



### ABSTRACT

A total of 245187 records of the first three lactations of 131126 Holstein cows in tropical conditions were used to estimate the genetic (co)variance components between age at calving and its respective milk productions. A multivariate analysis of six traits was performed. Heritability for age at calving  $(h_{AC}^2)$  and milk yield  $(h_{MY}^2)$  decrease from the first  $(h_{AC1}^2=0.254\pm0.007; h_{MY1}^2=0.198\pm0.003)$  to the second  $(h_{CA2}^2=0.242\pm0.006; h_{MY2}^2=0.184\pm0.003)$  and third lactation  $(h_{CA3}^2=0.232\pm0.006; h_{MY3}^2=0.168\pm0.008)$ . The genetic correlations (rg) between the AC1; AC2 and AC3 and between MY1; MY2 and MY3 were high and positive; however, the rg between age at calving and milk production were medium but negative. The first two eigenvalues accounted for 83% of the total genetic variance of the six traits. The corresponding eigenvectors were used as weighting factors, and two underlying expected breeding values (EBV) were estimated based on these components, allowing the identification of those sires that reduced ages at calving and increased milk yield in their daughters. A new approach was developed to estimate an equivalent of calving interval (CIeq) based on the differences between the EBV of the sire for two consecutive ages at calving.

KEY WORDS calving, genetic correlations, heritability, Holstein, milk production.

# INTRODUCTION

The age at first calving (AFC) represents a fundamental step for a cow since is the beginning of its productive life. AFC is a complex trait reflecting the maturity rate at which the animal reaches its incorporation into reproductive life as well as its fertility to achieve its first pregnancy and calving. A decrease in AFC can represent a reduction of 18% of the total cost of raising dairy replacements (Tozer and Heinrichs, 2001); however, within certain limits, greater reductions in AFC may be less effective. Even though in specialized dairy populations cows have first calving around 2 years of age, results have been published with the

tendency to reduce this indicator to 20-21 months (Ettema and Santos, 2004). However, according to a recent review of this subject (Curran *et al.* 2013), no clear answer is available, because an AFC at 21 months can produce significant economic benefits in some herds, and in others the response could be the opposite.

Evidence has been published on the evolution of AFC as a function of time. In Costa Rica, AFC in Holstein cows was 30 to 31 months with a tendency to decrease from 2006 to 2007 (Salazar-Carranza *et al.* 2013). In the United States of America (USA), AFC was reduced from 25.9 months in 1991 to 25.4 months in 2002 (USDA, 2009). In a more recent publication (Hare *et al.* 2006), convincing results

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showed a very marked decline of AFC in major dairy cow breeds of the USA. Nevertheless, these apparent advantages were annulled due to the increase in the first calving interval. Similar trends have occurred in South Africa (Makgahlela et al. 2008), Spain (González-Recio et al. 2004), and England (Wall et al. 2003). This unfavorable association may result in a collateral negative response in fertility traits due to higher selection emphasis for milk yield (Lucy, 2001). The results of a world survey on fertility in Holstein cattle (Sorensen et al. 2007) shown that fertility so far has been a real problem in the Holstein populations around the world. These authors suggest that action needs to be taken, both internationally as well as within each country, in order to change the decline of fertility. One year later Berglund (2008) present a general alternative to genetic improvement of fertility traits in dairy cattle and suggest that new traits and new methods need to be considered. In that context, the use of a multi trait setting could be a very useful procedure as was presented by Haas et al. (2007) using a character with a high level of  $h^2$  as body condition scores to improve reproduction performance. The statistical models used to estimate genetic parameters of several economic traits in dairy cattle, the AFC has been included as a fixed effect, which implicitly assumes no genetic variations in AFC; however, the available literature showed some variation in heritability (h<sup>2</sup>) values. In Holstein cattle under tropical conditions of Sudan, it has been reported for  $h^2$  AFC a range from 0.09 to 0.19 (Abdel *et al.*) 2007; Ibrahim et al. 2012; respectively). Different results are reported by Do et al. (2013) in Korean Holstein with  $h^2 = 0.11$ , in South Africa  $h^2 = 0.24$  (Makgahlela *et al.* 2008), in Thailand (Buaban et al. 2015) show  $h^2 = 0.25$  for the same trait and in the Mexican tropics, h<sup>2</sup> for AFC was 0.28 (Estrada-León et al. 2008). In Iranian Holstein cattle, h<sup>2</sup> values for AFC were 0.19 to 0.36 (Solemani-Baghshah et al. 2014; Toosi, 2002; respectively) have been reported. On the other hand, in 94 studies done in tropical regions with both beef and dairy cattle breeds, average h<sup>2</sup> for AFC was 0.31 (Lobo et al. 2000). All these references are coherent to shows the existence of a high additive genetic variance that can provide space for selection and breeding, therefore it seems that results from AFC can be used as a second trait in order to improve reproduction performance. Nevertheless, the publications cited in the previous paragraph have pointed out a negative relationship between AFC and the first calving interval and milk yield (MY) in the first two lactations.

The calving interval (CI) is estimated as a function of the difference between two ages at calving of the same cows, however these approaches will be a source of bias to genetic (co)variance estimation between AFC, CI and MY, because some cows are culling for milk yield or fail to be pregnant for second time.

Our objectives were to estimate the genetic parameters for age at calving during the first three calving and their corresponding milk yield in Holstein cattle under Cuban tropical conditions, using a six traits sire model with all data available, irrespectively if the cows were recorded for all traits. To make inferences about CI as an indicator of reproductive performance, we developed a new approach to estimate a calving interval equivalence (CIeq) based in the differences between estimated sire breeding values (EBV) for the first three AFC.

## MATERIALS AND METHODS

Data consisted of 245187 records of the first three lactations of 131126 Holstein cows (daughters of 1765 sires; 2526 in the pedigree) calved from January 1985 to December 2003 in 1106 dairy herds in Cuba. Distribution for the first three calving data is shown in Figure 1.



Figure 1 Data distribution according to age at calving-months of Holstein cows in the tropics

The cows were cared for under standards comparable to the guidelines established by the Canadian Council on Animal Care. Data of animals without date of birth or date of calving and herds with less than 20 records were deleted. Age at calving (ACi, where i=1, 2 and 3 calving) was estimated by the differences between calving data and birth dates. From each animal, the records of milk yield (MYi, where i=1, 2 and 3 lactations) were considered. The variables ACi and MYi were edited eliminating those records out of range of  $\pm 3.2$  standard deviations. Finally, a total of 93281, 76561, and 59421 records were available of first, second, and third calving, respectively. All the statistical analyses were performed using ASREML (Gilmour *et al.* 2009).

Several bi-character sire models were applied and the results were combined to estimate initial parameters and a multivariate analysis of six traits was performed simultaneously. With the results of this last model, the (co)variance components between ACi and MYi were estimated where *i* represent the first, second and third calving. A symbolic multi-trait general model can be described as follows:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_j \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_j \end{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_j \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_j \end{bmatrix} \mathbf{s}_1 \\ \mathbf{s}_j \end{bmatrix} + \begin{bmatrix} \mathbf{H}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{H}_j \end{bmatrix} \mathbf{h}_1 \\ \mathbf{h}_j \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_j \end{bmatrix}$$

Where:

 $y_i$ : vector of  $n \times ij$  of n records and  $j^{th}$  traits (j=1, 2...6) simultaneously analyzed.

 $\mathbf{b}_i$ : vector of  $\mathbf{n} \times \mathbf{ij}$  fixed effects of year and month of parity on each trait.

 $s_i$ : vector of  $\mathbf{n} \times \mathbf{ij}$  correlated random effects due to the additive genetic component. of the sire of each cows (1/4 of additive genetic variance).

 $h_i$ : vector of  $\mathbf{n} \times \mathbf{ij}$  correlated random effects due to the herd.

 $e_j$ : correlated random residual effect for  $j^{th}$  traits.

 $X_i Z_i$  and  $H_i$ : incidence matrices connecting the fixed and random effects with the data vector for each  $j^{th}$  trait.

In this model it is assumed that: (2)

$$\operatorname{Var}\begin{bmatrix}\mathbf{s_i}\\\mathbf{h_i}\\\mathbf{e_i}\end{bmatrix} = \begin{bmatrix}\mathbf{G_i} & \mathbf{0} & \mathbf{0}\\\mathbf{0} & \mathbf{H_i} & \mathbf{0}\\\mathbf{0} & \mathbf{0} & \mathbf{R_i}\end{bmatrix}$$

(3)

In which 
$$\mathbf{G}_{i} = \begin{bmatrix} \sigma_{s_{1}}^{2} & \sigma_{s_{j}} \\ \sigma_{s_{ji}} & \sigma_{s_{j}}^{2} \end{bmatrix} \otimes \mathbf{A};$$
  
$$\mathbf{H}_{i} = \begin{bmatrix} \sigma_{h}^{2} & \sigma_{h} \\ \mathbf{\sigma}_{h_{j1}} & \sigma_{h_{j}}^{2} \end{bmatrix} \otimes \mathbf{I}_{h} \text{ and } \mathbf{R}_{i} = \begin{bmatrix} \sigma_{e_{1}}^{2} & \sigma_{e_{1j}} \\ \sigma_{e_{j1}} & \sigma_{e_{j}}^{2} \end{bmatrix} \otimes \mathbf{I}_{h}$$

Where:

 $\sigma_{s_i}^2 \ ; \ \sigma_{h_i}^2 \ ; \ \sigma_{e_i}^2 \ ; \ \sigma_{a_{ji}} \ ; \sigma_{hj_i} \ \text{ and } \ \sigma_{e_{1j}} \ : \ \text{(co)variance ma-}$ 

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trices for genetic, herd, and residual random effects; genetic covariance among  $j^{th}$  traits; environmental covariance herd effects between  $j^{th}$  traits and covariance of residual effects among  $j^{th}$  traits, respectively.

 $I_h$  and  $I_n$ : matrices of order h (number of herds) and n (number of animals).

A: relationship matrix between all sires.

 $\otimes$  : symbol of product.

With these parameters, heritability for each trait  $(h_j^2)$ and genetic correlations  $(\mathbf{r}_g)$  between each pair of **j** traits were estimated by linear functions of the corresponding components using classical formulas (Falconer and McKay, 1996). The coefficients of genetic variation ( $CV_G$ ) were estimated by the square root of the genetic variance of each trait divided by the mean of the same trait and expressed in percentage. The expected breeding value (EBV) for each sire and each trait were estimated as twice the solution of the multi-character model described previously.

## **RESULTS AND DISCUSSION**

In general, the results found in milk production were higher, and the age at first calving was a little more reduced (Table 1) than the values reported in other studies in Holstein cattle of Cuba (Palacios-Espinoza *et al.* 2007) but with lower numbers of records and different statistical model.

The milk production levels reflected lower productivity with respect to Holstein cattle exploited under intensive conditions in arid regions of Sudan (Abdel et al. 2007), in northern Mexico (Palacios-Espinosa et al. 2001), and Brazil (Carneiro et al. 2008) with an average of 3475 to 8000 kg per lactation. Similarly, in Turkey 5541 kg per lactation were reported for first calving cows at 29.8 months (Teke and Murat, 2013). While in the USA Holstein cows reached their first, second, and third calving at 26.9, 40.1 and 53.3 months, respectively (Hare et al. 2006), in Brazil and Colombia ages at first calving of 29.5 and 32.1 months respectively have been reported by Cerón-Muñoz et al. (2004). These differences were related to nutritional deficiencies due to the fact that in the Cuban production system the animals are feed only with low quality pastures. These are the same pattern described three decades ago by Abubakar et al. (1986) with Holstein cattle used in many South American countries.

The main results of the multi-character genetic model are shown in Table 2. The variance components increased as parity number increased, while heritability ( $h^2$ ) showed an opposite trend. The herd was the main source of variation accounting for 16 to 33% for age at calving and from 23 to 48% for milk production. Genetic variances were low and relative to average estimates presented in Table 1, the genetic variation coefficients were from 5 to 7% for age at calving and of 15 to 20% for milk production. Estimates of  $h^2$  for milk production ranged from 0.17 to 0.20 and were higher than previous results presented by Palacios Espinoza *et al.* (2007), due to a larger and better-structured database used in this study, which is reflected by the low standard errors of  $h^2$  for all traits.

Most references about  $h^2$  of age at calving basically referred to the first calving and our results were higher. In Holstein cattle of Brazil and Colombia,  $h^2$  estimated for age at first calving was 0.19 and 0.13, respectively (Cerón-Muñoz *et al.* 2004). No references were available for second and third calving in tropical conditions.

Item	First ca	lving (n=93281)	Second calving (n=7656	51) Third calving (n=59421)
Age at calving, months	:	32.4±4.9	47.2±6.9	61.1±8.1
Milk production in 305 days, kg	264	4.4±1096.6	2657.2±1304.89	2639.7±1382.9
Table 2 Estimates of variance compo	nents (var) and heritability Genetic var	for ages at calving (A Herd var	C) and milk yield (MY) of <b>Total y</b> a	
AC 1	5.609	7.364	22.085	
AC 2	8.631	8.582	35.669	0.242±0.006
AC 3	12.487	8.854	53.826	0.232±0.006
MY 1	166195	406220	839372	0.198±0.003
MY 2	236256	469031	128400	1 0.184±0.003
MY 3	300451	402230	178840	0 0.168±0.008

Table 1 Mean values and standard deviation for each variable of Holstein cows in Cuba

The higher genetic parameters of our study may be related to the fact that very low selection intensity is applied in this breed in Cuba, therefore all existent variability in these traits have been shown in this population.

The joint distributions between EBV together with the genetic correlation of the six traits under study are presented in Figure 2. In this figure the diagonal, shows the variations between EBV of 923 sires with at least 20 daughters; below the diagonal, the distribution of EBV for each pair of characters and above the diagonal are the genetic correlations ( $\mathbf{r}_g$ ) between each one of the six traits from the multivariate model.

Within the group of character belong to age at calving (upper left quadrant) and the other for milk production (lower right quadrant) all  $\mathbf{r}_{g}$  were positive, with higher values for adjacent calving. In general, according to these patterns the AC1; AC2 and AC3 can be considered as the expression of the same traits, but for MY1, MY2 and MY3 the relationship is positive but lower. The upper right quadrant the r<sub>g</sub> between age at calving and milk yield were negative, particularly those for the same calving which were marked in red in the Figure 2, however these not to be interpreted a priori, as an evidence of the antagonism indicated by Lucy (2001). It is worth highlighting the important variability (lower left quadrant) between the joint distributions of EBV for different traits which need to be analyzed because sires can represent an ideal way for a breeding process in this species. From this representation, it is clear that there is much redundant information that can be simplified by a principal component analysis (PCA) and the results are shown in Table 3.

The results indicated that 83% of the genetic variance for six traits could be explained by the linear combination of the first two principal components, a very important variable reduction as a demonstration of the advantages of using PCA. Coefficients of the first two eigenvectors have to be highlighted because they have a precise biological interpretation. The first principal components (PC1) is known as a size vector or general genetic level and in this study the coefficients linked to ages at calving contrasted with those of milk yield as a consequence of the pattern shown in Figure 2. In turn, the second principal component (PC2) is called shape vector explained 28% of the genetic variance with all coefficients were positive. This source of variability in PC2 will be very useful because is related to change of form of response in milk yield and age at calving as we will show below.

In practical terms the coefficients for both principal components were used as a weighing factor on the original EBV (standardized previously) to estimate two new EBV based on the eigenvector coefficients of PC1 and PC2 (EBVPC1 and EBVPC2, respectively). These results can be used to identify those sires that can produce benefits by lowering age at calving and increasing milk yield. Figure 3 shows a bi-plot with these new results.

Figure 3 shows the projection of each observation around coordinates 0.0 corresponding to the average of EBVPC1 and EBVPC2. The directions of change are represented by vectors AC1, AC2, and AC3 and by vectors MY1, MY2, and MY3, highly correlated within each one (small angle between them) and whose meaning was the same but in opposite directions in the context of the database. In this biplot, a group of the best selected sires (at least 40 daughters each) based on EBVPC1 and EBVPC2 were highlighted in bold.

When this group of elite sires was merged with EBV estimated from the original data, the results were  $-1.09 \pm 0.67$ ;  $-1.48 \pm 0.67$ , and  $-2.25 \pm 0.93$  months for AC1, AC2, and AC3, respectively, and  $+269 \pm 61$  kg,  $+389 \pm 133$  kg and  $+627 \pm 67$  kg in MY1, MY2, and MY3, respectively, which confirms the previous expectation. Similar references have not been found to compare the results of this approach, which demonstrates that in the conditions of this database, it is possible to decrease the age at calving without affecting the levels of milk production.



Figure 2 Representation of variations between expected breeding value of sires, relationships between themselves, and genetic correlations between traits of age at calving and milk production of Holstein breed in Cuba

Table 3 Estimates of eigenvalues and eigenvectors of the correlation matrix between the expected breeding values of 923 sires with at least 20 daughters of Holstein cattle in Cuba

Item	Eigenvectors							
	PC1	PC2	PC3	PC4	PC5	PC6		
Age at calving 1	0.4509	0.4047	-0.2391	-0.3540	-0.5346	-0.4053		
Age at calving 2	0.5000	0.3415	0.1092	0.0213	0.0201	0.7877		
Age at calving 3	0.4896	0.1666	0.4096	0.3832	0.4522	-0.4618		
Milk yield 1	-0.3161	0.3248	0.6808	-0.5708	0.0684	-0.0208		
Milk yield 2	-0.3926	0.5147	0.1368	0.6236	-0.4162	0.0007		
Milk yield 3	-0.2296	0.5668	-0.5298	-0.1118	0.5755	-0.0381		
Eigenvalues	3.330	1.668	0.804	0.117	0.066	0.004		
Explained variance, %	55.5	27.8	13.4	1.69	1.1	0.36		
Accumulated variance, %	-	83.3	96.7	98.3	99.4	100		



Figure 3 Expected Breeding Value distribution of 923 sires of Holstein cattle in Cuba with at least 20 daughters each one in terms of the first two principal components

The negative association with the first calving interval (CI) has been identified as a constraint to lowering the age at first calving (Nieuwhof *et al.* 1989; Marini *et al.* 2007).

The CI is calculated by the difference between the date and the age between two successive calving of the cows. That type of animals showed a higher or better reproductive performance respect to those having only a single calving, which may be due to problems of consistency and continuity in the recording system or culling of animals with poor production level; in any case, it may introduce a bias in the analysis of the variation causes of CI. In our study a multitrait sire model was applied in which EBV of each sire for AC1, AC2, and AC3 could be estimated with all available data and the existent variations were shown in Figure 2.



Figure 4 Frequency distribution of the Expected Breeding Value of 923 sires for the first and second calving interval (Figures 4a and 4c), relationship between them (Figure 4b) and the relationships with the Expected Breeding Value for milk yield in the first (Figure 4d) and second calving (Figure 4e)

By analogy to the classical way to CI estimation, it was possible to obtain an equivalent of this reproductive parameter as  $CIeq_1 = EBVAC_2 - EBVAC_1$  and Cieq2 = EB- $VAC_3 - EBVAC_2$  and Figure 4 shows the variations between EBV of sires for these new parameters.

Several aspects should be highlighted from this figure:

(1) Variations between EBV of sires were around 2.8 months for  $Cleq_1$  (Figure 4a) and 3.7 months for  $Cleq_2$  (Figure 4c). In reference to real CI of the total data estimated classically but not analyzed, these magnitudes represented between 19% and 26% for the first and second CI, respectively.

(2) The joint distribution of  $Cleq_1$  and  $Cleq_2$  showed (Figure 4b) that both were highly correlated (r=0.911), so they could be considered as the manifestation of the same trait. The genetic correlation was 0.50, approximately between the first and second CI of Holstein cows of the USA (Short *et al.* 1990) and in Swedish dairy cattle (Strandberg and Danell, 1989).

(3) The distribution of the EBV for  $Cleq_1$  and  $Cleq_2$  with the EBV for MY<sub>1</sub> (Figure 4d) and for MY<sub>2</sub> (Figure 4e) shown a beneficial relationship in the sense that a decrease in calving intervals (by their equivalents in  $Cleq_1$  and  $Cleq_2$ ) did not produce a decrease in milk yield.

The results of this approach using the possibilities of a multivariate analysis to estimate EBV in reproductive traits, particularly the calving interval equivalent as a function of the events that gave it origin (EBV for ages at calving) has shown that generalizing the antagonism between CI and milk yield does not apply in all cases and there are possibilities to identify sires with EBV positive for both trait. This trend is not coherent with the expectations, which is likely to be associated with the low performance of this breed in the current conditions of exploitation, compared to the high potential that Holstein breed shows in countries with intensive production systems.

# CONCLUSION

Holstein cattle performance in Cuban conditions can be classified as poor not only based on its levels of milk yield but also on its reproductive efficiency, showing the first three calving at very late ages. Our study demonstrates that genetic variation in ages at calving can be an important option. It is suggested that breeding programs of Holstein cows must be redirected and should give greater importance to these traits with respect to milk yield. The multivariate procedure applied is highly recommended because it has allowed extracting more information with the same available data, identifying a source of genetic variation that already existed in the population but whose expression had not been quantified.

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