

**Genetics and Reproduction** 

### Original

## **Relevance of Climatic Stress for Weaned Charolais Animals in Eastern Cuba**

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# ABSTRACT

Background: The accumulated heat load (AHL) of HTI in the 6 months before weaning, on weaning weight (WW) was evaluated in Charolais animals to express heat stress (HS), and assess its possible genetic component. Methods: A total of 19 466 WW records were studied at the Manuel Fajardo Company, in Jiguani, Granma, Cuba. The animals were born between 1988 and 2017, from 16 herds. The reaction norm model was used in the study, considering the fixed effect four-month period-years-herd (859 classes), calf sex (2), and the mother's calving number (11), a fixed co-variable, using a Legendre polynomial (L), order 3, to represent the WW curve response in the AHL trajectory. The random elements evaluated were residue and animal deviation, which produced a record modeled by an order of 1 L. The standardized genetic values (GV) were used to analyze the principal components, while the eigenvector coefficient was used to build an index of the results. **Results:** The regression analysis showed that WW decreased in b=-0.807±0.04 kg per AHL unit ( $R^2=90.2\%$ ). Three different zones were established: cold CCA<=439; neutral CCA>=440 to <=450; and hot CCA >=451, having a PD= 149.3; 140.5, and 134.1 kg, respectively. The GV variation was greater in the cold zone, but the correlations were very low (0.33), evidencing genotype-environment interaction (Ige). Conclusions: This study demonstrated the existence of a genetic base for HS with Ige manifestations, and changed the merit order in extreme conditions.

Keywords: Charolais, Cuba, heat stress, reaction norm, weaning weight (Source: AGROVOC)

# **INTRODUCTION**

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According to Thornton *et al.* (2022), the effects of climatic stress have become a serious obstacle accounting for losses of approximately 10% of the commercial value of milk and meat in the animal sector worldwide, thus explaining the major efforts made in this regard. The trends have been the same in the Caribbean and Central America (UNEP, 2008), where the collateral effects can also affect a large part of the habitat and geographical areas covering many big and small islands. In Cuba, climate change predictions are similar (Roura-Pérez *et al.*, 2020). According to Milera (2011), it will be seen all over agriculture, including cattle raising, affecting pasture and forage yields, as the main components of cattle nutrition. it explains the marked decrease in meat and milk production observed in the nation (Sabina Roque, 2017a, b).

Charolais has been one of the most significant French breeds raised in Cuba since the 1900s, and for over a century, its main herd settled in Jiguani, Granma province. Since then, it has adapted properly to the country's environmental conditions, keeping its meat-producing potential, which was corroborated in a comparative study done with the French Charolais (Renand *et al.*, 1997). these results show the high merit of this breed in terms of meat production. In the region, there is evidence of a worsening of climatic conditions over time (Suarez-Tronco *et al.*, 2021), which could also lead to heat stress (HS) on cattle, also demonstrated by Suarez-Tronco *et al.* (2022a, b) in nationally-bred animals (Criollo), living on the same farm, in different areas. These authors used an index that combines the air temperature and relative humidity, known as THI, by estimating significant negative effects on live weight at weaning (WW), and 18 months of age in this breed, with proven adaptation to the tropic. Therefore, studying the same issue in Charolais could be useful.

The HS results on Criollo cattle coincide with the reports of Santana *et al* (2015) in Brazil, which match the approach and general conclusions that the selection programs producing very specialized animals, show negative collateral effects in other economically important traits, and have a lower adaptative capacity (Rauw *et al.*, 1998).

There are suitable statistical methods to estimate the genetic components linked to HT from the original results of this topic, according to Ravagnolo and Misztal (2000), with monthly production data in Holstein, but the intrinsic characteristics of meat production systems demand different perspectives since the relevant economic traits are quantified as the final result of a process (for instance, live weight at a set age). The evidence available indicates the antagonism between extreme HS and WW levels (Santana *et al.*, 2015; Suárez Tronco *et al.*, 2022b) in breeds known to be well adapted to heat in Brazil (Nellore and crossings), and in Cuba, with the Criollo, whereas, Bradford *et al.* (2016) showed the same pattern in Aungus animals in the USA.

As to WW, it has a composite character depending on the animal's genotype and other components with an environmental nature related to calves, though genetically linked to their mothers' milk production. The two effects were included in a study conducted by Santana *et al.* (2015), who also used the THI accumulative effects (AHL<sub>THI</sub>) withstood by the animal in the months before weaning, to measure the relevance of HS. The authors demonstrated the existence

of negative relations, both phenotypical and genetic between  $CCA_{THI}$  and WW among three types of animals that adapted well to the conditions of Brazil. the same trend was reported by Suárez Tronco *et al.* (2022b) in Criollo animals, though the latter did not consider the maternal genetic effects in the analysis.

The study of co-variance components among the direct and maternal genetic effects showed quite a few difficulties due to data structure and distribution (Meyer 1992), which may even be more complex considering the estimates of these parameters on a climatic scale. However, it is necessary to find alternatives that help determine the direct genetic merit of animals against a set of environmental levels.

Accordingly, this study aims to evaluate the effect of HS on weight at weaning in Charolais animals and to estimate the genetic co-variance factors along the trajectory of AHL<sub>ITH</sub> six months before weaning.

# **MATERIALS AND METHODS**

**Production data:** The study was based on 22 960 records of WW in thoroughbred Charolais animals, collected from the database of the genetic evaluation program for this breed. Overall, 19 466 records from the Manuel Fajardo Company, in Jiguani, Granma, were selected, where a major herd of this breed is located. The data were cleaned by removing the records outside  $\pm 3.2$  WW deviation. The data set came from the progenies of 157 studs and 6 298 cows that calved between January 1988 and May 2017, from 16 herds belonging to the company. The pedigree included 34 451 animals.

**Climatic data:** These data were used by Suarez Tronco *et al.* (2021), consisting of the average monthly estimates of air temperature (Ta) in °C, and relative humidity (HR) expression in percentage, which combined into an index known as HTI, following the same procedure used by Suárez Tronco *et al* (2021): **HTI = 0.81\*Ta + (HR/100) \* (Ta – 14.4) + 46.4** where the terms were already defined. This indicator was used to estimate the accumulated heat load (AHL<sub>HTI</sub>) withstood by the animal 6 months before calving (wm), the formula used was,

$$CCAITH = \sum_{md=i}^{i-6} ITH_{md_1}$$

The results of AHL<sub>HTI</sub> were in 41 levels, between AHL<sub>HTI</sub> =429 and AHL<sub>HTI</sub> =470.

**Final data:** The climatic and productive records were put together and formed the database to be used (Table 1). The weaning months were grouped in four-month periods, which then were added to the years and herds to make the same age groups (SA), each of which had a minimum of 5 records represented by more than one stud.

### Table 1. Overall indicators of the database available

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	Indicator*
Number of animals	19 408
Number of animals in the Pedigree	34 151
Number of studs	157
Number of mothers	6 298
Number of age groups	858
Number of years	28 (1988 - 2017)
Number of AHL levels	41 (429 - 470)
Calving age (years)	11 (2 - 12)
Weaning weight (kg)	146 (28)
Age at weaning (days)	199 (15)

\*between parentheses: amplitude and standard deviation for weight at weaning.

The data were analyzed through random linear models by SAS *proc glm*, to estimate the minimal-quadratic constants for WW and describe the response curves throughout the environmental factors free from genetic effects. The basic model was the following:

$$\mathbf{y}_{PD} = \mathbf{fixed}_{i} + \mathbf{CCAITH}_{x_{j}} + \beta_{1} \operatorname{age}_{k} + \operatorname{age}_{l} + \mathbf{father}_{m} + \operatorname{cattle}_{ijklm}$$
(1)

Where:

 $y_{pd}$  is the dependent variable (weight at weaning).

fixed<sub>i</sub>: represent the effects of the same age group (858 levels) and the calf sex (2).

**AHLHTI**  $x_j$ : is the fixed effect of j<sup>th</sup> levels of accumulated heat load (42 levels).

 $\beta_1$  age<sub>k</sub> : is a linear co-variable of age at weaning.

### agep1

: is the effect of the 1<sup>th</sup> class of age at calving in years (11 categories).

#### fatherm

: is the random effect of m<sup>th</sup> calf's father (157 levels).

cattleijklm: is the common residual effect in every observation.

The genetic analysis was similar to the one previously reported by Suarez *et al.* (2022), using ASreml 4.2 (Gilmour *et al.*, 2015), with the following animal model in the reaction norm (RN),

$$\mathbf{Y}_{ijkl} = \mathbf{G}\mathbf{C}_i + \mathbf{fixed}_j + \sum_{r=0}^{3} \Phi_r \mathbf{b}_{lr} + \sum_{r=0}^{1} \Phi_r \mathbf{a}_{kr} + \mathbf{W}_p + \mathbf{e}_{ijkl}$$
(2)

Where:

 $Y_{ijkl}$  represents the l<sup>th</sup> observation of WW in each animal. GCi is the i<sup>th</sup> herd-year-season weaning combination; fixed<sub>j</sub> includes the fixed effects of the cow's calving age, calf's sex, and a

fixed linear co-variable of age at weaning. The climatic variable CA<sub>HTI</sub> was modeled according to a fixed regression equation  $\mathbf{b}_1$  expressed through a Legendre polynomial  $\Phi$  order  $\mathbf{r} = 3$  which defines the response curve of all the population from the  $AHL_{HTI}$  on WW;  $W_p$  represents the permanent environmental random effect of the mother. Moreover,  $\mathbf{a}_{\mathbf{kr}}$  is the random deviation of each animal regarding the response curve mentioned throughout the trajectory of AHL<sub>HTI</sub>, in this case, modeled by a Legendre polynomial  $\Phi$ , order  $\mathbf{r} = 1$ , expressed in a -1 to +1 scale. (Both  $\Phi$ are automatically given by asreml). This  $\mathbf{a}_{\mathbf{k}\mathbf{r}}$  is the solution to model 1 and equals the genetic merit of the animal of the reaction norm for the intercept  $(a_i)$  and the slope  $(a_sP)$ , termed genetic function  $\mathbf{f}_{g} = [\mathbf{a}_{i} \ \mathbf{a}_{s}]$  for this TS indicator. Finally,  $\mathbf{e}_{ijkl}$  is a random residual effect common to all observations, and not correlated to the fixed effects. A similar model including the father of the cow used as a random effect was not significant.

In this model, 2 of RN, it is assumed that

$$\sigma_{y}^{2} = \{ \mathbf{A} \otimes \mathbf{K}_{\mathsf{G}} = \Phi \begin{bmatrix} \sigma_{i}^{2} & \sigma_{is} \\ \sigma_{si} & \sigma_{s}^{2} \end{bmatrix} \Phi' + \mathbf{I}_{w} \sigma_{p}^{2} + \mathbf{I}_{n} \sigma_{e}^{2} \}$$

in which  $\sigma_y^2$  is the genetic variance, A is the kinship matrix denominator;  $\mathbf{I}_w$  and  $\mathbf{I}_n$  are identity matrix order w (number of mothers) and **n** (number of observations), and  $\sigma_{e}^{2}$  is the residual variance. In this model, it is necessary to implement an additional procedure suggested by Jamrozik and Schaeffer (1997) through the  $K_g$  matrix, which allows for the estimation of covariance components in every point of the scale of climatic variable AHL<sub>HTI</sub>, expressed in terms of Legendre polynomial, order r = 1, which previously indicated that it contained the elements about a linear equation with a specific meaning.

 $\sigma_i^2$  and  $\sigma_s^2$  correspond to the genetic variances for the intercept (overall genetic level of WW in the AHL<sub>HTI</sub> scale), and the slope, respectively, representing the animal's reaction norm to modify the variance evolution of WW along the trajectory of different AHL<sub>HTI</sub> levels. In other words, this measures the animal's genetic capacity at a greater or lesser tolerance to heat stress.

 $\sigma_{is} = \sigma_{si}$  represent the genetic co-variance between the genetic level and tolerance to heat stress.

Matrix K<sub>G</sub> is 2x2, compatible with the matrix  $\Phi$  from AHL<sub>HTI</sub> (41x2), so their manipulation is helpful to estimate the genetic co-variances and the genetic values of each animal at every point of the trajectory of TS levels quantified through AHL<sub>HTI</sub> Using a classic formula (Falconer and McKay, 1996), the estimates are,

For genetic variance in each *i* point of the AHLHTI scale
$$\sigma_{yi}^2 = \Phi_i K_G \Phi'_i$$
For genetic co-variance in each i and j point of the AHLHTI scale $\sigma_{ij} = \Phi_i K_G \Phi'_j$ 

For genetic co-variance in each i and j point of the AHL<sub>HTI</sub> scale

For heredity in each i point of the AHL<sub>HTI</sub> scale

$$\mathbf{h}_{i}^{2} = \frac{\sigma_{yi}^{2}}{\sigma_{y1}^{2} + \sigma_{w+}^{2} \sigma_{e}^{2}}$$
$$\mathbf{r}_{g_{ij}} = \frac{\sigma_{ij}}{\sqrt{\sigma_{yi}^{2} * \sigma_{yj}^{2}}}$$

For genetic correlations between the i and j points of the  $AHL_{HTI}$  scale

As a solution to model 1 (previously shown), a genetic function was obtained ( $\mathbf{f_g}=[\mathbf{a_i} \ \mathbf{a_s}]$ ) in each of the 34 151 animal records and their predecessors without recorded history, whose elements were common to a linear genetic regression equation; that is, a 34 151x2 matrix. Based on these results and the matrix  $\mathbf{\Phi_i}$  of the genetic component, whose elements can be identified as  $\mathbf{L_i}$  and  $\mathbf{L_s}$  for the intercept and the slope, respectively, the genetic value (VG<sub>i</sub>) can be estimated in every *i* point of the AHL<sub>HTI</sub> trajectory.

### $\mathbf{GV}_i = [\mathbf{L}_i \quad \mathbf{L}_s] * [\mathbf{a}_i \quad \mathbf{a}_s]'$

This procedure is useful to determine all the parameters and  $VG_i$  from each animal along every AHL<sub>HTI</sub> trajectory point. These results were analyzed through principal component analysis (PCA), using Matlab (2019) and the eigenvector coefficients corresponding to the most significant eigenvalues, and used as weighting factors of VG<sub>i</sub> for synthesis of VG<sub>i</sub> results in one index, in all the AHL<sub>HTI</sub>, following the proposal of Togashi and Lin (2006). This new index (**Ipct**<sub>i</sub>) is represented as follows for every i<sup>th</sup> principal component:

$$\mathbf{I}_{\mathsf{pct}_1} = \mathbf{ev}_1' * \mathsf{GV}_1 + \mathbf{ev}_2' * \mathsf{GV}_2 + \dots \mathbf{ev}_n' * \mathsf{GV}_n$$

where  $ev_i$  are the eigenvector coefficients of each principal component and the VG<sub>i</sub> had already been defined and expressed in a standard way.

### **RESULTS AND DISCUSSION**

The analysis made according to model 1 of fixed effects demonstrates that  $AHL_{HTI}$  the animals underwent for 6 months before weaning (figure 1), showed a more or less stable response in the first 11 years represented, which changed from 2010 when an  $AHL_{HTI}$  increasing trend was observed in keeping with the climatic changes expected for the country (Roura-Pérez *et al.*, 2020). On the contrary, WW decreased significantly throughout the whole period, showing the same response as the others.

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Figure 1. Weaning weight and accumulated heat stress timeline

The AHL<sub>HTI</sub> effects on WW were highly significant, and the values adjusted due to all the effects in model 1, are shown in figure 2. The response curve is apparently negative and the regression analysis of the constants square minimal of weighted WW for the number of records in each AHL<sub>HTI</sub> showed that decreased (b=-0.807±0.04 kg) per AHL<sub>HTI</sub> unit (R<sup>2</sup>=90.2%). Even with the high adjustment level, three areas may be differentiated: cold (AHL<sub>ITH</sub><=439), neutral AHL<sub>HTI</sub>>=440 to <=450), and hot AHL<sub>ITH</sub>>=451), which were included as substitutes for the AHL<sub>ITH</sub> effect, and the same previous model was applied. The results were highly significant, with WW estimates of 149.3; 140.5, and 134.1 kg in the cold, neutral, and hot zones, respectively. The magnitude of this depressive effect is higher than the one indicated by Suárez Tronco *et al.* (2022a) in Criollo cattle, at the same company, while the overall impact of the negative response could be inferred, considering that the database represented, 27.7% of the animals stayed in the hot zone.



Figure 2. Minimal quadratic constants of AHL<sub>HTI</sub> effect on the weaning weight in Charolais animals

The results of the genetic model showed the existence of a major genetic variability in terms of animal tolerance to changes in the  $AHL_{HTI}$  levels. Heredity (h<sup>2</sup>) showed a decreasing curve as the level of heat undergone by the animals raised in the 6 months before weaning (figure 3). This form of response with maximum h<sup>2</sup> values on the two ends of the  $AHL_{HTI}$  scale was the typical value reported in other studies using random regression models in meat and dairy cattle (see the excellent discussions of Meyer, 2005 and Li *et al.*, 2020, respectively), which may be explained by intrinsic problems of Legendre polynomials when the data are distributed unequally. In comparative terms, the estimates in Figure 3 followed the same pattern reported by Suárez *et al.* (2022b) in Criollo cattle, in this region, whereas Santana *et al.*, (2005) showed similar trends, but higher estimates in crossbred animals with different proportions of B. *indicus* in Brazil.

The parameters shown in Figure 3 suggest the existence of greater space for any selection process when the environment is more favorable (AHL<sub>HTI</sub> cold zone). However, these possibilities are limited because the genetic correlations dropped to levels below 0.8, when the AHL<sub>HTI</sub> >=450, which is the limit set (Robertson, 1959) to indicate the existence of genotype-environment interaction (Ige).

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Figure 3. Heredity estimate\* and genetic correlation for weaning weight in Charolais animals along the accumulated stress for 6 months before weaning \*Standard errors of H<sup>2</sup> varied between 0.01 and 0.03.

The existence of Ige is an actual limitation demonstrated in several animal production scenarios (see the excellent review of Fodor *et al.*, 2023). According to these authors, interaction linked to climatic environmental effects is one of the most frequently studied, in which the reaction norm models (RN), such as the one used in this research, have been widely used. The inclusion of the RN could provide GV results in all the animals, in all the AHL<sub>HTI</sub> trajectory points, which underwent PCA resulting in 89.1% and 10.9% variability in the 41 GV estimates van be explained by the first two eigenvalues, so their corresponding coefficients can be used as a weighting factor for Ipc (Togashi and Lin, 2006), reported in the previous section. This new indicator facilitates all the selection work without the occurrence of major losses of the original information. Published evidence demonstrates the usefulness of this tool (Savegnago *et al.*, 2013; Boligon *et al.*, 2016) to cope with the issue of Ige. Figure 4 shows GV variability in all the animals, according to the results observed in the cold, neutral, and hot zones (on the diagonal), and the correlations between such GV (above the diagonal). Besides, it shows the estimated GV variability, according to the Ipc indicated.



Figure 4. Variability of Genetic Factors\* in animals, according to the results observed in the cold, neutral, and hot zones, and the overall index and correlations and evolution of GV along the AHL<sub>HTI</sub> scale in the best 300 animals.

\*The GV on the diagonal, the correlations above the diagonal, and the distribution of GV along the AHL<sub>HTI</sub> trajectory, under the diagonal (details in the text).

Several elements are highlighted. First, GV was greater in the cold zone than in the neutral and hot zones, whose magnitude accounts for 19.3%, 12.1%, and 10.4% of the minimal quadratic means, previously indicated in these zones. However, the correlations were very low (0.33), particularly at the end levels. caused by Ige. In the lower side of the distributions, GV evolution is represented throughout the  $AHL_{HTI}$  trajectory in the best 300 animals selected in each zone. In the cold zone, there is a clear decreasing trend as TS increases, whereas, in the hot zone, there is a marked amplitude reaching negative animals as an Ige demonstration.

In the lower right side (figure 4), there is total genetic variability in the database at all  $AHL_{HTI}$  levels, expressed in a single Ipe indicator, which has the highest correlations with the rest of the zones. Here, variability matches  $0,189\pm0.001$ ;  $0.151\pm0.001$ , and  $0.119\pm0.001$  WW per each  $AHL_{HTI}$  unit, according to the regression analysis. The best 300 animals were selected based on their Ipc and GV distribution in the same animals in each  $AHL_{HTI}$  point in the lower left side (figure 4). Distribution simulates the same pattern in the neutral zone, but its width in the extreme zones does not include the negative values, which is additional evidence of the advantages of this procedure to make Ipc.

The results could be useful for the breeding program in this breed, though, for practical reasons, the most recommendable idea is to examine the variations between parent studs, as the optimum way that favors all the population. For example, the best 30 studs were selected according to their genetic merit by Ipc, and the results are shown in Figure 5.



Figure 5. Genetic values of the best 30 studs selected according to their Ipc and evolution in the climatic stress scale 6 months before weaning

Among them, 21 studs with 30-730 progenies were identified, which showed different heat stress tolerance responses shown by their progenies in the 6 months before weaning. Two major behavior patterns could be made. On one hand, there was a main group of studs named Robusts, whose progenies maintained a uniform behavior through  $AHL_{HTI}$ , which must be the response based on lessening the effects of Ige. On the other hand, there was a Plastic group, whose response could be contrasting. This type of result corresponds to the evidence published by Santana *et al.*, (2015) and Suárez Tronco *et al.*, (2022b), in Brazilian and Cuban meat-producing animals.

# CONCLUSIONS

This study showed the existence of a significant depressive effect on weaning weight in Charolais animals in Cuba caused by accumulated heat stress in the 6 months before weaning. The results indicate the existence of a genetic base for tolerance to this climatic effect, with genotypeenvironment interactions (Ige) that change the merit order of the best animals in extreme conditions, from hot to cold zones. The magnitude of this depressive effect is -15.4 kg (10.4% of the mean) in the animals from the cold zone, which is not unfavorable at all, considering that near 28% of the sample was in that climatic zone. The utilization of a reaction norm model is highly recommendable for the genetic evaluation procedure in this breed to identify Robust studs, whose progenies maintained a uniform behavior through  $AHL_{HTI}$ , which must be the response based on lessening the effects of Ige.

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### AUTHOR CONTRIBUTION STATEMENT

Research conception and design: MAST, AMB; data analysis and interpretation: MAST, MRC.; AMB; redaction of the manuscript: MAST, AMB.

### **CONFLICT OF INTEREST STATEMENT**

The authors state there are no conflicts of interest whatsoever.