Inbreeding levels and their effects on phenotypic expression in Holstein cattle

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Abstract:

The objective of the present study was to calculate the inbreeding levels in the Holstein population of Mexico and to evaluate their effect on the production of milk, fat, protein and final conformation points. The pedigree information was made up of 326,238 animals, to which inbreeding was calculated through the modified recursive algorithm (INBUPGF90). Inbreeding trends of animals born from 1990 to 2018 were obtained through a regression analysis, and the effect of inbreeding on productive characteristics was evaluated with an analysis of variances, for which phenotypic information from 68,779 animals was included. Six groups were formed according to the level of inbreeding (1= <1%, 2= ≥1 and <2%, 3= ≥2 and <3%, 4= ≥3 and <4%, 5= ≥4 and <5%, and 6 ≥ 5%). The results showed that, for each percentage point of increase in inbreeding, the production of milk, fat and protein decreases by 88, 3.16 and 2.57 kg (P<0.0001). At low levels of inbreeding (<5%), no effect on fat and protein production was detected. However, when inbreeding increased to more than 5 %, the
loss in production was 12 kg of fat and 9 kg in protein. It was also observed that the animals with the lowest average conformation have low levels of inbreeding (<1%) and the highest levels did not show significant differences between them, which confirms that functional conformation is less sensitive to the effects of inbreeding than other characteristics of economic interest. It is recommended to promote selection programs based on optimal contributions to maximize genetic gains and control inbreeding levels.

**Key words:** Inbreeding, Inbreeding depression, Phenotypic expression.

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**Introduction**

Inbreeding is caused by the crossing of related animals\(^{(1)}\) and represents the probability that, at any locus of an individual, there are identical genes by descent\(^{(2)}\). This affects the modification of the expression of genotypes, a phenomenon known as inbreeding depression. In livestock species, inbreeding depression increases the risk that individuals suffer from some genetic diseases, decrease their fertility\(^{(3)}\) and that their productive and health aptitude is affected\(^{(4)}\); in addition, it can affect the performance of any characteristic under selection\(^{(5)}\).

Some of the genetic explanations for the causes of inbreeding depression are the effects of overdominance, incomplete dominance, epistasis and genotype-by-environment interaction\(^{(1,2)}\). The hypothesis that supports the effects of overdominance indicates that inbreeding increases the frequency of homozygotes, which reduces the frequency of heterozygotes and the expression of their superiority. The hypothesis of incomplete dominance states that an increase in inbreeding is reflected in a greater frequency of homozygotes and with this, the presence of recessive deleterious alleles reduces\(^{(6)}\), which are eliminated from populations after a few generations. This is the mechanism that is considered to have the greatest frequency and effect on populations\(^{(7)}\).

The third hypothesis proposes a gene interaction (epistasis), which under conditions of inbreeding, creates unfavorable combinations of genes and, consequently, the productive potential of animals reduces\(^{(5,7)}\). The genotype-by-environment interaction is another factor that can explain inbreeding depression since the more heterozygous an individual is, the less sensitive they are to environmental stress compared to homozygous individuals. This
interaction primarily affects fitness-related traits\(^{(5)}\). The mechanisms of genetic action
described above have a low impact when measuring the effects in individual loci, but in
polygenic characters the performance of the individual can be significantly decreased\(^{(8,9)}\).

In dairy cattle, globalization, technological advancement and the innovation of genetic tools
have intensified the selection process, which has caused an increase in the mating of related
animals, causing a decrease in the diversity of genetic material\(^{(10)}\) that is directly associated
with an increase in inbreeding rates and a decrease in animal performance. In Holstein cattle,
an increase in the percentage of inbred animals has been estimated over the years and
although inbreeding rates have not presented drastic changes (approximately 0.11 to 0.21 %
per year, which corresponds to an average increase from 0.59 to 0.96 % per generation)\(^{(11,12)}\),
the decrease in the generation interval has promoted a decrease in inbreeding per
generation\(^{(13)}\), this being greater in males than in females due to the selection pressure exerted
on few sires used intensively\(^{(14)}\). In the livestock field, high inbreeding levels have caused
significant losses in the production of milk\(^{(15)}\) and its components (fat and protein)\(^{(16)}\), in
longevity\(^{(15,17)}\), in characters of conformation\(^{(16)}\) and fertility\(^{(18)}\), causing significant economic
losses for farmers\(^{(5,16)}\).

The objective of the present study was to calculate the inbreeding levels in the Holstein
population of Mexico, both females and males, and to evaluate their effect on the levels of
production of milk, fat, protein and final conformation points.

**Material and methods**

The pedigree information used to estimate inbreeding levels consisted of 326,238 animals of
the Holstein breed registered by the Holstein Association of Mexico. To estimate the
inbreeding index, a modified recursive algorithm was used, which, in the case of unknown
ancestors, incorporates as an inbreeding value the average of the animals born in the same
year, an algorithm implemented in the INBUPGF90 program developed by Aguilar and
Misztal\(^{(19)}\); which has as a principle the method suggested by Wright (1922), which, through
the following equation, considers the probability that the gametes of the father and mother
carry the same genes:

$$ Fx = ((1/2)^{(ns + nd + 1)})(1 + Fa) $$

where: \( Fx = \) inbreeding coefficient of the animal \( x \), \( ns = \) number of generations from the father
of the animal to the common ancestor, \( nd = \) number of generations from the mother of the
animal to the common ancestor, \( Fa = \) inbreeding coefficient of the common ancestor.
Subsequently, inbreeding trends by year of birth (from 1990 to 2018) were obtained through a linear regression analysis. The analysis included a total of 321,466 records; of which, 91% were females. To animals born in the period under study, production information (milk, fat and protein) adjusted to 305 days and the score of final conformation points obtained in the first round were included. Animals that did not have productive records were removed from the study. Finally, the database was made up of a total of 68,779 animals. The productive and conformation information was collected by the production control system of the Holstein Association of Mexico.

In order to evaluate the general effect of inbreeding and year of birth on the characteristics studied (milk, components and conformation), linear regression analyses were performed. To determine at what level of inbreeding, effects on the characters of economic importance are observed, females were classified into 6 groups, determined by the level of inbreeding expressed as a percentage. Group 1 included animals with <1%, group 2 those with ≥1 and <2%, group 3 those with ≥2 and <3%, group 4 those with ≥3 and <4%, group 5 those with ≥4 and <5%, and group 6 those with a level ≥5%. Through an analysis of variance, the comparison of means of the productive characteristics and of final conformation points was made for each of the groups formed by the level of inbreeding. To evaluate the trends of the effect of inbreeding by class, orthogonal contrasts were performed. The comparison of means and contrast test were performed with the LSMEANS-GLM procedure and regressions through the REG procedure, both with the SAS® 9.3 package (20).

**Results and discussion**

The mean and standard deviation of inbreeding for animals born from 1990 to 2018 in the Holstein population of Mexico was 2.60 ± 2.57, with a rate of increase per year of 0.07 (P<0.001), being lower for males (0.05) than for females (0.07) (Figure 1). The trend by sex differs from that observed in the North American Holstein population, where the rate of inbreeding is lower in females than in males (21), a trend that can be explained by the greater selection pressure exerted on the latter specimens. In the Holstein population of Mexico, the lower rate of inbreeding of males could be explained by the selective importation of sires (which turn out to be little inbred) from various populations, especially the North American one. The rate of change of inbreeding presented in males of the population under study in the decade from 1990 to 2000 coincides with those calculated in Holstein populations of France, the Netherlands and the United States (0.12) (11), and is approximately half of that reported in Holstein cattle of Canada (0.26) (22), as well as males and females of the United States (0.22 and 0.21) (21) for the same decade.
For the two decades that followed, the rate of change in the study population was lower (0.03 from 2000 to 2009 and of 0.06 from 2010 to 2018) (Table 1). The low rate of inbreeding from 2000 to 2009 in females (0.03) and the zero increase in males can be explained by the efficient implementation of selection programs based on the optimal genetic contributions of future generations, which consider the estimated genetic values and genetic relationships between selected individuals\(^{(23)}\).

**Table 1: Rate of change in inbreeding by time period of the Holstein population of Mexico, classified by sex**

<table>
<thead>
<tr>
<th>Holstein population of Mexico</th>
<th>Periods of year of birth</th>
<th>1990-1999</th>
<th>2000-2009</th>
<th>2010-2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td>0.17</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td>0.12</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>General population</td>
<td></td>
<td>0.17</td>
<td>0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The use of selection programs based on optimal contributions in Mexico has been promoted mainly by artificial insemination companies, which provide direct service to farmers. The crossbreeding strategies employed in selection programs based on optimal contributions and the globalization of artificial insemination companies could explain the introduction of genetic material from other countries that had not been used in Mexico. According to pedigree information, from 1996 to 2000, daughters of sires from Italy, Spain, France, Belgium and Australia began to be born; event to which the decrease in inbreeding in the following decades can be attributed. In the study period, the same pattern of behavior was
observed in the Holstein population of Canada and the United States, in which the rate of inbreeding increased in the 90s and decreased in the early 2000s\(^{(21,24)}\). However, the rates of inbreeding of other populations were higher compared to the Holstein population of Mexico; for example, the Canadian Holstein population showed an increase of 0.08 per year, from 2000 to 2009 and of 0.23 from 2010 to 2016\(^{(22,24)}\); while the Holstein population of the United States showed increases of 0.11 and 0.27 for the same decades\(^{(21)}\).

In Mexico, as in the United States and Canada, the same behavior was observed, since inbreeding increased between two and three times in the 2010s (from 0.03 to 0.06, from 0.11 to 0.27 and from 0.08 to 0.23, respectively), compared to the previous decade, although the difference between Mexico and Canada or the United States remained. The increase in inbreeding of sires used in Mexico in the 2010s was very noticeable and may be due to the use of sires that were selected through genomic selection, since the introduction of this technological tool has decreased the presence of recessive deleterious genes, but at the same time, has affected the diversity of haplotypes in the genome of dairy cattle populations\(^{(25)}\). This increase coincides with that observed in other North American populations in the same years\(^{(21,24)}\).

In various populations, it has been shown that inbreeding can cause a decrease in the efficiency of characters of economic importance\(^{(3,26)}\). In the Holstein population of Mexico, it was found that, for every percentage point of increase in inbreeding, the production of milk, fat and protein decrease by 88, 3.16 and 2.57 kg \((P<0.0001)\). In the Holstein population of the United States, a lower loss for milk production (73 kg) than that found in the present study was found, and lower for fat (-1.08 kg) and protein (-0.97 kg)\(^{(27)}\). In the analysis of the effect of the level of inbreeding on the production of milk, fat and protein, as well as final conformation points (Table 2), the results show that animals with a higher level of inbreeding (group 6, with inbreeding level ≥5%) are statistically different from animals with a lower percentage, with differences between the extreme classes of -444 kg, -17 kg and -11 kg of milk, fat and protein, respectively. These results are consistent with those found by Maiwashe et al\(^{(28)}\), who mention that the production of milk and its components are affected by the increase in inbreeding coefficients, being reflected in the average annual performance of the population. In the population studied, it was observed that, for milk production, there are three statistically different classes, those with <3% (groups 1, 2 and 3), those from 3 to <5% (groups 4 and 5) and ≥5% (group 6), implying for the latter a decrease of 260 kg per lactation compared to the average of groups 4 and 5. The effect of inbreeding on the composition of milk (fat and protein) at low levels (<5%) does not have a negative effect. However, when the inbreeding exceeds 5%, the loss in production of fat is 11 kg, and in protein 10 kg.

It is also important to mention that the trends in the effect of inbreeding on productive characteristics were not linear in any of them (Table 2), suggesting the idea that there are threshold values of inbreeding for it to be expressed in deterioration of the productive
potential of animals\textsuperscript{(3,18)}. Contrary to what was observed in the productive characters, the animals with the lowest levels of inbreeding (<1\%) were those that presented the lowest average in final conformation points and the highest levels (>1\%) showed no significant differences between them, which suggests that the functional conformation is less sensitive to the effect of inbreeding. Studies conducted on Holstein cattle of Ireland showed that inbreeding does not have large negative effects on all conformation characters, and those that are affected show detrments at high levels (>12.5 \%)\textsuperscript{(18)}.

Table 2: Comparison of means of milk, fat, protein (kg) production and final conformation points by level of inbreeding of animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of inbreeding</th>
<th>Number of animals</th>
<th>Milk $\Delta\eta$</th>
<th>Fat $\Delta\eta$</th>
<th>Protein $\Delta\eta$</th>
<th>Final points $\Delta\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1%</td>
<td>21,734</td>
<td>11,699$^a$</td>
<td>384$^a$</td>
<td>344$^a$</td>
<td>79.59$^a$</td>
</tr>
<tr>
<td>2</td>
<td>$\geq 1$ y &lt;2%</td>
<td>11,656</td>
<td>11,754$^a$</td>
<td>380$^a$</td>
<td>343$^a$</td>
<td>80.33$^b$</td>
</tr>
<tr>
<td>3</td>
<td>$\geq 2$ y &lt;3%</td>
<td>11,184</td>
<td>11,636$^a$</td>
<td>379$^a$</td>
<td>340$^a$</td>
<td>80.30$^b$</td>
</tr>
<tr>
<td>4</td>
<td>$\geq 3$ y &lt;4%</td>
<td>9,211</td>
<td>11,519$^b$</td>
<td>376$^a$</td>
<td>339$^a$</td>
<td>80.22$^b$</td>
</tr>
<tr>
<td>5</td>
<td>$\geq 4$ y &lt;5%</td>
<td>6,410</td>
<td>11,511$^b$</td>
<td>378$^a$</td>
<td>343$^a$</td>
<td>80.38$^b$</td>
</tr>
<tr>
<td>6</td>
<td>$\geq 5%$</td>
<td>8,604</td>
<td>11,255$^c$</td>
<td>367$^b$</td>
<td>333$^b$</td>
<td>80.41$^b$</td>
</tr>
</tbody>
</table>

Means with unequal superscripts present significant statistical differences ($P<0.001$).
Linear ($\Delta$) and quadratic ($\eta$) significant trends, ($P\leq0.05$).

With the results obtained in the present study, the negative effects that inbreeding can have when it is at levels higher than 5\% were shown and that the introduction of genetic selection tools can modify the inbreeding levels in a positive way in the expression of some characteristics, such as those of conformation, in those associated with the longevity and lifetime production of the animal\textsuperscript{(29)}, but at the same time it may affect the expression of others\textsuperscript{(27)}; so it would be important to promote selection programs based on optimal contributions of animals to maximize genetic gains and control inbreeding levels at rates below 1\% per generation\textsuperscript{(30)}. It is important to mention that the effects of inbreeding are not limited to productive or conformation characteristics, its effects on reproductive characteristics also affect the profitability of milk-producing companies. Smith et al\textsuperscript{(4)} found that an increase of one percentage point of inbreeding can increase by 0.55 d the age at first calving, decrease the productive life of the animals by 6 d and the production by 4.8 d. Mc Parland et al\textsuperscript{(18)} showed a negative effect of high levels of inbreeding (up to 12.5\%) on the reproductive performance of animals, observing a 2\% increase in the incidence of dystocia, 1\% more in the incidence of stillbirth, an increase of 8.8 d in the calving interval and 2.5 d in the age at first calving, a 1.68\% decrease in the pregnancy rate, when females go from a inbreeding level of 6.25 to 12.5\%\textsuperscript{(3)}.  

The estimation of inbreeding coefficients is an important indicator of the optimal use of genetic resources, since it evaluates the presence of loci that can affect the productive performance of animals within a population. Its calculation from the pedigree information has turned out to be a tool to be considered in the selection process and has allowed to evaluate its effect on phenotypic expression in various populations. Also, the use of molecular tools can significantly help to know the details at the molecular level that inbreeding entails\(^{(1)}\), to provide the possibility of predicting early rates of genetic improvement and thus minimize the effects associated with high levels of inbreeding\(^{(31)}\).

However, in genomic selection programs, the widespread use of sires has led to a reduction in genetic diversity within populations with high productive performance\(^{(12)}\), so it is necessary to establish optimal contribution selection schemes based on genomic values that maintain low to moderate levels of inbreeding, especially in the selection of breeding animals\(^{(23,32)}\). Recent studies suggest the incorporation of the estimation of the inbreeding coefficient in genetic value prediction procedures; for example, include it as a covariate or consider it in the inverse of the additive relationship matrix in the estimation of genetic values of the BLUP evaluations, as well as in the estimation of their reliability, since, if not included, the variance of the prediction error can increase, or the reliability can be over or underestimated\(^{(33)}\).

**Conclusions and implications**

The results obtained show that low levels of inbreeding do not affect the phenotypic expression of productive characters and that their effect on the productive characteristics studied is not linear. Levels above 5% are associated with the decrease in characteristics of economic interest such as the production of milk, fat and protein. In addition, the increase in inbreeding in the population will increase the probability that lethal genes or genetic diseases associated with recessive genes can be expressed within the population. On the other hand, the way in which the genetic improvement industry is structured has promoted that it is the animals high in inbreeding who show the most functional conformation, which can have desirable repercussions for farmers. Therefore, it is recommended to design comprehensive genetic improvement programs that include technology, reproductive, health and productive life characters to control the level of inbreeding of the population and that consequently the expression of productive characteristics is not compromised.
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Conflict of interest

The authors declare that there are no conflicts of interest.

Literature cited:


