  is a diagonal matrix with diagonal elements corresponding to the inverse of ERCs. The matrices  $X$  and  $Z$  are incidence matrices that relate phenotypes to the corresponding effects.

Variance components and solutions for a random regression model were estimated using the software WOMBAT (Meyer, 2007) for French data and using the BLUPF90 software suite (Miszta et al., 2014) for the Dutch and the Spanish ones. Reliabilities for levels and slopes were obtained with MiXBLUP (ten Napel et al., 2021) in all countries. The meta-analysis was performed with the software BLUPF90.

## Results & Discussion

For all level traits, both in thermo-neutral and under heat-stress conditions, heritabilities were mostly consistent with national estimates, although lower for Spain and the Netherlands. The heritability of SCS for France was surprisingly high (0.34). In thermo-neutral conditions, the genetic correlations between the three countries were high for all level traits, with values ranging from 0.89 and 0.97 (Table 1). These estimates for level traits were consistent with those estimated by Interbull in the MACE evaluations for Holstein breed (April 2023 MACE evaluation, [www.interbull.org/ib/maceev\\_archive](http://www.interbull.org/ib/maceev_archive)). Under heat-stress conditions, a slight decrease in estimated genetic correlations was observed, although they remained high with values between 0.81 and 0.97, and in agreement with the estimates in thermo-neutral conditions (Table 2). Therefore, we consider the approach used in this meta-analysis as a valid approach

for international genetic evaluations for heat tolerance traits.

For all milk production and SCS level traits, the genetic correlations estimated between the three countries were nonetheless high (above 0.8). These results suggest that countries with limited data under heat-stress conditions could benefit from the information available in Southern countries through international evaluations.

**Table 1.** Heritabilities (diagonal) and genetic correlations (off-diagonal) between countries for level traits under thermo-neutral conditions for milk production and SCS. Standard deviations are within brackets.

Trait	Country	FRA	SPA	NLD
MY	FRA	0.18 (<0.01)	0.96 (0.01)	0.94 (0.02)
	SPA		0.19 (<0.01)	0.92 (0.02)
	NLD			0.25 (0.02)
FY	FRA	0.25 (<0.01)	0.97 (0.01)	0.90 (0.02)
	SPA		0.13 (<0.01)	0.89 (0.02)
	NLD			0.21 (0.01)
PY	FRA	0.15 (<0.01)	0.96 (0.01)	0.89 (0.02)
	SPA		0.12 (<0.01)	0.90 (0.02)
	NLD			0.20 (0.01)
SCS	FRA	0.34 (0.01)	0.95 (0.02)	0.88 (0.02)
	SPA		0.08 (<0.01)	0.89 (0.03)
	NLD			0.15 (<0.01)

MY: milk yield; FY: fat yield; PY: protein yield; SCS: somatic cell score.

FRA: France; SPA: Spain; NLD: the Netherlands

Several hypotheses can be drawn regarding the drop of the estimated genetic correlations from thermo-neutral conditions to heat-stress conditions. First, from a physiological point of view, a hypothesis to explain this drop is that the impact of heat-stress on gene expression differ according to the environment. The differences between farming conditions in the three countries involved in this study support this hypothesis. A second hypothesis is that this drop is due to the lower accuracy of the national EBVs estimated under heat-stress conditions. A large part of the performances used in the estimation of variance components and EBVs were recorded under thermo-neutral conditions, leading to lower accuracies for the EBVs under heat-stress conditions. These two hypotheses

are non-exclusive. However, the latter seems the most likely since the genetic correlations within country were close to 1 for all level traits.

**Table 2.** Heritabilities (diagonal) and genetic correlations (off-diagonal) between countries for level traits under heat-stress conditions for milk production and SCS. Standard deviations are within brackets.

Trait	Country	FRA	SPA	NLD
MY	FRA	0.18 (<0.01)	0.92 (0.01)	0.89 (0.02)
	SPA		0.25 (0.01)	0.86 (0.02)
	NLD			0.25 (0.02)
FY	FRA	0.25 (<0.01)	0.97 (0.02)	0.87 (0.03)
	SPA		0.13 (<0.01)	0.85 (0.04)
	NLD			0.20 (0.02)
PY	FRA	0.17 (<0.01)	0.89 (0.01)	0.81 (0.02)
	SPA		0.17 (<0.01)	0.83 (0.02)
	NLD			0.21 (0.01)
SCS	FRA	0.30 (<0.01)	0.96 (0.02)	0.88 (0.02)
	SPA		0.09 (<0.01)	0.88 (0.03)
	NLD			0.16 (<0.01)

MY: milk yield; FY: fat yield; PY: protein yield; SCS: somatic cell score.

FRA: France; SPA: Spain; NLD: the Netherlands

For slopes, genetic parameters and genetic correlations between countries could not be estimated accurately. The estimation of heritabilities for milk production and SCS slopes resulted in unreliable results, with values ranging from 0.04 for PY in the Netherlands to 0.93 for FY in France, and for SCS from 0.003 in the Netherlands to 0.90 in Spain. The estimated genetic correlations between countries were much lower than expected and, in some cases, even negative, with values between 0.04 and 0.53 for MY, -0.17 and 0.09 for FY, 0.03 and 0.30 for PY, and between -0.87 and 0.24 for SCS.

Additional analyses were performed to evaluate the impact of the THI chosen on the genetic parameters. Large variations were found in estimated variances at different THI within countries. For example, residual variance for MY expressed in (kg/THI)<sup>2</sup> increased from 0.9 at THI 60 to 6.9 at THI 70 with French data, and in the Netherlands from 4.9 at THI 68 to 8.6 at THI 70.

Several reasons could explain these difficulties in properly estimating the genetic variability of slope traits. The slopes were defined as the derivative of the Legendre polynomial curve at a given THI. Therefore, they might be sensitive to errors in the estimation of the rate of decline under heat-stress conditions. In addition, the heritabilities of the slopes could not be estimated. Their values were guessed but could be different from the true values. Furthermore, two main issues for the deregression may have ensued from the structure of our datasets. First, the hypothesis of a single-trait model could not be satisfied. Few sires had many daughters with at least one record measured under heat-stress conditions because of the low frequency of days above heat-stress and because of the infrequent (monthly) milk recording. Therefore, the estimations of the slopes relied on phenotypes recorded at lower THI and the EBVs of most sires were indirectly predicted from the levels at lower THI. Second, EBVs for slopes were associated with low accuracies, which may have adversely affected the quality of the deregression. All bulls included in the meta-analysis were required to have a minimum reliability of 25% and at least 20 daughters with performances, but these performances were not necessarily recorded under heat-stress, but these requirements were probably not sufficient for the slopes. Bohmanova et al., (2008) encountered similar difficulties in their comparison of heat-stress EBVs of sires evaluated in the North-East and South-East of the USA. EBVs were similar in both regions only for sires having many phenotyped daughters, with correlations between EBVs increasing from 0.58 for sires with at least 100 daughters to 0.81 for sires with at least 700 daughters.

## Conclusion

In conclusion, high genetic correlations were obtained for the traits based on levels, and thus even at high THI. These results show the interest in developing international

collaborations to evaluate heat tolerance in Holstein. Countries of Northern part of Europe, where few performances have been recorded under heat-stress conditions so far, could benefit from the information available in Southern countries and have access to reliable predictions under heat-stress conditions through international genetic evaluations.

However, the meta-analysis showed some limits for the slopes of decay. We were not able to capture the genetic (co)variability associated to these traits with our approach. Other approaches should be investigated to better measure the decline in performances due to heat-stress.

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Test-day records of French, Spanish and Dutch Holstein cows were extracted from the respective genetic evaluation data bases (BDNZ, France; CONAFE, Spain; and CRV, the Netherlands).

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