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Deliverable 3.3

Report on the effect of heat stress of pregnant dam on the performances of her progeny

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1. Summary

The objective of Deliverable 3.3 within the RUMIGEN project is to investigate **how maternal heat stress impacts the performance of dairy cows' offspring**, focusing specifically on production and fertility traits. Institut de l'Elevage (Idele, France), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE, France), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC, Spain), Instituto Regional de Investigacion y Desarrollo Agrolimentario y Forestal de Castilla-La Mancha (IRIAF, Spain) collaborated to explore whether the effects of heat stress experienced by the dam during gestation persist across generations. This work supports RUMIGEN's aim to create breeding strategies resilient to climate change, contributing to the development of climate-adaptive livestock.

This deliverable integrates livestock performance data with meteorological data to analyse maternal heat stress effects. The approaches relied on different thermal loads at various stages of gestation and the modelling of maternal and inter-transgenerational effects. The analysis focused on 305-day milk yield (MY305), fat yield (FY305), and protein yield (PY305) and conception rate (CR) in Holstein and Montbeliarde cows, assessing the influence of temperature throughout gestation on production and fertility. They showed the following results:

- Up to now, we observed only slight impacts of maternal heat stress on progeny MY305.

- A negative effect of heat stress suffered by dams in early gestation on progeny production

- There are clear evidences of inter-transgenerational effects in the Spanish Holstein population. These effects are more important for fat and protein than for milk.

- For third-generation (G3) cows, the effect of heat stress experienced by dams, granddams, and great-granddams was noticeable, especially in first lactation using month of birth as a proxy of thermal load. Daughters of females born during heat-stressed periods in fall (September-October) exhibited lower milk production. However, when temperature was used, the impact of heat stress in different periods of the gestation correlated with decreased in protein and fat production in G3 cows. The impact increased with lactation number of G3 cows.

- The addition of a maternal environmental component to the production model for Spanish cows revealed the existence of an additional source of variation which is partially confounded with the additive genetic component. This finding suggests that maternal stress, potentially magnified with each generation, may influence progeny performance, especially in successive lactations.

2. Introduction

Epigenetics effects on dairy cows have been recurrently investigated in association with different environmental stressor such as metabolic (Gonzalez-Recio et al., 2012) and heat stress (Laporta et al., 2020) and as elements that trigger and induce changes in gene expression and therefore contributing to phenotypic variation. Considering that dairy cows spend most of their lives lactating except for a period of 6-8 weeks of dry period before calving for each lactation, they are exposed to stressors since the periconception and most part of the gestation. Except for primiparous cows, gestation occurs during lactation, which exposes the embryos to a maternal intrauterine environment characterized by a metabolic stress that could have a long-life effect on the performance of those embryos as well as the performance of the future generations (Ouellet et al., 2021). With climate change, **heat stress has imposed an additional component to the maternal intrauterine environment** caused by the environmental thermal stress under which cows are producing.

Maternal intrauterine environment has two major mechanisms of action (Laporta et al., 2020; Kathib et al., 2024). On one hand, maternal environment may have an *in utero* detrimental effect on the embryo at different stages and/or the fetus that may damage its normal development and compromise its future performance, immune-response and fertility during its productive life (Laporta et al., 2020; Ouellet et al., 2021). On the other hand, we speak of epigenetic effects if there is an epigenetic change induced in the embryo and fetus by the maternal environment (Ouellet et al., 2021). In addition, if those epigenetic changes can affect not only the embryo's development but also the embryo's primordial germ cells that constitute the first wave of epigenetic changes. **Epigenetic changes** may or may not be transmitted to future generations, depending on whether they affect germlines, particularly in females (Fitz-James and Cavalli, 2022) and the existence of reprograming that occurs in mammalian species (Braz et al., 2022; Kahtib et al., 2024). **If those changes are transmitted, we speak of inter or transgenerational genetic effects**.

Currently there are a lot of discussion about the differences between inter and trans. Intergenerational epigenetic changes refer to epigenetic changes that pass on its immediate generation and induce changes that affect future performances of the next generation germlines or embryos while the environmental agent that induces changes persist across generations (Braz et al., 2022; Fitz-James and Cavalli, 2022; Kahtib et al., 2024). We speak of transgenerational epigenetic when changes persist across generations when there is no exposure to the environmental agent that trigger those changes (Braz et al., 2022; Kahtib et al., 2024). Thus, to disentangle inter and transgenerational effects, in the females' lines, we need a third generation carrying out the change but that is beyond the point where the environmental stimulus still exists.

Separating maternal effects on the embryo/fetus, from an epigenetic induced change by maternal environmental constraint as well as separating inter from transgenerational effects is a real challenge for quantitative geneticist without having epigenomic information.

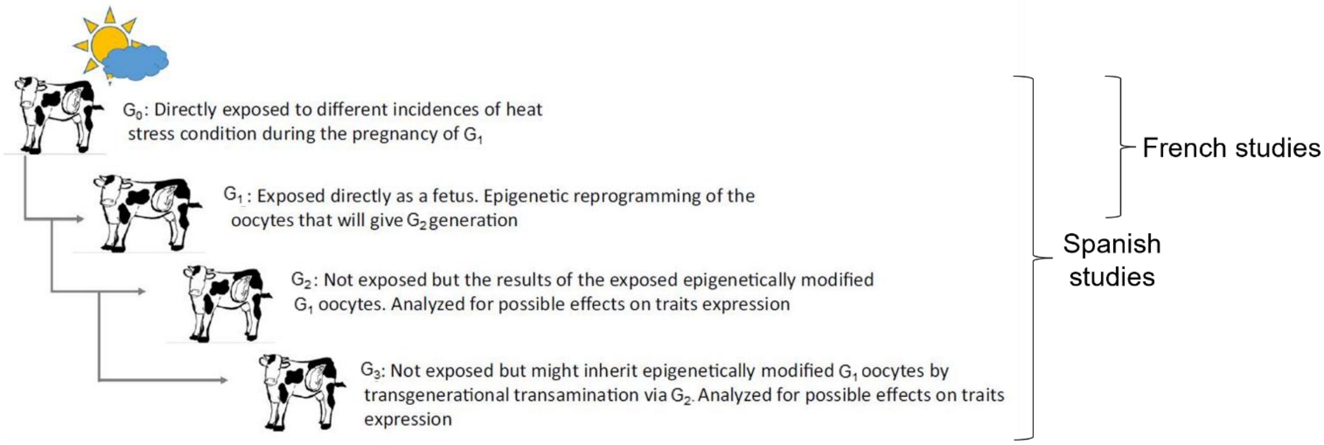


Figure 1: Generations considered in French and Spanish studies (from Weller et al. 2021)

One of the main objectives of RUMIGEN is to provide breeding tools adapted to future environmental conditions, and thus to adapt the selection criteria for taking climate change into account. For this purpose, France (Idele, INRAE), Spain (INIA, IRIAF) and the Netherlands (WU and WR) collaborate in WP3 ("Impact of trade-offs on productive lifespan") to define several traits related to heat tolerance. To achieve this purpose, large-scale data from commercial farms were combined with meteorological data.

WP3 is organized into three tasks:

- Task 3.1: Identify impacts of heat stress on performance and define new traits associated with heat tolerance.
- Task 3.2: Investigate the effects of climate change on trade-offs between production and other traits such as health and reproduction.
- Task 3.3: Study the impact of maternal heat stress during gestation on progeny.

Tasks 3.1 and 3.2 have been covered in deliverables D3.1 and D3.2, respectively. This deliverable D3.3 focuses on Task 3.3, where Spain (INIA-CSIC, IRIAF) and France (Idele, INRAE) are committed to assessing the effects on female progeny of maternal heat stress during gestation, especially on production and fertility traits, as well as to determine if these effects persist across generations.

This deliverable describes the contributions from both the Spanish and French teams. **French studies focused on maternal effects of heat stress whereas the Spanish studies have put more emphasis in investigating inter-transgenerational effects** (**Figure 1**).

3. Description of livestock and weather data

3.1. Breeds

The breeds investigated in this study are a cosmopolitan breed, **Holstein**, and one local breed, **Montbeliarde**. Data for Holstein are available in France and Spain. Data for Montbeliarde are only available in France.

3.2. Production and fertility traits

For production traits, **cumulative milk production over 305 days** (MY305), **cumulative fat yield over 305 days** (FY305) and **cumulative protein yield over 305 days** (PY305) from test-day records were studied. For fertility traits, the **conception rate** (CR), defined as the success rate of first artificial insemination (AI), was studied.

For France, all data were extracted from genetic evaluation data bases (Base Nationale de Données Zootechniques, or BDZN). For Spain, the lactational records as well as pedigree file were provided by the Spanish Friesian Confederation (CONAFE).

For both countries, the extracted datasets used for this deliverable covered approximately a period of 10 years, starting from 2010. Detailed description of the data and edits for each approach is presented in Appendix 1. Depending on the studies, the period started around 2010 until around 2020, including data for first up to third parity cows in both countries.

3.3. Meteorological information

Meteorological data were provided by Météo-France (Safran database) from 1959 to 2020 for France and by the National Meteorological Agency (AEMET) from 2000 to 2020 for Spain. Weather records were available for 9,892 8 by 8 km squares for France and for 1,993 Spanish weather stations distributed throughout each national territory. Each herd was associated to the closest weather station, according to the ZIP code of the farm. The average distance to the associated weather station was of 8.4 km in Spain and less than 5.7 km in France (grid of 8x8 km).

The **temperature humidity index** (THI) – calculated using the NRC formula (1971), THI = (1.8*T+32)-(0.55-0.0055*RH)*(1.8*T-26), with T being the average daily temperature (degrees Celsius) and RH being the average daily relative humidity (percent) – or **temperature only** were used to describe the environmental conditions at the time of conception and/or during gestation.

Statistics on meteorological information are available in D3.1 (Appendix 3).

4. Maternal heat stress effects on progeny performance

Several approaches were investigated to search for evidence of maternal heat stress effects on progeny performance, combining **different types of performance** and **different indicators of *in utero* meteorological conditions** (thermal loads).

Performances were either **estimated breeding values** (EBVs) or **raw phenotypes**. Thermal loads 1) included factors such as the **month of birth** (of the dam, granddam or great-granddam of the progeny), the **average temperature post-conception** (for 30, 60 or 90 days), the **average temperature pre-birth** (for 30, 60 or 90 days), the **average THI per month of gestation** or the **number of days with THI values ≥ 65 per trimester of gestation** (potential heat stress) or 2) were included in a **maternal environmental random effect** as an additional source of variation related to the dams on the performance of their progeny.

4.1. Maternal effect of heat stress on performance of French cows

4.1.1. Effects of THI during each month of gestation on cow phenotypes

4.1.1.1. Method

This study was carried out on first-lactation French Holstein and Montbeliarde and is estimated as the **deviation between observed and predicted performance** using i) the **genomic value of the progeny** obtained by summing the effects of the SNPs it carries (i.e. the Direct Genetic Value, namely DGV) and ii) the sum of the **fixed effects** used in the official French evaluation for the trait under consideration. Part of this difference between observed and predicted performance can be attributed to environmental effects not yet included in the model. In this study, the environmental effect considered was the **different levels of THI during the gestation of the dam** (average THI per month of gestation).

The model is as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\alpha}\mathbf{g} + e$$

where **y** is the vector of the observed performance of the female offspring for a given trait; **β** is the vector of the fixed effects specific to each trait and the meteorological variable, the monthly average THI during the foetal life, by classes (]0;40],]40;45],]45;50],]50;55],]55;60],]60;65],]65;80]). The other fixed effects were herd-year, age at calving, month of calving-year and gestation rank of the dam for the production traits, and herd-year, weekday, semen sex-year, gestation rank of the dam, age at insemination (for heifers only), interval between calving and insemination (primiparous only), and age at calving (primiparous only) for the conception rate.

X is the incidence matrix for the fixed effects.

g is the vector of female offspring DGV produced by the official genomic evaluations, fitted as a covariate. The individual DGV was calculated for each trait by summing the predicted effects of the SNPs carried by the progeny. To avoid any direct dependencies between the DGV and the performance, these SNP effects were previously estimated in large training sets from March 2018 that did not take into account the performances of the females retained in our analyses.

α is the fixed coefficient of regression on DGV.

Statistical analyses were performed using the GLM procedure of SAS software version 9.4 (SAS Inst., Cary, NC).

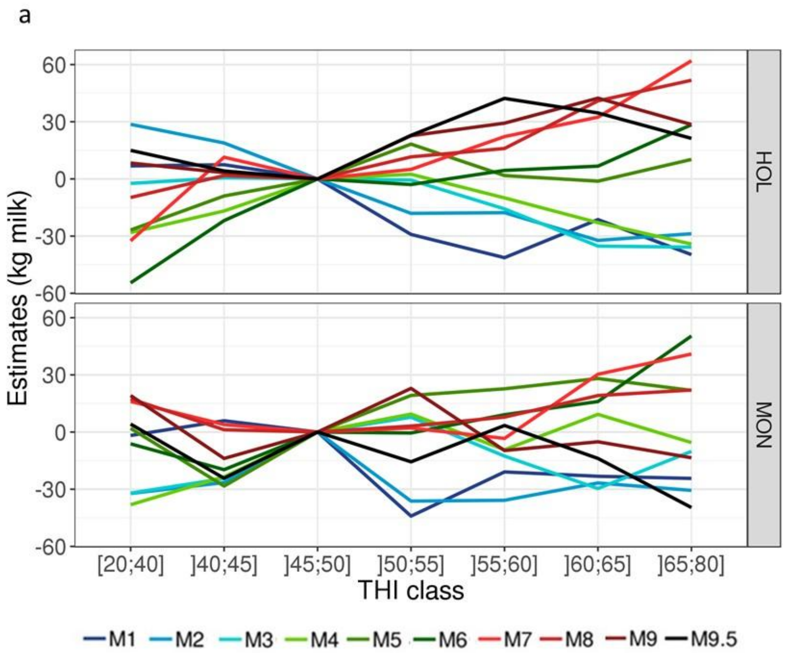
4.1.1.2. Results and discussion

The p-values of the effect of the average THI by month of gestation of the dams on their progeny performances are presented in **Appendix 2** (Figure 2.1). To account for multiple testing, the effects were deemed significant if the nominal p-value was smaller than 0.0001, corresponding to an overall p-value of 4% at the scale of the group of traits.

The mean THI per gestational month did not present a statistically significant effect on the offspring's conception rate (CR), neither for heifers, nor for cows (Appendix 2, Figure 2.1). Conversely, the mean THI per gestational month presented a statistically significant effect on production traits.

The **effect of environmental THI at each month of embryonic or foetal development** on 305-day milk (MY) are shown in **Figure 2** and in **Appendix 2** for fat (FY) (Figure 2.2) and protein (PY) (Figure 2.3) yields.

Whatever the trait considered, **the effect of THI during the *in utero* development on subsequent performances is low**. For instance, for MY305, the difference between the extreme effects of THI was 96 kg. This difference represented **1.2% of the average MY305 for Holstein** and was observed between the coldest and the hottest situations for the seventh month. The highest difference for **Montbeliarde** MY305 was 72 kg, so **1.1% of the average MY305**, and was observed between the coldest and the hottest situations for the sixth month. For Holstein, a **high-THI has a negative effect on production traits only when it occurs during the first months of the gestation of the dam**, **whereas the effect is positive when a high-THI occurs during the last months of the gestation of the dam**. For the Montbeliarde breed, the trend is the same but these high-THI effects are weaker and many of them had no significant effect on the traits.



b

		[20;40]	]40;45]	]50;55]	]55;60]	]60;65]	]65;80]
HOL	M1	ns	ns	***	***	**	***
	M2	**	**	*	*	***	**
	M3	ns	ns	ns	ns	***	***
	M4	ns	ns	ns	ns	*	***
	M5	ns	ns	**	ns	ns	ns
	M6	**	*	ns	ns	ns	***
	M7	ns	ns	ns	**	***	***
	M8	ns	ns	ns	*	***	***
	M9	ns	ns	**	***	***	**
	M95	ns	ns	*	***	***	ns
MON	M1	ns	ns	**	ns	ns	ns
	M2	**	*	*	*	ns	ns
	M3	**	*	ns	ns	*	ns
	M4	**	ns	ns	ns	ns	ns
	M5	ns	*	ns	*	**	*
	M6	ns	ns	ns	ns	ns	***
	M7	ns	ns	ns	ns	*	**
	M8	ns	ns	ns	ns	ns	ns
	M9	ns	ns	ns	ns	ns	ns
	M95	ns	*	ns	ns	ns	***

Figure 2: a) Effect of the average temperature-humidity index (THI) during each month of fetal development on first lactation's 305-day milk yield for Holstein (HOL, top) and Montbeliarde (MON, bottom). b) Significance levels associated to each class of THI by month of gestation.

M1: from d1 to d30 of the gestation of the dam; M2: from d31 to d60 of the gestation of the dam; …; M9: from d241 to d270 of the gestation of the dam; and M9.5: from d250 to d279 of the gestation of the dam

***: p≤0.001; **: p≤0.01; *: p≤0.05; ns: not significant at a level of 5%

Although the THI for different gestational periods were tested independently, they are not independent. Due to the bovine gestation length and the temperate climate in mainland France, among Holstein and Montbeliarde cows that experienced a possible heat stress (THI > 65) during the seventh, eighth or ninth month of gestation, none of them experienced an average THI above 65 in their first three months of gestation. In addition, cows that experienced a high THI at the end of the gestation are cows that begun their gestation during cool period.

Therefore, the positive effects of high THI during the last months of gestation can likely be interpreted as the positive effects of the absence of high THI at the beginning of dam's gestation. We tested some models that included not one but two average monthly THIs, one at the beginning and one at the end of gestation. These monthly THIs were considered either as two different fixed effects, or as a combination of fixed effects. Due to the partial dependence of the THI at the beginning and end of the gestation period, some combinations were not possible. Nevertheless, these models did not show results different from those presented in this deliverable, that is, a negative effect of high THI on production only when it occurred at the beginning of the gestation whereas a high THI at the end of the gestation had rather positive effects.

4.1.2. Effects of the number of days with THI values above 65 during trimesters of gestation on cow phenotypes

4.1.2.1. Method

This second French analysis had two objectives: 1) study the impact of *in utero* heat stress, using the **number of days with THI values above 65** (NB_THI≥65) **per trimester of gestation** instead of the average monthly THI; 2) better disentangle the effects of heat stress during gestational period, to confirm which period is the most sensitive.

A two-step approach has been carried out for each breed x parity separately.

First, the following model was applied to all cows in the database, for each breed x parity:

$Y_{ijklmn} = \mu + b1\ EBV\_dam_j + b2\ EBV\_sire_k + Age\_Calving_l + Month\_Calving_m + Herd \times Year_n + e_{ijklmn}$ [1]

where Y_{ijklmn} is the performance, EBV_dam_j and EBV_sire_k are the estimated parental breeding values, b1 and b2 are regression coefficients associated with these EBVs and the three environmental effects (age at calving, month of calving and herd x year) are fixed effects.

As the data sets were large, the model was run in bootstrap mode, by repeating the model fit 40 times on a randomly drawn sub-sample of 1000 herds x years.

Subsequently, each subset was **divided into groups according to the birth month of the cows**. The residuals of each cow with model [1] were analysed separately for each birth month using the following model:

$e_{ijklmn} = c_1\ NB\_THI{\geq}65\_T1 + c_2\ NB\_THI{\geq}65\_T2 + c_3\ NB\_THI{\geq}65\_T3 + \dot{\omega}_{ijklmn}$ [2]

where NB_THI≥65_T1, NB_THI≥65_T2, NB_THI≥65_T3 represent the number of days with a daily average THI higher than 65 experienced by the cow *in utero* during the 1^{st}, 2^{nd} and 3^{rd} trimester of gestation, respectively. Indeed, pregnant and lactating cows are likely to show clinical signs of heat stress beyond this THI threshold. The regression coefficients c_1, c_2, c_3 represent the effect of one day with average daily THI over 65 during a given trimester of gestation on the performance.

This approach enables the pre-correction of cow performances for the effects affecting the entire population in the initial step, while analysing the impact of *in utero* thermal stress on each trimester of gestation separately per birth month, which improves the interpretation of results, as the hottest season occurs within the same trimester for all cows in a given group.

The models were applied to **cumulative milk production traits over 305 days** (MY305, FY305, PY305) **for Holstein and Montbeliarde cows in first, second and third lactation**.

4.1.2.2. Results

For MY305, the r-squared of model [1] ranges from 0.69 to 0.76 in Holstein and from 0.57 to 0.59 for Montbeliarde while that of model [2] was close to zero.

The results are presented for three birth months – September, December and March – representing periods of heat stress occurring during different trimesters of gestation. For cows born in September, the first trimester of gestation corresponds to winter, the second to spring and the third to summer. For cows born in December, the first trimester of gestation corresponds to spring, the second to summer and the third to autumn. For cows born in March, the first trimester of gestation corresponds to summer, the second to autumn and the third to winter.

The distribution of the value of the coefficients of the *in utero* THI variables of the 40 sub-bases fitted models is shown graphically in the **Figure 3** below for MY305 in Holstein (see **Appendix 3** for Montbeliarde). The results were similar for FY305 and PY305 and thus are not presented.

Given the weather conditions in France, heat stress is most prevalent in summer (late spring and early autumn), which explains that the variability of the estimated regression coefficients is lower for the summer quarters than for the other seasons, indicating more accurate estimates. For this reason, the interpretation of the coefficients corresponding to the other seasons is less relevant in our context and we focused on the trimester coinciding with summer (in yellow in **Figure 3**).

For both breeds and all parities, and whatever the trimester of gestation, **the distribution of estimated regression coefficients determining the impact of the number of days with heat stress is centred on very low values (close to zero).** The medians of the regression coefficients corresponding to summer ranged from -5.5 and -7.9 kg per day of heat stress for Holstein and Montbeliarde cows in third lactation born in March (potential heat stress during 1st trimester) to +1.9 and +2.4 kg per day of heat stress for primiparous Holstein and second-lactation Montbeliarde cows born in September (potential heat stress during 3rd trimester).

For Holstein cows, as in the previous study, a maternal heat stress during gestation tends to have a negative effect (-1.6 kg/day with THI>=65) **on the first lactation of the daughters when it occurs at the beginning of the gestation** (trimester 1) **and a positive effect** (+1.9 kg/day with THI>=65) **when it occurs at the end** (trimester 3). For first-lactation Montbeliarde cows, the results are not consistent with those of the previous study and should be taken cautiously because the number of cows taken into account was very small.

The estimated effect of heat stress during gestation on cumulative milk production over 305 days of the offspring was negligible compared to the production level of these cows. The highest estimated effect per day with THI above 65 corresponded to less than 1 ‰ of the average MY305 of the population.

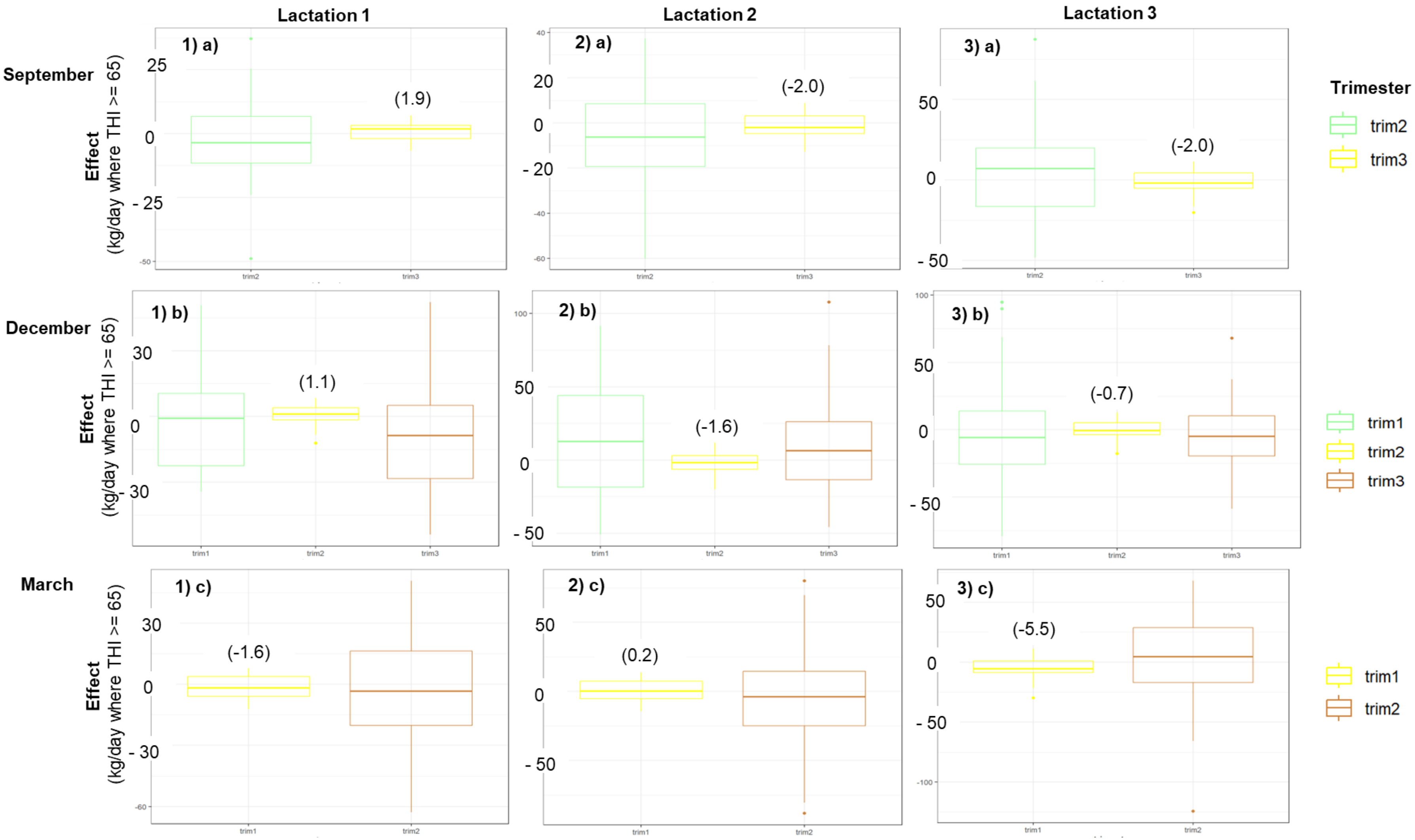


Figure 3: Distribution of estimated regression coefficients of NB_THI>65_T1 (trim1), NB_THI>65_T2 (trim2) and NB_THI>65_T3 (trim3), in kg of milk per day with THI above 65, for first (1), second (2) and third (3)-lactation Holstein cows born in September (a), December (b) and March (c).

Median value between brackets for trimester occurring during summer (in yellow)

4.1.3. Discussing French results

Our results show, when they exist, **effects of *in utero* heat stress on subsequent performances smaller than generally found in similar studies**. Indeed, the results reported in the literature for the first lactation MY305 can reach values from 200kg to 900kg (Barash et al., 1996 for Israeli cattle; Brown et al., 2015 and 2016, Laporta et al., 2020, and Pinedo and de Vries, 2017 for US cattle; van Eetvelde et al., 2017 for Belgian cattle).

According to our data, **high THI tends to be unfavourable for progeny production when it occurs at the beginning of the gestation of the dam**, during embryonic and early foetal life, but tends to be **favourable when it occurs at the end of the gestation of the dam**. This negative effect of heat stress at the beginning of the gestation and positive effect of heat stress at the end of the gestation of the dam on offspring's production performances were observed in most studies analysing the seasons of conception or the season of birth in warm regions (Barash et al., 1996; Brown et al., 2015, 2016; Pinedo and de Vries, 2017). Conversely, the authors who focused their analyses on the effect of heat stress at the end of the gestation of the dam highlighted an unfavourable effect of heat stress during this period (Kipp et al., 2021; Laporta et al., 2020; Dahl et al., 2016; Monteiro et al., 2016).

The studies available in the literature have most often investigated the effects of heat stress early in gestation (warm or thermoneutral months of conception, sometimes excluding other months of birth as in Brown et al., 2015; 2016) or late in the dam's gestation (month of calf birth, effect of THI on late-gestation stages) but rarely over the whole period as we have done.

The estimated effects were too small to study their genetic determinism. Therefore, such a quantitative genetic approach was not carried out on the French data.

4.2. Exploring a maternal component in Spanish cow performance

4.2.1 Material and Method

The total number of records used appear in Table 1 on a lactation bases. Average milk, protein and fat production per lactation are shown in Table 1.

Table 1. Number of raw data, edited data, average kg of milk, kg of protein and kg of fat per lactation

	N raw	N edited	Milk (kg)	SD	Protein kg	SD	Fat kg	SD
Lact 1	1581002	1268548	9594.3	1836.5	312.9	60.0	350.5	77.8
Lact 2	1268939	919518	11171.6	2301.3	364.5	73.3	411.6	100.5
Lact 3	871277	503087	11734.9	2410.8	377.7	76.4	434.7	106.9

Van Vleck and Bradford (1965) highlighted the existence of a maternal component in the milk production of cows that generated an overestimation of the additive genetic variance when ignored. More recently, Tal et al. (2010) discussed the effect of ignoring epigenetic sources of variation on the additive variance, provided that it is not clear if that effect stays in the error term or it is absorbed in the additive part. With these two findings in mind, we have explored the **existence of an additional source of variation related to the dams of cows on the production traits of cows**. To do so, we compared two single traits models:

$$Y_{ijkm} = HYS_i + RPM_j + RPLA_k + a_m + e_{ijkm}$$ Model 1a

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°101000226.

Where Y_{ijkm} is the record of the 305-day milk, protein and fat yield of the m cow; HYS_i is the systematic effect of Heard-year-season; RPM_j is the systematic effect of the region-period-month at calving; $RPLA_k$is the random effect of region-period-lactation age at calving; $a_m$is the the additive value of the cow m; e_{ijkm} is the random residual effect of the model.

$Y_{ijkmn} = HYS_i + RPM_j + RPLA_k + a_m + D_n + e_{ijkmn}$ Model 1b

This model (1b) is similar to the previous model except that we have included a maternal environmental random effect of the dam of cows for production traits.

4.2.2 Results and discussion

Table 2 shows the estimate of the additive variance, error variance for Model 1a and Model 1b as well as the maternal environmental variance in Model 1b. Regardless of the trait, the additive variance as well as the residual variance have experienced a reduction when a maternal environmental component was fitted.

Table 2. Estimates of additive genetic variance (Va), error variance (Ve), environmental maternal variance (V_D), heritability (h²) and ratio of environmental maternal and additive variance (D²) for milk, fat and protein in the first three lactations

Milk	Model	Va	Ve	V_D	h^2	$D^2=V_D/Va$
Lact1	a	646,560	1,077,700		0.38	
	a+D	643,780	1,072,800	5,976	0.37	0.009
Lact2	a	705,380	1,996,200		0.26	
	a+D	696,180	1,982,500	19,861	0.26	0.028
Lact3	a	634,420	2,411,700		0.21	
	a+D	583,750	2,346,200	45,225	0.20	0.077
Fat						
Lact1	a	921.89	1722.0		0.35	
	a+D	915.5	1712.4	13.56	0.36	0.015
Lact2	a	1212.7	3252.0		0.27	
	a+D	1198.6	3229.6	32.08	0.27	0.021
Lact3	a	1156.7	4020.5		0.22	
	a+D	1056.4	3914.9	67.91	0.21	0.064
Protein						
Lact1	a	479.64	1061.5		0.31	
	a+D	476.39	1056.6	6.95	0.31	0.014
Lact2	a	572.63	1854.4		0.23	
	a+D	563.26	1839.7	21.03	0.23	0.037
Lact3	a	538.14	2249.3		0.19	

	a+D	502.35	2180.5	43.81	0.18	0.087

Heritability estimates decrease with lactation number for the three traits. Heritability estimates in models 1b including the maternal component (a+D) do not show large changes compared to Model 1a. However, the ratio between the maternal component and the additive genetic variance (D²) increases with lactation number for the three traits. The maternal environmental component is an indicator of the existence of an additional source of variation which is partially confounded with the additive genetic variance. This extra source of variation could be explained by an epigenetic source of variation caused by a maternal environmental constraint during the gestation of the cow that affect is future performance as an adult cow.

Regardless of the cause, if cows have suffered any maternal environmental stress during their *in utero* development that has triggered or not an epigenetic mark, it would be plausible that the impact of such component increases according to the number of lactation of the cows when the demands to maintain gestation and lactation increases (Laporta et al., 2020; Ouellet et al., 2021). In any case, these results suggest that EBVs for production traits are an interesting potential "phenotype" to explore epigenetic effects on production traits as proposed by Weller et al. (2021) and Macciota et al. (2023). In addition, these results also suggest that, if maternal effects represent – to some extent – an epigenetic source of variation, this is partially contained in the additive as well as in the residual component of the model, as suggested by Tall et al. (2010).

It is true that we have not associated the maternal component with any specific stressor. For this model we have assumed the existence of any perturbation affecting the *in utero* environment generated during the gestation of the cows.

4.3. Inter-transgenerational effects of heat stress on performance of Spanish cows

Inter-transgenerational effects were explored under two approaches differing on the definition of thermal load. Thermal loads are represented by month of birth as a proxy of thermal stress during the periconception, embryo development and fetus. Both make use of **EBVs of the cows as phenotypes** based on the idea that, as stated in the previous section, **EBV capture part of epigenetic inheritance**.

4.3.1 Effects of month of birth of the dam, granddam and great-granddam on the progeny's EBVs

4.3.1.1. Material and Methods

The total number of records per lactation were shown in **Table 1** (cf. 4.2.1). In addition to production traits, we have also studied effects of dams and grandams' month of birth on the conception rate at first insemination of heifers. For this approach, we only used EBVs from cows whose dams, granddams and great-granddams had EBVs predicted based on their own production and fertility records. First, second and third-lactation EBVs were first obtained running genetic evaluation models similar to the one used by CONAFE for routine genetic evaluations, but considering each lactation separately (Model 1a, cf. 4.2.1).

Models aim to capture the inter-transgenerational effect of granddams on the phenotypes of their granddaughters and of great-granddams on the phenotypes of their great-granddaughters. Therefore, we need to go back at least to the great-grandmother's generation to ensure a potential transgenerational maternal effect transmitted through the maternal line, as shown in **Figure 1**. Thus, we have four generations present in the data files: G_0 represents the great-granddams, G_1 the granddams, G_2 the dams and G_3 the cows whose phenotypes (EBVs) are modeled. The EBVs are lactational EBVs. The models are as follows:

$$EBV_{(G3)} = BMC_{(G3)} + \mathbf{BMD}_{(G2)} + b_{1*}birthdate_{(G2)} + b_{g2*}\,EBV_{(G2)} + b_sYBS + e \quad \text{Model 2a}$$

$$EBV_{(G3)} = BMC_{(G3)} + \mathbf{BMgD}_{(G1)} + b_{1*}birthdate_{(G1)} + b_{g1*}\,EBV_{(G1)} + b_sYBS + e \quad \text{Model 2b}$$

$$EBV_{(G3)} = BMC_{(G3)} + \mathbf{BMggD}_{(G0)} + b_{1*}birthdate_{(G0)} + b_{g0*}\,EBV_{(G0)} + b_sYBS + e \quad \text{Model 2c}$$

To evaluate the effect of thermal stress during the gestation of the dams and granddams by their granddams and great-granddams on the performances of the G3 cows, thermal loads are represented by **month of birth** of dams (G_2), granddams (G_1) and great-granddams (G_0) of G3 cows. For example, $BMD_{(G2)}$ is the effect of dams' month of birth on their daughters' phenotypes. It represents the environmental conditions during the granddams gestation of the dams. In addition, we fit the linear effect of the birthdate of the dam to correct for genetic trend and of the $EBV_{(G2)}$ of the dam to avoid confounded effects between BMD and the genetic level of dams (Weller et al., 2021). In addition, the year of birth of sires of G3 cows (YBS) was fitted as a covariable to account for genetic trend. Model 2b and 2c are similar to 2a except that months of birth refer to granddam (BMgD) and great-granddam (BMggD). Similarly, birthdate and EBVs are related to granddams (G_1) and great-granddams (G_0) in those models. These models have been applied to production traits (kg of milk, kg of protein and kg of fat) as well as conception rate for the first insemination of heifers.

4.3.1.2 Results and discussion

Figure 4, **5** and **6** show the least square solutions of the effect of months of births of dams, of granddams and great-granddam on kg of milk, fat and protein of G3 cows, for the first, second and third lactation respectively.

As we can observe in **Figure 4**, **5** and **6**, the patterns of the quantity vs milk quality are more remarkable on G3 cows during their first lactation, for milk, fat and protein than in the second and third lactation contrary to Laporta et al. (2020) findings. G3 cows that are daughters, granddaughters and great-granddaughters of females born particularly in September and October tend to produce the least milk. Those females went through a **late gestation during summer period with a detrimental effect on the production traits of future generations**. Thus, fetal stress suffered by the dam, granddam and great-granddam affect production of the G3 cows. However**, the effect of great-granddams appears to have a larger magnitude that the effect of dams and granddams**. The older the generation the higher the probability of being females with more lactations, therefore they could be females that were gestating while they were lactating as well and suffer more metabolic stress in addition to heat stress (Gonzalez-Recio et al., 2012). One interesting result for the G0 dams is that we observed an effect at the periconception moment, suggesting that G0 suffering an environmental event of heat stress affect the G1 embryo as well as the germ cell of the embryo (G2), suggesting the presence of **heritable epigenetics effects**. Contrary to Laporta et al (2020), we observe a **pattern of inter-transgenerational effects on the first lactation of G3 cows** but it was not that evident for the following lactations. In addition to the period described, we could observe that for protein and fat content there is also an effect of the environmental conditions during the periconception period. It means that dams, granddams and great-grandams born in spring, therefore conceived in July or August, have a negative impact on fat and protein production of the G3 cows as an inter-transgenerational effect. The results suggest the existence of inter-transgenerational effect that have being transmitted from the G0 to G2 generation and observed in G3. **Even though the pattern exits the effects using EBVs and month of birth are almost negligible**. For example, for milk yield the maximum estimated G0 birth month effect was 9 kg in from great-granddam born September and November what is to say spending the last three months of gestation under heat stress. We need to reproduce these models describing the thermal loads as a function of temperature as we do in the following section (4.3.2).

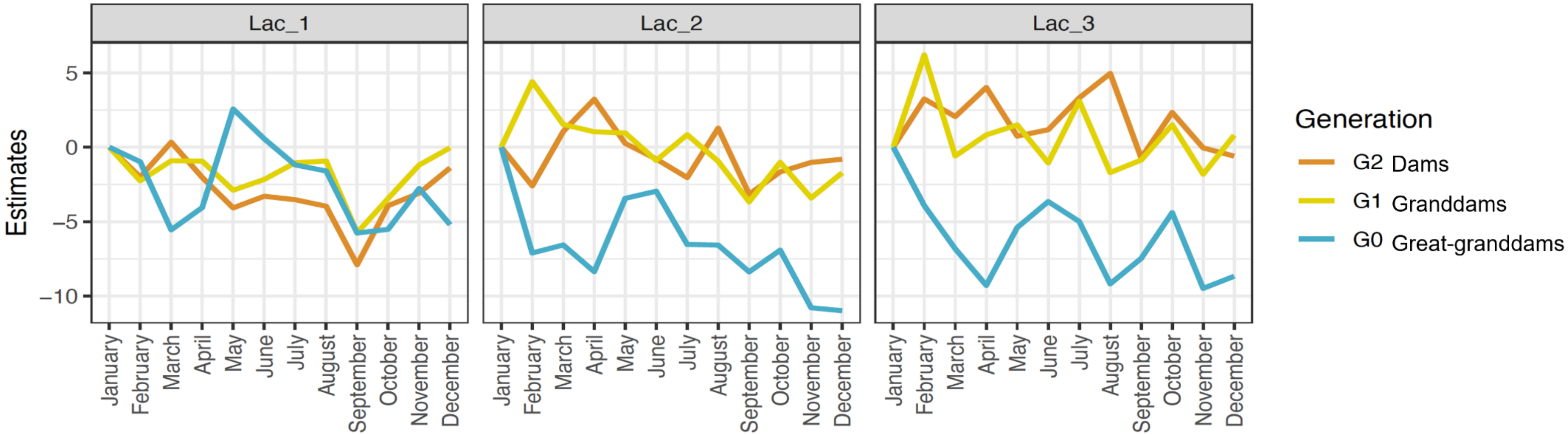


Figure 4. Least square solutions for the effect of month of birth of dams, granddams and great-granddams on 305-days milk production of cows (G3) for the first, second and third lactation

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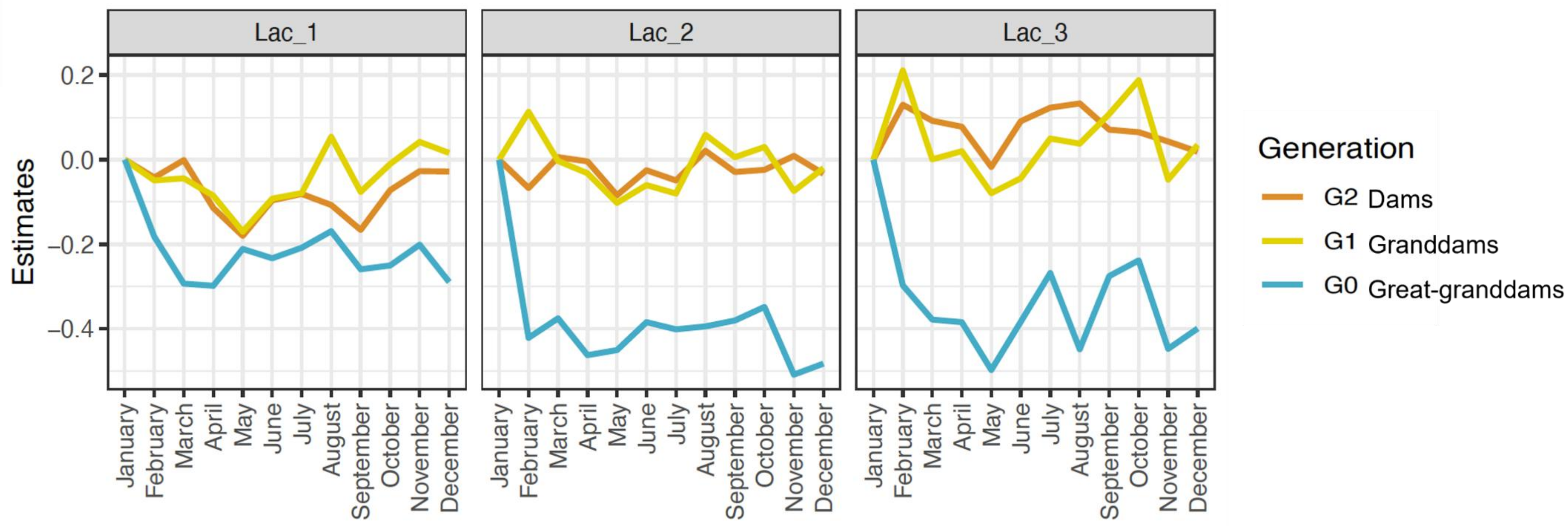

Figure 5. Least square solutions for the effect of month of birth of dams, granddams and great-granddams on 305-day <u>kg of protein</u> of cows (G3) for the first, second and third lactation

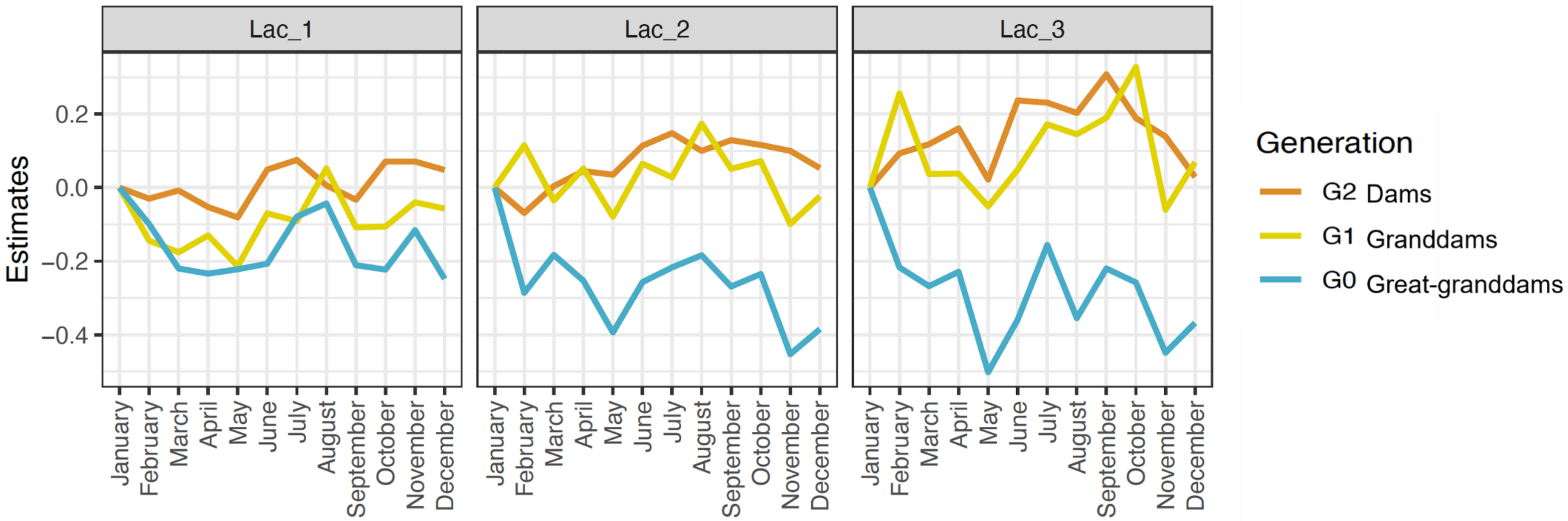

Figure 6. Least square solutions for the effect of month of birth of dams, granddams and great-granddams on 305-day kg of fat of cows (G3) for the first, second and third lactation

Table 3 shows the results of the impact of Month of birth of dams and granddams on the conception rate of their daughters and granddaughters, the G3, as heifers. We may observe that, while G_0 cows were conceiving in summer, a period of heat-stress events, the G_1 cows' gametes suffer an epigenetic mark that is transmitted to G_2 oocytes and affect G_3 cows' conception rate at first IA. Some experimental studies have been searching for evidence of epigenetic effects on the reproduction performances of those embryos as cows due to maternal heat-stress on embryos during the last part of gestation. A retrospective study of those experiments failed to find differences in reproductive performances between cows subject to *in utero* heat stress and those that benefited from *in utero* comfort during the last 46 days of gestation (Laporta et al., 2020). As mentioned by Ouellet et al. (2021), the evolution of primordial germ cells to oogonia happens during the first trimester of gestation. Thus, **events occurring during that first part of the gestation will impact reproduction performance of cows**. Somehow, those findings provided support to our results. The epigenetic effects we have found in our study occur systematically at conception and/or the first period of gestation, therefore it is plausible that the key period when modeling the effect of THI as a mechanism generating epigenetic marks affecting fertility traits should focus on the first third of the gestation. Though the pattern is clear and it is statistically significant, the magnitude of the effect is limited. The magnitude of the effect capture by Month of birth of granddams is systematically larger than the one capture by the month of birth of dams.

Table 3. Least square solutions for month of birth of dams and grand-dams on conception rate at first inseminations of cows (G_3).

Month of birth	**Conception* Month**	**N**	**Dams**	N	**Grand-dams**
January	April	21783	0.0	9699	0.0
February	May	19321	9.935e-05	8696	-6.876e-04
March	**June**	**20843**	**-6.736e-04**	**9181**	**-1.277e-03***
April	**July**	**19520**	**-1.195e-03****	**8647**	**-2.130e-03****
May	**August**	**20164**	**-7.328e-04***	**8571**	**-2.284e-03***
June	**September**	**20639**	**-8.286e-04***	**8992**	**-1.590e-03***
July	October	22038	5.218e-06	9598	2.309e-04
August	November	23051	1.565e-05	9958	3.595e-04
September	December	22052	2.408e-04	9747	8.435e-04
October	January	22239	6.819e-04	9809	8.723e-04

November	February	21042	3.328e-04	9516	8.681e-04
December	March	22484	2.955e-04	10007	-7.630e-04

As a subproduct of previous models, we have the least square solutions of the effect of the *in utero* environment of G3 cows providing the phenotype. This *in utero* environment is represented by Month of birth of G3 cows. As already mentioned, they were included in the model to avoid confounding the maternal *in utero* environment with inter/transgenerational effects. These solutions are equivalent to those obtained by French models.

Table 4 contains the least square solutions for 305-day kg of milk, protein and fat during the first lactation, and conception rate (CR) for the first lactation.

Table 4. Least square solutions of cow's month of birth (BMC$_{(G3)}$) for production traits according to their month of birth.

Cow's month of birth	**Conception* Month**	**Kg of Milk**	**Kg of Protein**	**Kg of Fat**	**CR**
January	April	0.0	0.0	0.0	0.0
February	May	-0.990	**-0.09**	**-0.19**	2.489e-04
March	**June**	-2.163	**-0.14**	**-0.26**	**-6.346e-04**
April	**July**	-7.026	**-0.32**	**-0.47**	**-8.857e-04***
May	**August**	-2.888	**-0.16**	**-0.49**	**-8.047e-04***
June	**September**	-2.567	**-0.05**	**-0.35**	**- 3.979e-04***
July	October	4.915	0.09	-0.12	4.247e-04
August	November	8.704	0.27	0.18	3.182e-04
September	December	8.963	0.23	0.12	6.355e-04
October	January	6.867	0.16	0.17	2.716e-04
November	February	5.664	0.11	0.07	2.930e-04
December	March	3.141	0.07	0.03	3.774e-04

* This column has been included to facilitate the interpretation of results

As we can see in **Table 4**, the negative impact on production traits as well as CR of the **environmental constraints generated *in utero* because of an increase in temperature** (assumed to be represented by month of birth of the cows) is **more relevant in cows that were conceived during summer**. According to these results, cows conceived in summer produced up to 16 kg less milk, 600 gr less protein and 660 gr less fat and have 0.1% less probability of getting pregnant than those being conceived in cooler months. This effect is statistically significant but biologically negligible. It is much smaller than the one described in other Holstein populations (Laporta et al., 2020) and of same order of magnitude as the French results.

4.3.2 Effects of temperature post-conception and pre-birth of the dam and the granddam on the progeny and grand-progeny's EBVs

4.3.2.1 Method

Models are similar to the previous ones but in this case, thermal loads are accommodated by the **mean of average daily temperatures deviated from average comfort temperature** estimated in D3.1 **(24 ºC) during 30, 60 and 90 days post-conception and pre-birth** of the dams ($TLD_{(G2)}$) and granddams ($TLgD_{(G1)}$) of G3 cows. The other effects are as described before. When TLD or TLDg were bellow 24, the difference was set to 0. So far, we only have results for production traits.

$$EBV_{(G3)} = BMC_{(G3)} + b_{T1}*(\mathbf{TLD_{(G2)}\text{-}24}) + b_1*birthdate_{(G2)} + b_{g2}* EBV_{(G2)} + b_sYBS + e$$

$$EBV_{(G3)} = BMC_{(G3)} + b_{T2}* (\mathbf{TLgD_{(G1)}\text{-}24}) + b_1*birthdate_{(G1)} + b_{g1}* EBV_{(G1)} + b_sYBS + e$$

4.3.2.2 Results and discussion

Table 5 shows the results of the analysis incorporating thermal loads as the average of the mean temperatures minus comfort temperature (24ºC) during the 30, 60 and 90 days of the periconception period (post-conception) and during the last 30, 60 and 90 days of gestation. Results are shown for milk, protein and fat during the first three lactations.

In general, losses of production increase with the length of the modeled period. Thus, thermal stress up to 90 days post-conception and/or during the last 90 days of gestation of dams and granddams have a more negative impact on production traits of G3 cows than shorter periods. The magnitude of the impact increases with lactation number as well as with the generation, in the opposite direction. Therefore, we observe that thermal stress during the conception and at the end of gestation of the granddams generates a larger impact on production traits of G3 cows than those same periods for dams of G3. Whether or not this is an artifact of the model or a biological finding is something we need to pursue.

The **decrease of milk of G3 cows per degree of increase of average temperatures during the gestation of the dams varied between 3.2 kg/ºC** for the first 30 days of the periconception period **and 5.8 kg/ºC** during the last 90 days of the gestation. If we interpret these results in the range of temperatures during those periods, it will mean that **G3 cows could loose between 23 and 38 kg of milk in their first lactation because of the environmental conditions during the first 30 days of conception or the last 90 days of gestation**, **suffered by their dams while they were gestated.** During their second lactation of G3 cows their loss ranged between 25 and 72.8 kg of 305-day milk. Those figures are negligible compared to the average milk production of Holstein cows now. However, the impact on 305-day kg of protein and kg of fat of G3 cows is more relevant. The difference in losses of kg of protein in G3 cows caused by the thermal stress during the 90 days post-conception of their dams could be up to almost 5 kg of protein for the whole lactation which represents **1.3% of the phenotypic mean of the trait during the second lactation**. Moreover, the impact of the same conditions on G3 cows' fat content will be of about 6 kg or **1.5 % of the mean of the traits for cows in their second lactation**.

If we look at **the granddams' effect, the estimated losses seem to be higher than the estimated dams' effect**. G3 cows whose granddams suffered high temperatures above comfort during a period of 90 days post-conception have experienced losses of up to 136 kg of milk (**1.2% of the mean 305-day milk**), 8.8 kg of protein (**2.4 % of the mean 305-day kg of protein**) and 12.4 kg of fat (**3% of the mean 305-day kg of fat**).

We have estimated losses due to epigenetic changes as if those changes were caused just in specific periods. However, it would be plausible that the effects occur during the whole gestation and therefore **the losses of G3 cows would be expected to be larger** than if we model their effects affecting just one of the periods (Macciota et al., 2023).

Table 5. Effect (kg/ºC) of heat stress during dams and granddams' gestation on 305-day milk, protein and fat production of G3 cows during the first three lactations. Periods are 30, 60 and 90 days of periconception (post-conception) and at the end of the gestation (pre-birth).

		Post-Conception			**Pre-Birth**		
		30	60	90	30	60	90
Lactation N	Range T (ºC)[1]	[24, 31.8]	[24, 30.6]	[24, 29.7]	[24, 32.2]	[24, 30.8]	[24, 29.7]
Milk Yield (kg/ºC)							
1rst	Dam	-3.2±1.1**	-4.0±1.4**	-4.6±1.9*	-2.8±1.0*	-4.1±1.2*	-5.8±1.6*
	gDam	-3.8±1.9*	-6.3±2.4*	-8.8±2.9***	-2.4± 1.8	-5.0±2.2*	-8.0±2.8**
2nd	Dam	-7.5±1.3***	-9.3±1.6***	-12.7±2.3***	-6.1±1.2***	-8.8±1.5***	-11.2±1.9***
	gDam	-10.5±2.3***	-15.2±2.9***	-20.03±4.2***	-7.9± 2.2***	-9.3±2.6***	-11.2±3.5**
3rd	Dam	-4.0±1.8*	-6.3±2.3**	-10.2±3.1***	-3.6±1.7*	-7.0±2.0***	-9.9±2.7***
	gDam	-5.3±3.2	-8.5±4.0*	-16.4±5.7**	-8.5± 3.2**	-13.1±3.6***	-18.9.1±4.9***
***Protein Yield** (kg/ºC)							
1rst	Dam	-0.28±0.03	-0.35±0.04	-0.45±0.05	-0.26±0.02	-0.34±0.03	-0.44±0.04
	gDam	-0.47±0.05	-0.64±0.07	-0.81±0.09	-0.36±0.05	-0.50±0.06	-0.70±0.08
2nd	Dam	-0.44±0.03	-0.56±0.05	-0.76±0.06	-0.39±0.05	-0.51±0.04	-0.65±0.05
	gDam	-0.76±0.06	-1.0±0.08	-1.32±0.11	-0.59±0.06	-0.71±0.07	-0.94±0.01
3rd	Dam	-0.33±0.05	-0.47±0.06	-0.67±0.09	-0.31±0.05	-0.43±0.06	-0.59±0.08
	gDam	-0.61±0.09	-0.83±0.11	-1.19±0.16	-0.54±0.08	-0.71±0.10	-0.96±0.13
***Fat Yield** (kg/ºC)							

1rst	Dam	-0.32±0.04	-0.41±0.05	-0.57±0.07	-0.35±0.04	-0.45±0.05	-0.58±0.06
	gDam	-0.71±0.07	-0.95±0.09	-1.29±0.1	-0.57±0.07	-0.86±0.08	-1.17±0.1
2nd	Dam	-0.49±0.05	-0.65±0.07	-0.9±0.0.9	-0.45±0.05	-0.57±0.06	-0.72±0.07
	gDam	-1.0±0.09	-1.35±0.12	-1.85±0.17	-0.70±0.09	-0.97±0.11	-1.42±0.15
3rd	Dam	-0.54±0.07	-0.78±0.09	-1.16±0.13	-0.50±0.07	-0.61±0.08	-0.78±0.11
	gDam	-0.90±0.13	-1.37±0.17	-1.93±0.24	-0.64±0.08	-0.92±0.15	-1.40±0.21

* All regression coefficients are equally significant p<0.001.

Results shown in **Table 5** suggest the existence of inherited epigenetics changes in the Spanish Holstein populations. These findings will support either inter or transgenerational effects depending on the persistence of the environmental factor that triggers the epigenetic change (Braz et al., 2022; Khatib et al., 2024). In **Table 5** we can see that ranges of temperature are similar across generations. Therefore, even though we observe similar patterns across generations, it is **difficult to assess if we should talk about inter or transgenerational effects**. The only way to reach any conclusion may come from genomic information.

4.4. Discussing Spanish results

In general, we could affirm that heritable epigenetic changes exist in the Spanish Holstein population. Modelling thermal loads as Month of birth to estimate epigenetic effects on production traits provides a pattern indicating that **dams, granddams and great-grandams that were under heat stress during the last three months of their gestations have a negative impact on their daughters, granddaughters and great-granddaughters milk production**. Similar patterns are described by Weller et al. (2021) and Machiotta et al. (2023). This pattern is clear during the first lactation. For fat and protein, the periconception period also provided an interesting pattern. Dams, granddams and great-granddams that were born in April-May – which means conceived in August – have a negative impact on the 305-day protein and fat content of G3 cows. For conception rate the critical periods occur around conception. In any case, **the estimated magnitudes of the effects for production as well as for conception rate are very small, almost negligible**. However, modelling thermal loads as a function of the deviation of mean average daily temperatures from the comfort during the periconceptional and pre-birth periods revealed more interesting results depending on the traits. The cost of energy to produce each component of the milk is very different, therefore it would not be strange to think that different epigenomic marks are associated to each trait and that the magnitude of the impact of epigenetic changes in general will differ depending on the traits considered. For example, Weller et al. (2021) did not find any significant epigenetic change for milk production but the opposite for protein and fat contents.

5. Conclusion

The different approaches have made it possible to characterize the effects of gestational heat stress on dairy cows' production performances in France and Spain.

For France, whatever the gestation period and heat stress indicators considered, the impact of heat stress during the dam's gestation did not result in a drop of more than 1.2% of the average 305-day milk for Holstein. The effect was not sufficiently strong to extend the studies to the individual level.
In Spain, the estimated magnitudes of the effects depended on the thermal load used. When the proxy used to model the environmental stress triggering epigenetic marks were the Month of birth of dams and granddams the effects were statistically significant but negligible. However, when thermal loads were represented by temperatures during post-conception and the last period of gestation, the suggested epigenetic inherited marks have a negative impact of up to 1.2 % of the mean of milk production, 2.4 % of the mean of total protein and 3% of total fat. The variance of the maternal environmental effect was found to be of 1.5 to 7.7% of the genetic variance, with an increase in impact according to the lactation number. However, this maternal environmental effect is not specifically related to heat stress.

Both French and Spanish results indicate a negative effects of heat stress in early gestation. However, only epigenotyping will determine whether this is the result of a maternal environmental effect or/and epigenetic change caused by a restrictive *in utero* environment.

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7. Appendices

Appendix 1: General description of the French performances

- **Effects of THI during gestation on cow phenotypes**

The analyzed data correspond to production and reproduction performances of genotyped females born to dams in gestation between 2015 and 2020. The dams and their offspring were purebred Holstein and Montbeliarde females, each pure breed was studied separately. The dams' gestations lasting less than 270 days were removed.

The progeny traits analyzed were as follows: three 305-d production traits: milk yield (MY), fat yield (FY) and protein yield (PY) and two fertility traits: conception rate after the first insemination for heifers (CRh) and cows (CRc). Conception rates resulting from an insemination with male-sexed semen were very limited and not randomly distributed among lactating cows, therefore they were discarded. Only purebred inseminations were considered. For heifers, only first inseminations performed between 12 and 36 months of age were kept. For primiparous, only cows that calved between 21 and 48 months of age and first inseminated between 15 and 700 days after calving were retained. All 305-d production were discarded for lactation of less than 250 days.

For each trait, the contemporary groups, the herd-year, with fewer than three cows were removed.

Descriptive statistics are presented in Table A1.

Table A1. Number of females and means and standard deviations (SD) by trait in Holstein and Montbeliarde.

	Holstein			Montbeliarde		
	Number	Mean	SD	Number	Mean	SD
Milk yield	214,151	7876	1467	69,848	6825	1268
Fat yield	214,151	315	58	69,848	265	52
Protein yield	214,151	254	48	69,848	228	44
Conception rate in heifers	419,624	57.3	49.5	149,987	58.3	49.3
Conception rate in cows	284,337	49.0	50.0	76,678	56.1	49.6

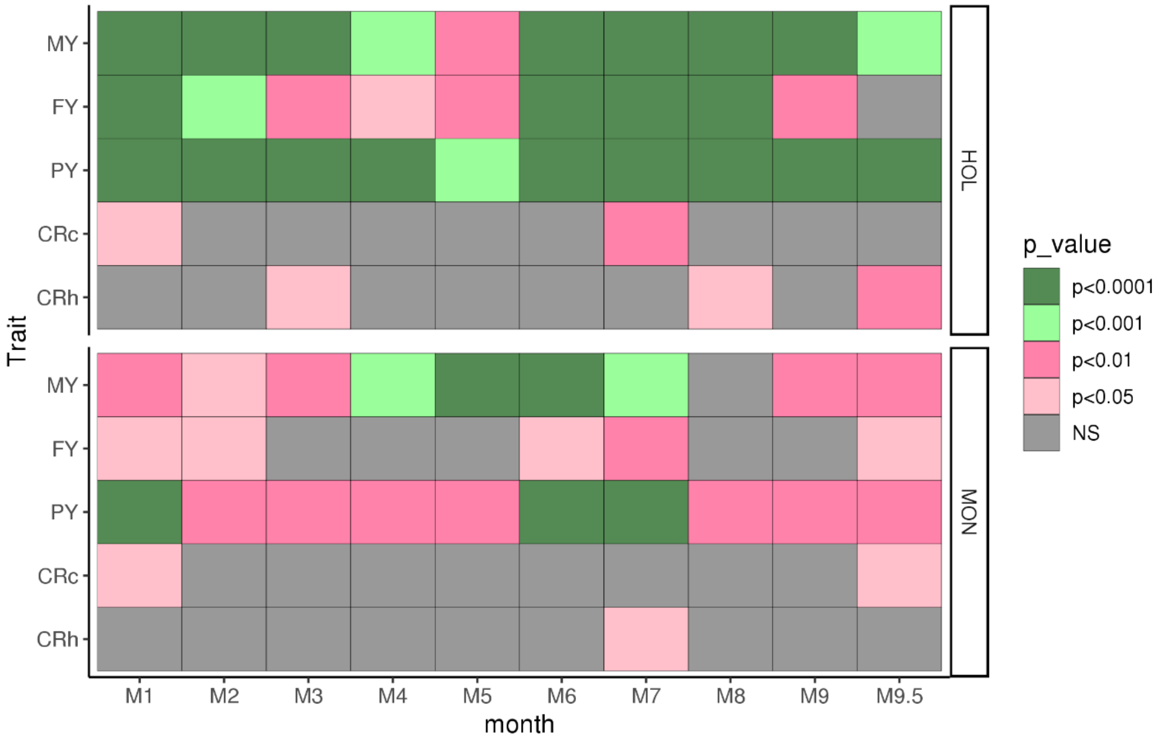
- **Effects of heat stress during gestation on cow phenotypes**

A partial extraction of data from the national database was carried out according to ICAR protocols for milk recording, geographical areas, and birth months. Lowland dairy areas where grazing is widely practiced were chosen, to ensure the greatest consistency between the weather data (recorded outdoors under shelter) and the animals' experiences. This corresponds to the following regions: North, Normandie, Brittany, Pays de la Loire, Poitou-Charentes and Lorraine.

Milk performances of at least 305 days of lactation and based on a minimum of 7 test-day records were selected, for Holstein and Montbeliarde in first, second and third lactation. After selection, the dataset consisted of 358 547 Holstein cows and 10 718 Montbeliarde cows in the period 2010 – 2020, including all parities

Appendix 2: Complementary results for the effects of THI during gestation on cow phenotypes

Figure A2.1. p-values of the effect of the average temperature-humidity index (THI) by month of the gestation of the dam on the progeny performances for both breed, Holstein (HOL, top) and Montbeliarde (MON, bottom).

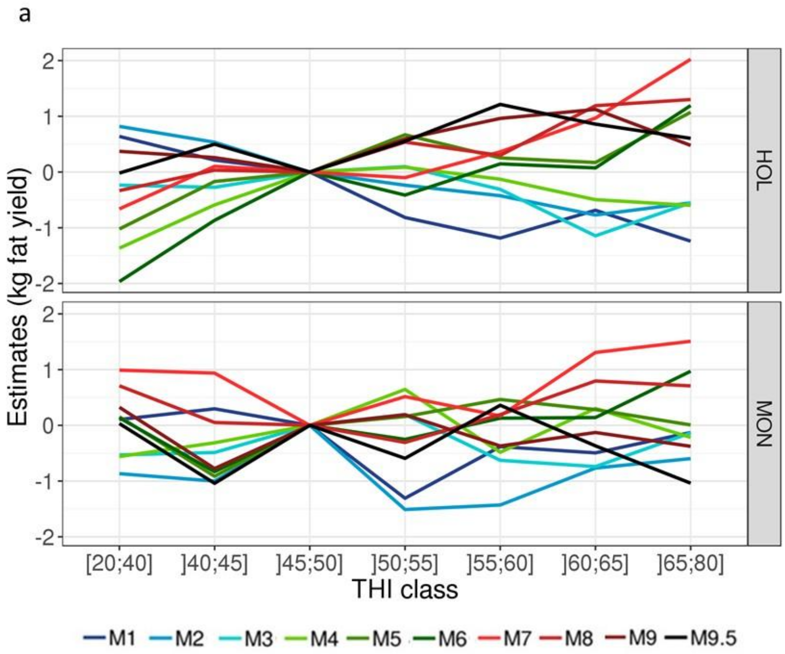


Results are given by colors with grey cells indicating that the null hypothesis (i.e. the absence of effect of the THI during the considered period) cannot be rejected at the 5% level of significance.

M1: from d1 to d30 of the gestation of the dam; M2: from d31 to d60 of the gestation of the dam; …; M9: from d241 to d270 of the gestation of the dam; and M9.5: from d250 to d279 of the gestation of the dam

NS: not significant at a level of 5%

Figure A2.2: a) Effect of the average temperature-humidity index (THI) during each month of fetal development on first lactation's 305-d fat yield for Holstein (HOL, top) and Montbeliarde (MON, bottom). b) Significance levels associated to each class of THI by month of gestation.



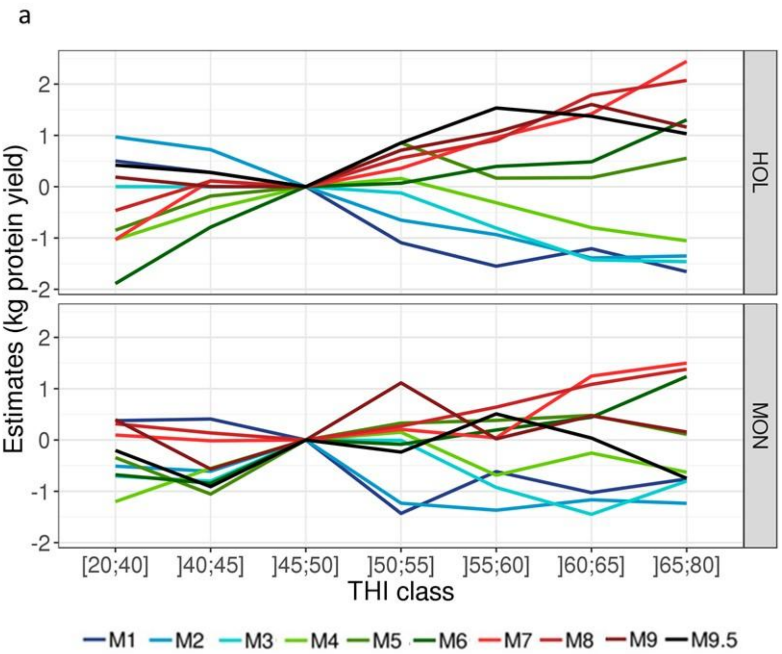
b

		[20;40]	]40;45]	]50;55]	]55;60]	]60;65]	]65;80]
HOL	M1	ns	ns	**	***	*	***
	M2	*	*	ns	ns	*	ns
	M3	ns	ns	ns	ns	***	ns
	M4	*	ns	ns	ns	ns	ns
	M5	ns	ns	**	ns	ns	***
	M6	**	*	ns	ns	ns	***
	M7	ns	ns	ns	ns	***	***
	M8	ns	ns	ns	ns	***	***
	M9	ns	ns	*	**	***	ns
	M95	ns	ns	ns	**	*	ns
MON	M1	ns	ns	*	ns	ns	ns
	M2	*	*	**	*	ns	ns
	M3	ns	ns	ns	ns	ns	ns
	M4	ns	ns	ns	ns	ns	ns
	M5	ns	*	ns	ns	ns	ns
	M6	ns	ns	ns	ns	ns	*
	M7	*	*	ns	ns	**	**
	M8	ns	ns	ns	ns	ns	ns
	M9	ns	ns	ns	ns	ns	ns
	M95	ns	*	ns	ns	ns	**

M1: from d1 to d30 of the gestation of the dam; M2: from d31 to d60 of the gestation of the dam; …; M9: from d241 to d270 of the gestation of the dam; and M9.5: from d250 to d279 of the gestation of the dam

***: p≤0.001; **: p≤0.01; *: p≤0.05; ns: not significant at a level of 5%

Figure A2.3: a) Effect of the average temperature-humidity index (THI) during each month of fetal development on first lactation's 305-d protein yield for Holstein (HOL, top) and Montbeliarde (MON, bottom). b) Significance levels associated to each class of THI by month of gestation.



b

		[20;40]	]40;45]	]50;55]	]55;60]	]60;65]	]65;80]
HOL	M1	ns	ns	***	***	***	***
HOL	M2	**	***	**	***	***	***
HOL	M3	ns	ns	ns	**	***	***
HOL	M4	*	ns	ns	ns	**	***
HOL	M5	ns	ns	***	ns	ns	**
HOL	M6	***	**	ns	*	**	***
HOL	M7	ns	ns	ns	***	***	***
HOL	M8	ns	ns	*	***	***	***
HOL	M9	ns	ns	***	***	***	***
HOL	M95	ns	ns	**	***	***	*
MON	M1	ns	ns	**	ns	*	ns
MON	M2	ns	ns	**	**	*	*
MON	M3	*	*	ns	ns	**	ns
MON	M4	**	ns	ns	ns	ns	ns
MON	M5	ns	**	ns	ns	*	ns
MON	M6	ns	*	ns	ns	ns	***
MON	M7	ns	ns	ns	ns	**	***
MON	M8	ns	ns	ns	ns	*	**
MON	M9	ns	ns	*	ns	ns	ns
MON	M95	ns	*	ns	ns	ns	*

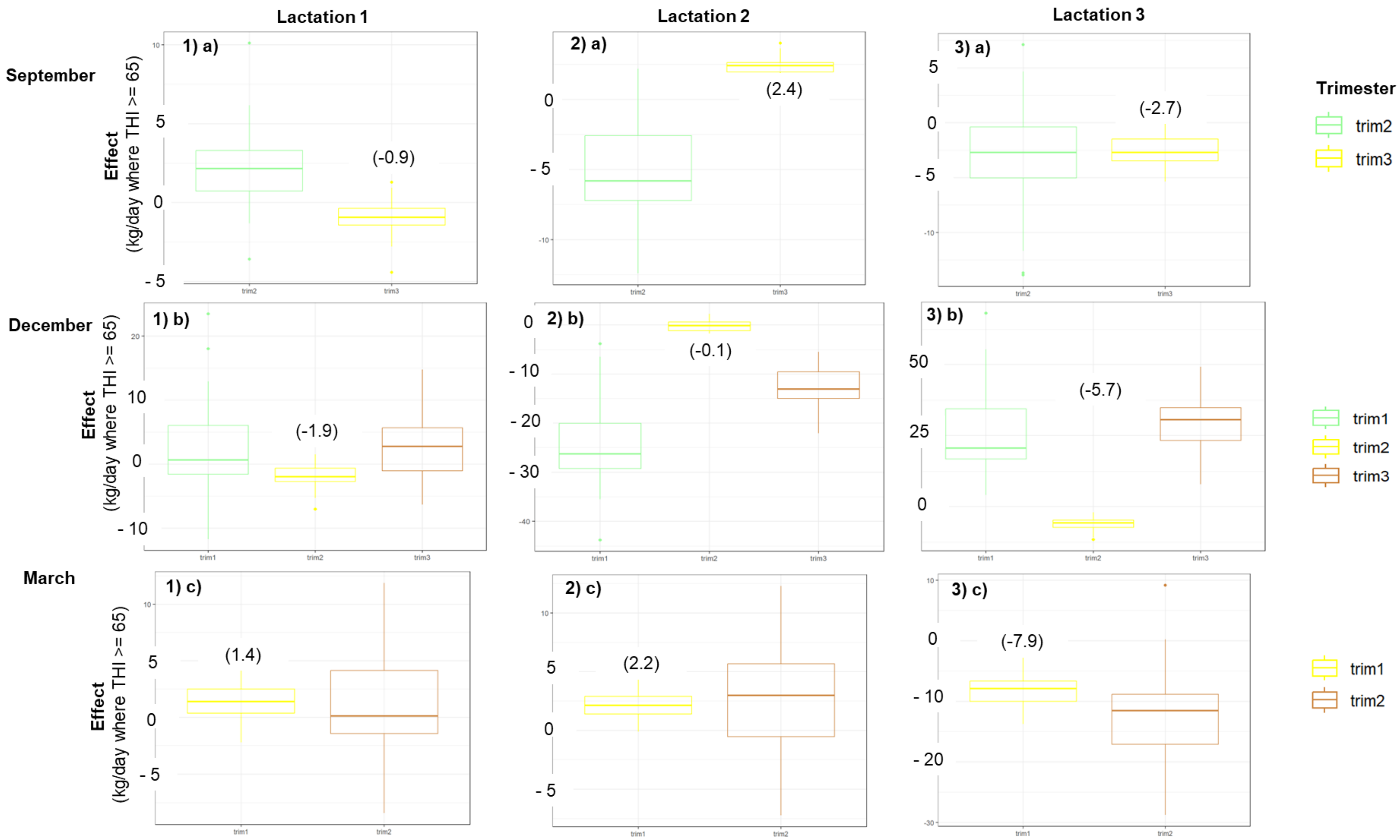
M1: from d1 to d30 of the gestation of the dam; M2: from d31 to d60 of the gestation of the dam; …; M9: from d241 to d270 of the gestation of the dam; and M9.5: from d250 to d279 of the gestation of the dam

***: p≤0.001; **: p≤0.01; *: p≤0.05; ns: not significant at a level of 5%

Appendix 3: Complementary results for the effects of heat stress during gestation on cow phenotypes

Figure A3: Distribution of estimated regression coefficients of NB_THI>65_T1 (trim1), NB_THI>65_T2 (trim2) and NB_THI>65_T3 (trim3), in kg per day with THI above 65, for first (1), second (2) and third (3)-lactation Montbeliarde cows born in September (a), December (b) and March (c).

Median value between brackets for trimester occurring during summer (in yellow)


Lactation 1
Lactation 2
Lactation 3
September
December
March
Effect (kg/day where THI >= 65)
1) a)
(-0.9)
2) a)
(2.4)
3) a)
(-2.7)
1) b)
(-1.9)
2) b)
(-0.1)
3) b)
(-5.7)
1) c)
(1.4)
2) c)
(2.2)
3) c)
(-7.9)
Trimester
trim1
trim2
trim3

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