





Effects of breed and genomic inbreeding on milk, fat and protein lactation yields and fertility traits in pasture-based dairy cows in Argentina

Animal Research Paper

*Contributed equally to the study.

María José Beribe^{1,2,*}, Hugo Adrián Carignano^{3,4,*} , Mario Andrés Poli^{5,6} and Nicolás Lopez-Villalobos⁷ 

Cite this article: Beribe MJ, Carignano HA, Poli MA, Lopez-Villalobos N (2024). Effects of breed and genomic inbreeding on milk, fat and protein lactation yields and fertility traits in pasture-based dairy cows in Argentina. *The Journal of Agricultural Science* **162**, 181–189. <https://doi.org/10.1017/S0021859624000273>

Received: 24 July 2023
Revised: 15 April 2024
Accepted: 7 May 2024
First published online: 24 May 2024

Keywords:

calving to conception interval; cattle breed; genomic inbreeding values; milk production; run of homozygosity

Corresponding author:

Nicolás Lopez-Villalobos;
Email: n.lopez-villalobos@massey.ac.nz

¹Estación Experimental Agropecuaria Pergamino, Instituto Nacional de Tecnología Agropecuaria, Pergamino, Buenos Aires, Argentina; ²Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina; ³Instituto de Virología e Innovaciones Tecnológicas, Instituto Nacional de Tecnología Agropecuaria – Consejo Nacional de Investigaciones Científicas y Técnicas, Hurlingham, Argentina; ⁴Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina; ⁵Instituto de Genética, Instituto Nacional de Tecnología Agropecuaria, Hurlingham, Buenos Aires, Argentina; ⁶Facultad de Ciencias Agrarias y Veterinaria, Universidad del Salvador, Pilar, Buenos Aires, Argentina and ⁷School of Agriculture and Environment, Massey University, Palmerston North, New Zealand

Abstract

The objective of the current study was to evaluate the effects of breed and genomic inbreeding on 305-day lactation yields of milk, fat and protein; and fertility traits of pasture-based dairy cows in Argentina. The genomic inbreeding and heterozygosity of 890 first-lactation cows and 27 bulls were calculated through methods based on the genomic relationship matrix and run of homozygosity using 44 174 single-nucleotide polymorphisms. Cows were classified into four breed groups: Holstein, Holstein crossbred, Holstein–Jersey crossbred and Jersey crossbred. The effect of genomic inbreeding was not significant on production traits, but inbred cows increased 3.0 days calving to conception interval (CCI) per 1% genomic inbreeding. On average, purebred Holstein cows produced 1119 kg milk, 22 kg fat and 30 kg protein more than Jersey crossbred cows. In the case of the fertility traits, Jersey crossbred cows had 45 days shorter CCI than purebred Holstein cows. A possible reason for the non-significant effects of genomic inbreeding of production and fertility traits is that these effects were evaluated in a crossbred population in which rates of heterozygosity would operate to some extent in the opposite direction to rates of genomic inbreeding.

Introduction

The dominant breed of dairy cattle in Argentina is Holstein (Gastaldi *et al.*, 2020). Traditionally, the cows have been produced using dairy sires from the USA of high-genetic potential for milk production, which are suitable for indoor systems using total mixed rations (Lazzarini *et al.*, 2019). Contradictorily to the intensive indoor systems, in Argentina 149/155 of dairy farms are pastoral (Gastaldi *et al.*, 2020). Feeding is based mainly on grazing alfalfa (*Medicago sativa* L.) throughout the year, in combination with silage and concentrates (Gastaldi *et al.*, 2020). Some dairy farmers have used Jersey sires to incorporate Jersey × Holstein crossbred cows, which produce lower milk yields but higher proportions of fat and protein. In 2021, approximately 3 197 025 of 3 475 028 semen doses from dairy bulls that were marketed in Argentina were from Holstein, 0.05 from Jersey and the rest from other dairy breeds such as Brown Swiss and Guernsey (Cámara Argentina de Biotecnología de la Reproducción e Inseminación Artificial, 2021).

Inbreeding depression is the reduction of an individual's fitness due to fixation of deleterious recessive genes (Falconer and Mackay, 1996). Progeny that results from mating of genetically related animals results in high inbreeding coefficient. A inbreeding coefficient has been originally defined as the probability that an individual inherits two identical alleles at the same locus from the parents (Malécot, 1969) or as the correlation between two homologous alleles in uniting gametes (Wright, 1922). Therefore, inbreeding could also be reflected as an increase in autozygosity (i.e. homozygosity due to the inheritance of identical alleles by offspring) and a loss of genetic variability (deficit in heterozygosity). Inbreeding coefficients can be calculated through either ancestral pedigrees or genomic analysis. Parentage data can be used to construct the relationship matrix (*A*) among the individuals represented in the pedigree file. This matrix is symmetric, the elements of the diagonal represent the genetic relationship among the individuals of the pedigree and the diagonal elements represent the degree of inbreeding of each animal in the pedigree, where the value is 1 plus the expected inbreeding coefficient (Falconer

© The Author(s), 2024. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.



and Mackay, 1996). Analysis of inbreeding in large populations poses difficulty due to the need for a simple and accurate, but high-throughput method to calculate the inbreeding coefficient. Such calculations of inbreeding predict only the expected proportion of animal genome that is considered identical by descent (Nietlisbach *et al.*, 2017). Inaccurate estimates of inbreeding coefficients can arise if pedigrees are small or incomplete, missing parentage can underestimate actual rates of inbreeding (Nietlisbach *et al.*, 2017; Gutiérrez-Reinoso *et al.*, 2022).

Currently, the availability of molecular markers distributed throughout the whole genome enables the estimation of genome-based inbreeding coefficients without the need to record extensive genealogy or parental relationship among individuals (Leutenegger *et al.*, 2003). Therefore, genomic-based inbreeding coefficients demand less time and effort than pedigree-based measures. Besides, they can be more accurate due to their capacity to account for variation in the random process of meiotic recombination (Mendelian sampling). The simplest estimations of this realized inbreeding coefficient rely on single-nucleotide polymorphism (SNP) analysis of different genomic relationship matrix (GRM) formulations (equivalent to the F calculation when the A matrix is used) (VanRaden, 2008) or analysing multi-SNP consecutive DNA stretches, known as run of homozygosity (ROH) (Keller *et al.*, 2011; Purfield *et al.*, 2012). Depending on the GRMs utilized, the single-SNP inbreeding coefficients could describe (1) deviations from Hardy–Weinberg equilibrium (HWE) (F_{HOM}), (2) correlations between uniting gametes (F_{UNI}) and (3) the variance of additive genetic values (F_{GRM}) (Villanueva *et al.*, 2021). On the other hand, the ROH-based inbreeding coefficient, calculated as the sum of the ROHs identified in an individual divided by the total genome length, ideally represents the proportion of the individual's autosomal genome that is autozygous (Howrigan *et al.*, 2011).

Similarly, the rate of heterozygosity of an individual can be calculated from genotypes and requires no knowledge of ancestry in contrast to pedigree inbreeding (Iversen *et al.*, 2019).

The effect of inbreeding on production and reproduction traits has been well documented in Holstein–Friesian dairy cattle, depicting that as inbreeding increases milk production, fertility is reduced (Cassell *et al.*, 2003; McParland *et al.*, 2007). Although a similar relationship has been reported in the Jersey breed, the effect of inbreeding has a greater influence on milk yield in the Holstein–Friesian breed (Maiwashe *et al.*, 2008; Pryce *et al.*, 2014). Other studies have reported that fat and protein yield follow the same trend as milk yield, but inbreeding has substantially less influence on change of milk yield, resulting in small regression coefficients (Dezetter *et al.*, 2015; Doekes *et al.*, 2019). Also, increases in inbreeding coefficients have been associated with larger concentrations of somatic cell counts (SCCs) in milk, with greater incidence seen in older animals (McParland *et al.*, 2007). Contrary to these findings, some studies have found no significant effect of inbreeding on SCCs (Rokouei *et al.*, 2010; Dezetter *et al.*, 2015).

Crossbreeding is the mating of individuals from different lines, breeds or populations (Lopez-Villalobos *et al.*, 2000). As a breeding strategy, crossbreeding offers the removal of the negative effects associated with inbreeding depression in particular traits associated with fitness and survival (Falconer and Mackay, 1996). Crossbreeding also offers a potentially attractive avenue for farmers to improve economic efficiency by using breed complementary and exploiting heterosis for milk production, fertility and survival (Buckley *et al.*, 2014). The heterozygosity rate has been indicated as a proxy for heterosis (Iversen *et al.*, 2019); in

F1 crosses all animals are expected to be heterozygotes (maximum heterozygosity rate), but in backcross and subsequent generations a reduction in heterozygotes frequency would be observed, altogether a heterosis decline. Therefore, given the interplay between crossbreeding, inbreeding and heterozygosity, quantification of these values in crossbred cattle would be relevant for management decisions in dairy cattle.

Comparisons between Jersey, Holstein and Jersey \times Holstein crossbred cows for production and reproduction traits in pasture-based production systems of Argentina have been reported in the literature (Baudracco *et al.*, 2011; Mancuso and Marini, 2012; Biga *et al.*, 2022). To our knowledge, there are no studies in the literature that report the rate of genomic inbreeding and heterozygosity calculated using genetic markers in a crossbred population and the effects of genomic inbreeding in dairy cattle in Argentina. The objective of the current research was to evaluate breed and genomic inbreeding on lactation milk yields, fat and protein content and fertility in pasture-based dairy cows from Argentina.

Materials and methods

Data

Study population

Records of 20 005 Holstein and Holstein \times Jersey cows born between 1994 and 2010 were obtained from 37 dairy farms. Dates of birth, services, calving, dairy controls, drying-off, transfers and rejection added up to a total of 755 141 records. The cows (4804/20 005 Holstein and 5201/20 005 Holstein \times Jersey) belong to 30 half-sisters families. In particular, the crossbred groups considered in this study were the following: H: purebred cows with 1.00 Holstein (639); HX: backcross to Holstein with 0.75 Holstein and 0.25 Jersey (64); HJ: first crossbred Holstein–Jersey with 0.50 Holstein and 0.50 Jersey (157); JX: backcross to Jersey with 0.25 Holstein and 0.75 Jersey (30).

The dairy farms are located in the central dairy basin of Argentina (central-eastern region). On each farm, two daily milkings were performed within a semi-stabled feeding system with cows outside grazing alfalfa during the hot summer months and oats or barley in the winter. The diet was systematically supplemented with maize silage, soybean meal and concentrates elaborated in house containing 0.16–0.18 of crude protein.

The cow's reproductive management system involves continuous calving, where calving periods are uniformly distributed throughout the year.

Genotyping and quality control

A total of 970 cows (718 purebred Holstein and 252 crossbred Holstein \times Jersey) and 29 bulls (24 Holstein and 5 Jersey) were chosen for genotyping as described elsewhere (Carignano *et al.*, 2018). Briefly, quality control of the genotypic data was carried out using a set of tools and routines provided by PLINK v1.9 (Purcell *et al.*, 2007). Individuals with a genotype call rate (CR_{IND}) $<90\%$ were excluded from further analysis. SNPs with a call rate (CR_{SNPs}) $<90\%$, a deviation from HWE $P < 1.10^{-8}$ and minor allele frequency <0.01 were removed from the study. After the quality control, the data set for genomic inbreeding calculations comprised of 44 174 SNPs and 917 individuals (890 cows and 27 bulls).

Genomic inbreeding and heterozygosity coefficients

Genomic-based individual's inbreeding coefficients (F) were calculated using different approaches as follows:

F_{ROH} : The inbreeding coefficient F_{ROH} for each cow was calculated using the *-homozyg* routine implemented in PLINK version 1.9 (Purcell *et al.*, 2007). The runs of homozygous genotypes for each individual were defined using sliding windows of 50 SNPs across the genome, requiring 25 homozygous SNPs spanning a ≥ 1000 kb distance. A sliding window hit ('homozygous') contained at most one heterozygous SNP and none missing calls. An SNP was included in an ROH segment if the hit rate (proportion of 'homozygous' windows that overlap that position) was > 0.05 . The final ROH segments were constrained to a maximum interval between two consecutive SNPs of 500 kb, allowing one heterozygous genotype and a minimal density of 1 SNP/100 kb.

The coefficient F_{ROH} was defined as the proportion of the autosomal genome in runs of homozygosity:

$$F_{ROH} = \frac{L_{ROH}}{L_{aut}}$$

where L_{ROH} is the total length of all ROH segments identified in an individual and L_{aut} is the length of the autosomal genome covered by SNPs (i.e. between the first SNP and the last SNP per chromosome for all autosomal chromosomes).

F_{GRM} : The inbreeding coefficient F_{GRM} for each cow was calculated using the diagonal elements of the GRM according to VanRaden (2008):

$$F_{GRM} = \frac{1}{N} \sum_{i=1}^N \frac{(X_i - 2P_i)^2}{h_i} - 1$$

where X_i is the genotype coded as the number of reference allele copies for the i th SNP; P_i is the observed frequency of this allele (i.e. the allele whose homozygous genotype was coded as '0'), N is the total number of markers and $h_i = 2P_i(1 - P_i)$ is the expected heterozygosity.

F_{HOM} : This inbreeding coefficient was based on the homozygous excess and is defined as

$$F_{HOM} = \frac{O(hom) - E(hom)}{N - E(hom)} = 1 - \frac{1}{N} \sum_{i=1}^N \frac{X_i(2 - X_i)}{h_i}$$

where $O(hom)$ is the observed number of homozygous markers of the individual and $E(hom)$ is the expected number of homozygous markers under the HWE calculated from the allele frequencies estimated on the sample. The expected number of homozygotes is calculated assuming HWE as $1 - 2P_i(1 - P_i)$.

F_{UNI} : The inbreeding coefficient F_{UNI} is based on the correlation between uniting gametes following the method proposed by Ritland (1996):

$$F_{UNI} = \frac{1}{N} \sum_{i=1}^N \frac{X_i^2 - (1 + 2P_i)X_i + 2P_i^2}{h_i}$$

where $2P_i(1 - P_i) = 1$ if X_i is heterozygous and $= 0$ if it is homozygous.

The inbreeding coefficients F_{GRM} , F_{HOM} and F_{UNI} were calculated using the *-ibc* routine implemented in GCTA v1.24 software (Yang *et al.*, 2011).

Heterozygosity was measured as the proportion of heterozygous marker genotypes for each individual as $(N - O)/N$, where

N is the number of non-missing genotypes and O is the number of homozygous genotypes for a given animal (Purcell *et al.*, 2007).

Phenotypic traits

The initial production data set included 316 855 monthly herd tests from 48 367 lactations belonging to 20 005 cows. The lactation curves for daily milk, fat and protein production for each cow–lactation were modelled using a sixth-order Legendre polynomial. Predicted daily yields were then used to calculate 305-day lactation yields of milk (MY305), fat yield (FY305) and protein yield (PY305), for each lactation of each cow (Beribe, 2020).

Two fertility traits were calculated, calving to first service interval (CFSI) and calving to conception interval (CCI). CFSI was calculated as the number of days between calving date and the first service date, and CCI was calculated as the number of days between calving date and conception date.

Finally, for this study the values of MY305, FY305, PY305, CFSI and CCI were merged with the 890 first-lactation cows that had genotypic data. Not all cows that were genotyped and passed quality control for genetic markers had the full set of phenotypic traits.

Statistical analysis

The effects of breed and inbreeding were estimated using the following mixed model:

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta x_l + \tau z_m + \varepsilon_{ijklm}$$

where:

y_{ijklm} is any of the traits evaluated: MY305, FY305, PY305, CFSI and CCI.

α_i is the fixed effect of breed group with four classes (H: proportion of Holstein = 1, HX: proportion of Holstein = 0.75, HJ: proportion of Holstein = 0.5 and JX: proportion of Holstein = 0.25).

β_j is the fixed effect of calving season with five classes: November–January, February–April, May–June, July–August and September–October.

γ_k is the random effect of the contemporary group, defined as the group of cows that started lactation in the same herd and year.

δ is the regression coefficient of the dependent trait on age at calving x_l .

τ is the regression coefficient of the dependent trait on the genomic inbreeding coefficients z_m (expressed as a percentage).

ε_{ijklm} is the random residual associated with observation y_{ijklm} .

Calving season was defined based on monthly average temperatures and ensuring a representative number of records in each class (>19 records).

Least-squares means and standard errors were obtained for each breed group and calving season and used for multiple mean comparisons using Fisher least significant difference test.

Analyses were performed using the MIXED procedure of SAS v9.4 (SAS® Institute Inc., 2013, Cary, NC, USA).

Comparisons of mean rates of genomic inbreeding and heterozygosity among the different breed groups were performed using individual *t*-tests.

Results

Descriptive statistics for the production and fertility traits and measures of genomic inbreeding coefficients are presented in

Table 1. The fertility traits presented greater variability (higher coefficient of variation [CV]) than production traits. For example, CCI was in the range of 18–673 days (CV = 64%), whereas FY305 was in the range of 156–281 kg (CV = 12%).

The distributional properties of F_{GRM} , F_{HOM} and F_{UNI} coefficients were similar; median at -2.69 (F_{GRM}), -1.31 (F_{HOM}) and -2.26 (F_{UNI}) with (min to max) values at $(-13.90$ to $46.11)$, $(-18.72$ to $16.04)$ and $(-11.38$ to $24.68)$, respectively. The range of the F_{ROH} values was 0.00 – 19.00 , with positive values for the mean and median. The range of heterozygosity was from 0.29 to 0.39 with median and mean values of 0.35 and low standard deviation (Table 1).

Scatter plots and distributions (histograms) of each of the genomic inbreeding coefficients and pairwise Pearson's correlation coefficients are presented in Fig. 1. All correlation coefficients were statistically significant ($P < 0.05$). The correlation between F_{ROH} and F_{UNI} was strong positive (0.70), but the correlation between F_{ROH} and F_{HOM} was stronger (0.86). No correlation between F_{ROH} and F_{GRM} (-0.08) was observed. Among the inbreeding coefficients derived from the genomic matrix, a weak negative correlation was found between F_{GRM} and F_{HOM} (-0.36). The correlations between F_{GRM} and F_{UNI} , and between F_{HOM} and F_{UNI} were moderately positive.

Mixed models were used to study the effects of breed and genomic inbreeding on production and fertility traits. For milk production traits, F -statistics indicated that breed and calving age explained the majority of the variation (Table 2). Besides, calving season had a significant effect on CFSI. In the case of F coefficients, only F_{ROH} had a significant effect for CCI ($P = 0.019$). The rest of the genomic F coefficients evaluated did not influence MY305, FY305, PY305 or CFSI in the studied population (data not shown). A simple linear regression analysis of CCI on F_{ROH} showed that CCI increased by 4.87 days per 1% increase of F_{ROH} ($P < 0.0001$; Fig. 2(a)), but when corrected (full model) by breed, season calving and age at calving the partial regression coefficient was 3.0 days per 1% inbreeding. Similarly, regression analysis of MY305 on F_{GRM} showed that MY305 reduced by 22.7 kg per 1% of F_{GRM} (Fig. 2(b)). However, when the effect of F_{GRM} was evaluated considering the full model the partial regression coefficient was not statistically significant.

Effects of genomic F_{GRM} , F_{HOM} and F_{UNI} on the productive and fertility traits were not significant (data not shown).

The effect of breed group on productivity and fertility was evaluated through least-squares analysis (Table 3). In general, milk yield increased as the proportion of Holstein breed increased in the population. The differences between first-lactation purebred Holstein and JX crossbred cows were 1119 kg milk, 22 kg fat and 30 kg protein ($P < 0.001$). The breed groups' mean comparisons for CFSI were not significantly different (Table 2). However, a trend ($P = 0.099$) was observed to reduce CCI as the proportion of Jersey increased; JX cows had 45 days shorter CCI than H purebred.

The relationship between heterozygosity and F_{ROH} across the different breed groups is presented in Fig. 3. First crossbred HJ cows which are close to 0.50 H and 0.50 Jersey had the highest heterozygosity and the lowest F_{ROH} coefficients, whereas purebred H cows tended to have the lowest values of heterozygosity and highest values of F_{ROH} coefficients.

In accordance with Fig. 3, crossbred HJ cows had the lowest ($P < 0.05$) rate of inbreeding, measured by the F_{ROH} coefficient, and the highest ($P < 0.05$) rate of heterozygosity.

Discussion

The current study investigated the effect of breed and genomic inbreeding coefficients on production and fertility traits in pasture-based dairy cows from commercial farms of Argentina. The average rate of milk production found in the current study (5687 kg milk, 213 kg fat and 197 kg protein per cow) was similar to that reported for the Holstein breed in Argentina (5760 kg milk, 207 kg fat and 193 kg protein per cow, respectively) (FCA, 2016). Subtle differences in cows' productivity between the current research and national herd could be explained by variation in food supplementation intensity and farm management conditions as shown in Lazzarini *et al.* (2019).

The mean CFSI was 87.5 ± 43.2 days, which was similar to values reported in USA for Holstein cows (81 ± 4 days; Mullen *et al.*, 2015) and UK Holstein and Jersey cows (71 ± 1.9 days; Coffey *et al.*, 2016). The average CCI (155 ± 99.3 days) was in

Table 1. Descriptive statistics for production and fertility traits and different measures of genomic inbreeding of pasture-based dairy cows from commercial herds of Argentina

Trait ^a	N	Mean	Median	SD	CV	Min	Max
MY305 (kg)	833	5687	5631	84.9	15	3295	8375
FY305 (kg)	816	213	213	26.3	12	145	308
PY305 (kg)	816	197	195	26.8	14	156	281
CFSI (days)	890	87.5	75.5	43.2	49	15	297
CCI (days)	890	155.0	126.0	99.3	64	18	673
F_{GRM}	890	-1.95	-2.69	6.2		-13.9	46.1
F_{HOM}	890	-2.06	-1.31	6.3		-18.7	16.0
F_{UNI}	890	-2.01	-2.26	3.5		-11.4	24.7
F_{ROH}	890	5.22	5.39	3.5		0.00	19.0
Heterozygosity	890	0.35	0.35	0.02		0.29	0.39

N, number of cows; SD, standard deviation; CV, coefficient of variation (%); Min, minimum value; Max, maximum value.

^aMY305, 305-day milk production; FY305, 305-day fat production; PY305, 305-day protein production; CFSI, calving to first service interval; CCI, calving to conception interval; F_{ROH} , coefficient of genomic inbreeding calculated based on proportion of the autosomal genome in runs of homozygosity, F_{GRM} , coefficient of genomic inbreeding obtained from the diagonal elements of the genomic relationship matrix, F_{HOM} , coefficient of genomic inbreeding calculated based in the homozygous excess and F_{UNI} , coefficient of genomic inbreeding calculated based on the correlation between uniting gametes. Heterozygosity was calculated as the proportion of heterozygous marker genotypes for each individual.

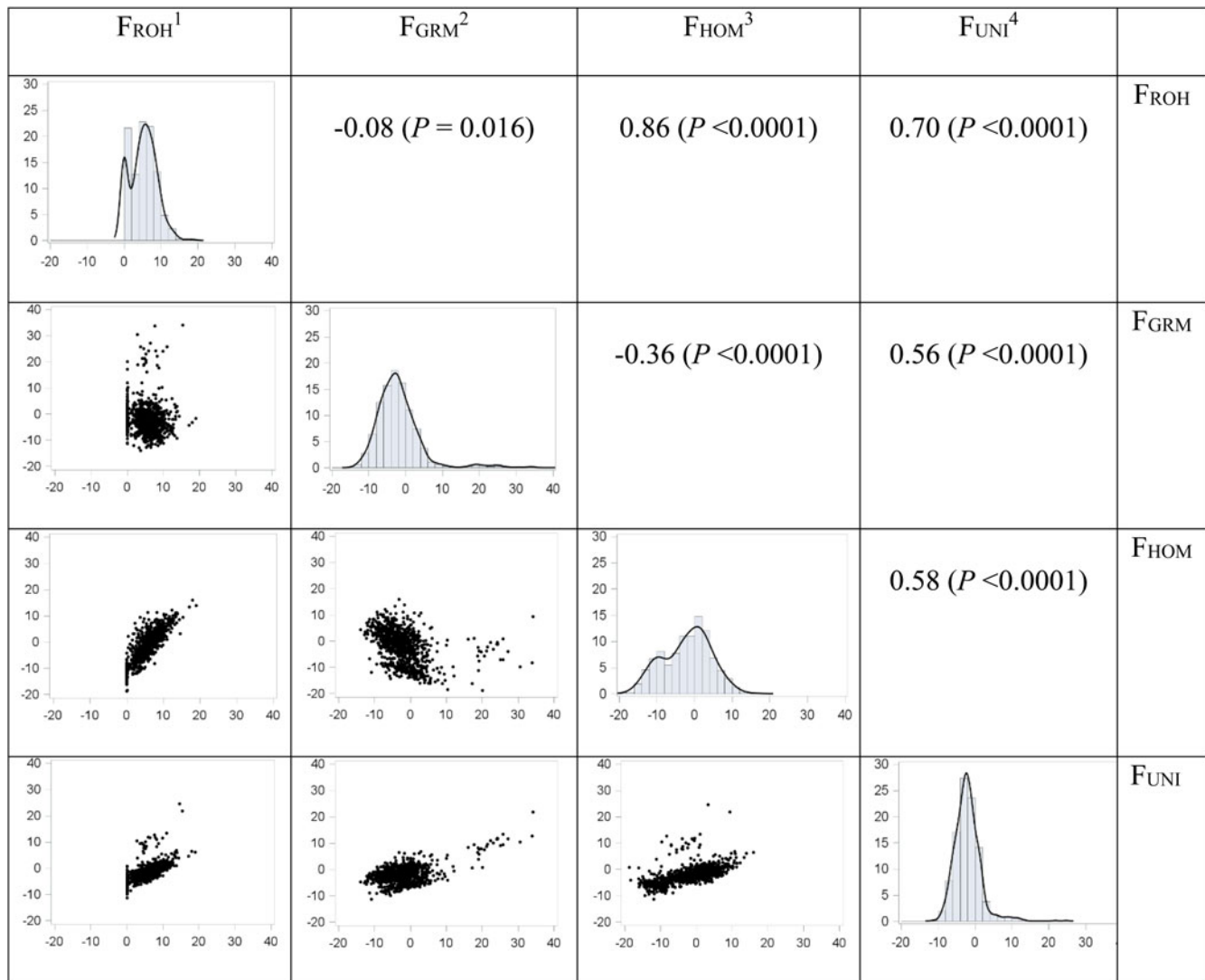


Figure 1. Pearson correlations and P -values (within brackets) (above diagonal), scatter plots (below diagonal) between each pair of the four genomic inbreeding estimators and densities (diagonal) of the inbreeding estimators of dairy cows in commercial herds of Argentina. $^1F_{ROH}$, coefficient of genomic inbreeding calculated based on proportion of the autosomal genome in runs of homozygosity; $^2F_{GRM}$, coefficient of genomic inbreeding obtained from the diagonal elements of the genomic relationship matrix; $^3F_{HOM}$, coefficient of genomic inbreeding calculated based in the homozygous excess; $^4F_{UNI}$, coefficient of genomic inbreeding calculated based on the correlation between uniting gametes.

line with those reported in the USA (115–177 days) and Argentina (92–157 days) (Dutour and Melucci, 2011). However, much shorter CCI values have been reported in Canada (102–108 days), New Zealand (90–101 days) and UK (100–115 days) (reviewed by Dutour and Melucci, 2011). The differences between our results and other studies are explained by the differences in genetic, management and feeding programmes.

Despite the coefficient of inbreeding being an important variable for breeding mating management in dairy herds, aiming to reduce inbreeding depression, there is currently no consensus on which genomic inbreeding coefficient estimator is appropriate (Dadousis *et al.*, 2022). Furthermore, the evaluation of genomic inbreeding coefficients in crossbred cattle populations has been scarcely reported. In the current study, the range of genomic inbreeding coefficient values for F_{GRM} , F_{HOM} and F_{UNI} were (as percentage) [–13.90 to 46.11], [–18.72 to 16.04] and [–11.38 to 24.68], respectively. These values did not meet Wright's original definitions of inbreeding, since these estimates of genomic

inbreeding are outside of the ranges allowed by probabilities (0–100%). Alternatively, these estimators can be interpreted as measures of genotypic variability (heterozygosity) in the current population compared to a base population (Villanueva *et al.*, 2021). However, it is important to note that the allelic frequencies in the base population are often unknown and must be estimated from the current population assuming HWE (Villanueva *et al.*, 2021; Dadousis *et al.*, 2022). In this regard, in a study by Villanueva *et al.* (2021) F_{GRM} , F_{UNI} and F_{HOM} were analysed through simulations using different scenarios of allelic frequencies in the base and current populations. They described that F_{GRM} and F_{UNI} could indicate variability increasing (genomic inbreeding < 0) in the current population when in fact it has decreased (genomic inbreeding > 0), or vice-versa. Also, F_{GRM} and F_{UNI} could indicate that more variability than present in the base population has been lost (genomic inbreeding $> 100\%$), which makes no sense. On the other hand, by definition, F_{ROH} has only positive values, ranging from 0 to 19% in the current research. Among

Table 2. *F*-values and *P*-values (within brackets) for factors affecting production and fertility traits of pasture-based dairy cows from commercial herds of Argentina

Trait ^a	Effect			
	Breed	Calving season	Age at calving	F_{ROH}
MY305	22.52 (<0.001)	0.82 (0.485)	16.63 (<0.001)	0.62 (0.430)
FY305	9.01 (<0.001)	0.84 (0.503)	12.20 (<0.001)	0.05 (0.821)
PY305	14.53 (<0.001)	1.03 (0.391)	14.86 (<0.001)	0.58 (0.447)
CFSI	1.37 (0.252)	2.52 (0.040)	2.68 (0.102)	1.90 (0.168)
CCI	2.10 (0.099)	2.07 (0.083)	0.24 (0.622)	5.44 (0.019)

F_{ROH} , coefficient of genomic inbreeding calculated based on runs of homozygosity.

^aMY305, 305-day milk production; FY305, 305-day fat production; PY305, 305-day protein production; CFSI, Calving to first service interval; CCI, calving to conception interval.

several alternative estimates of genomic inbreeding coefficients, it is accepted that F_{ROH} values are more accurate and/or comparable to classical inbreeding coefficient based on pedigree information to determine inbreeding in several livestock species and humans, as they reflect the shared ancestry of genomic haplotypes (Curik *et al.*, 2014; Purfield *et al.*, 2017; Yengo *et al.*, 2017; Yoshida *et al.*, 2020).

Overall, based on the F_{ROH} , the rate of inbreeding present in this sample of purebred and crossbred cows is similar to the rates of inbreeding reported in other studies; 1.93–3.05% in Irish Holstein–Friesian (McParland *et al.*, 2007), 3.6% in US Holstein (Cassell *et al.*, 2003) and 5.00 in French Holstein (Dezetter *et al.*, 2015).

In our study the effects of inbreeding and crossbreeding on milk production and fertility traits were evaluated using a single model. A decrease in milk, fat and protein yields with a decreasing breed proportion of Holstein is in line with previous research in New Zealand (Lembeye *et al.*, 2016), Ireland (Prendiville *et al.*, 2011) and Argentina (Baudracco *et al.*, 2011; Mancuso and Marini, 2012; Biga *et al.*, 2022). The productive performance of the HJ crossbred cows was lower than the productive performance

of the purebred Holstein and higher than the JX crossbred cows. Because there were not purebred Jersey cows it was not possible to estimate heterosis effects. Crossbreeding systems have been adopted widely in the New Zealand dairy industry because of the favourable heterosis for milk production and fertility resulting in improved farm profitability (Lopez-Villalobos *et al.*, 2000). All crossbred cows in the current study tended to have shorter CFSI and CCI than purebred Holstein but differences were not significant. These results can be used for modelling studies to evaluate the effects of these fertility rates on farm profitability under pasture-based conditions of Argentina.

The effects of inbreeding, evaluated as the regression coefficient of the milk production and fertility traits on the estimators of genomic inbreeding (F_{GRM} , F_{HOM} and F_{UNI}) considered in the current research were not significant. These results are in disagreement with previous reports by Cassell *et al.* (2003) in US Holstein cows and McParland *et al.* (2007) in Irish dairy cattle. The only trait that was affected by inbreeding, considering the F_{ROH} estimator, was CCI ($P < 0.05$), which agrees with the effect of inbreeding on the lengthening of the calving interval by up to 0.31 days per 1% increase in inbreeding (Fuerst and Sölkner,

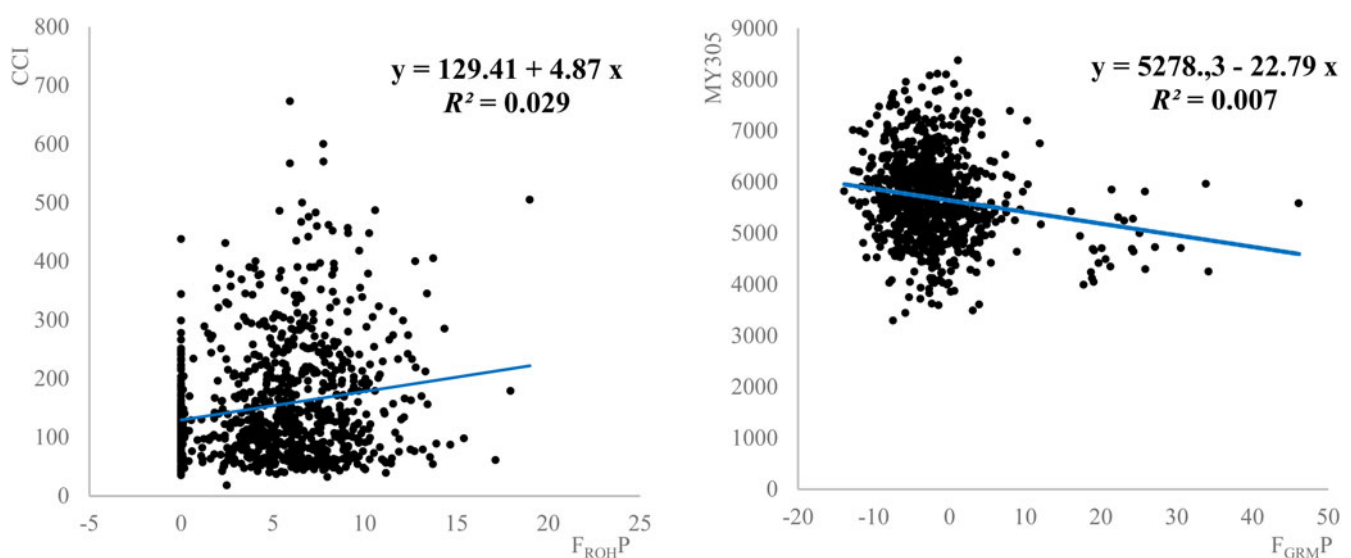


Figure 2. Pearson correlations and *P*-values (within brackets) (above diagonal), scatter plots (below diagonal) between each pair of the four genomic inbreeding estimators and densities (diagonal) of the inbreeding estimators of dairy cows in commercial herds of Argentina. ¹ F_{ROH} , coefficient of genomic inbreeding calculated based on proportion of the autosomal genome in runs of homozygosity; ² F_{GRM} , coefficient of genomic inbreeding obtained from the diagonal elements of the genomic relationship matrix; ³ F_{HOM} , coefficient of genomic inbreeding calculated based in the homozygous excess; ⁴ F_{UNI} , coefficient of genomic inbreeding calculated based on the correlation between uniting gametes.

Table 3. Least-squares means and standard errors for production and fertility traits, and means and standard errors of inbreeding coefficients of purebred Holstein and crossbred Holstein × Jersey cows from pasture-based commercial herds of Argentina

Trait ^a	Breed ^b				P-value ^c
	H 639	HX 64	HJ 157	JX 30	
MY305 (kg)	5932 ± 67.0	5566 ± 122.0	5125 ± 117.0	4813 ± 159.0	<0.001
FY305 (kg)	219 ± 2.2	215 ± 4.1	203 ± 3.9	197 ± 5.3	<0.001
PY305 (kg)	205 ± 2.3	195 ± 4.1	182 ± 3.9	175 ± 5.3	<0.001
CFSI (days)	90 ± 3.0	77 ± 6.5	81 ± 5.9	81 ± 8.9	0.252
CCI (days)	160 ± 6.3	151 ± 14.0	140 ± 12.2	115 ± 19.4	0.099
F_{ROH}	6.6 ± 0.11	3.7 ± 0.18	0.020 ± 0.0004	5.2 ± 0.76	
F_{GRM}	-3.4 ± 0.16	-0.2 ± 0.51	-0.9 ± 0.35	21 ± 1.8	
F_{HOM}	0.8 ± 0.17	-6.7 ± 0.38	-11.2 ± 0.18	-6 ± 1.2	
F_{UNI}	-1.3 ± 0.09	-3.5 ± 0.19	-6.0 ± 0.14	7 ± 1.4	
Heterozygosity	0.35 ± 0.0004	0.36 ± 0.0004	0.38 ± 0.0003	0.34 ± 0.0004	

^aMY305, 305-day milk production; FY305, 305-day fat production; PY305, 305-day protein production; CFSI, calving to first service interval; CCI, calving to conception interval. F_{ROH} , mean of genomic inbreeding coefficient calculated based on proportion of the autosomal genome in runs of homozygosity. Heterozygosity was calculated as the proportion of heterozygous marker genotypes for each individual.

^bH, purebred cows with 1.00 Holstein; HX, crossbred Holstein with 0.75 Holstein and 0.25 Jersey; HJ, crossbred Holstein–Jersey with 0.50 Holstein and 0.50 Jersey; JX, crossbred Jersey with 0.25 Holstein and 0.75 Jersey.

^cP-value for breed group effect.

1994; Smith *et al.*, 1998). In this study, the Holstein sires were imported from the US Holstein population using frozen semen, which is a common practice to produce herd replacements. The apparent constriction of no significant effect of inbreeding on milk and fertility traits in this study compared with the significant effects reported by Cassell *et al.* (2003) in US Holstein cows and McParland *et al.* (2007) in Irish Holstein–Friesian cows can be explained considering that in this study we evaluated the effect of inbreeding using a crossbreed population.

The apparent no effects of inbreeding on MY305, FY305, PY305 and CFSI are likely attributed to the fact that the effect of genomic inbreeding was evaluated across breed groups. Crossbreeding and inbreeding have opposite effects (Falconer and Mackay, 1996); inbreeding is the mating of animals that are

more closely related than the average in a population increasing homozygosity, which increases the frequency of unfavourable genotypes, and crossbreeding is the mating of animals that are less related than the average in a population increasing heterozygosity, which allows the expression of heterosis (interactions between alleles in the same locus). Table 3 shows that the HJ crossbred cows, which have the maximum rate of heterozygosity (0.38) in fact, had the lowest mean rate of inbreeding (0.02%) measured according to the F_{ROH} formula. Furthermore, F_{ROH} would credibly measure autozygosity (homozygosity genomic segments produced by identity by descent) and is nowadays considered the most consistent coefficient and the one that best captures inbreeding depression in livestock (Keller *et al.*, 2011; Peripolli *et al.*, 2017; Caballero *et al.*, 2020, 2022; Lozada-Soto

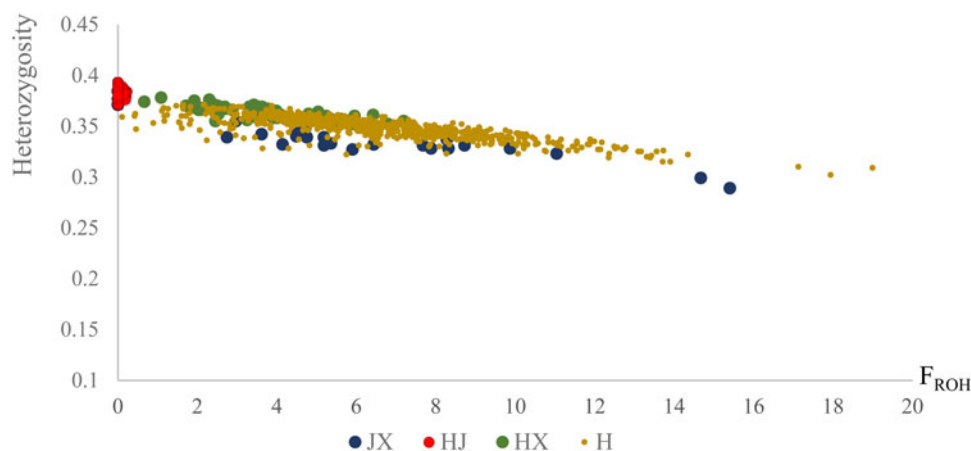


Figure 3. Relationship between heterozygosity and F_{ROH} in purebred Holstein and crossbred Holstein × Jersey cows from pasture-based commercial herds of Argentina. H, purebred cows with 1.00 Holstein; HX, crossbred Holstein with 0.75 Holstein and 0.25 Jersey; HJ, crossbred Holstein–Jersey with 0.50 Holstein and 0.50 Jersey; JX, crossbred Jersey with 0.25 Holstein and 0.75 Jersey. ¹Heterozygosity was calculated as the proportion of heterozygous marker genotypes for each individual. ² F_{ROH} , coefficient of genomic inbreeding calculated based on proportion of the autosomal genome in runs of homozygosity.

et al., 2022). Based on the available information so far, F_{ROH} shows the highest correlation with the classical F pedigree-based (Purfield et al., 2012, 2017; Ferencaković et al., 2013; Marras et al., 2015; Caballero et al., 2022; Dadousis et al., 2022).

Other studies have reported no significant effects of inbreeding on production and fertility traits (Hodges et al., 1979) using a small sample of cows, but concordantly with the results obtained in the current study. In a much larger study, Hofmannová et al. (2019) conclude that inbreeding has a negligible influence on the breeding values for conception.

This study reports significant effects of breed on milk production and fertility using data from a large commercial herd. These results can be used for simulation studies that assist the design of crossbreeding programmes (Lopez-Villalobos et al., 2000; Clasen et al., 2020) that increases farm profitability. This study also reports rates of genomic inbreeding and heterozygosity calculated using genetic markers. It was found that, in general, the effects of genomic inbreeding on milk production and fertility traits were not significant, except on the CCI. A possible reason for these non-significant effects is that these effects were evaluated in a crossbred population in which rates of heterozygosity would operate to some extent in the opposite direction to rates of genomic inbreeding.

Acknowledgements. The authors acknowledge the dairy farms for giving access to phenotypic records and allowing collecting biological samples (Project PAE147-ANCyPT). H. A. C. acknowledges the support from CONICET (Consejo Nacional de Investigaciones en Ciencia y Tecnología).

Author contributions. M. J. B.: conceptualization, data curation, methodology, formal analysis, visualization, investigation, writing – original draft; H. A. C.: conceptualization, data curation, methodology, formal analysis, investigation, writing – review and editing; M. A. P.: project administration, review and editing; N. L.-V.: conceptualization, methodology, validation, writing – review and editing, supervision. The final manuscript has been read and approved by all authors.

Funding statement. The present study was partially supported by project Instituto Nacional de Tecnología Agropecuaria (INTA, Hurlingham, Argentina) 2023-PD-I108.

Competing interests. None.

Ethical standards. The procedures followed for extraction and handling of samples followed the guidelines described in the Care and Use of Experimental Animals of the National Institute of Agricultural Technology (CICUA-INTA) manual. Farm owners' consent was obtained before animal sampling.

References

- Baudracco J, Lopez-Villalobos N, Romero LA, Scandolo D, Maciel M, Comeron EA, Holmes CW and Barry TN (2011) Effects of stocking rate on pasture production, milk production and reproduction of supplemented crossbred Holstein–Jersey dairy cows grazing lucerne pasture. *Animal Feed Science and Technology* **168**, 131–143.
- Beribe MJ (2020) Curvas de lactancia e identificación QTLs asociados a características productivas en ganado Holando y cruza Holando×Jersey (PhD thesis). Facultad de Ciencias Veterinarias Universidad de Buenos Aires, Ciudad autónoma de Buenos Aires, Argentina. Available at <http://hdl.handle.net/20.500.12123/11716> (accessed 29 June 2023).
- Biga P, Borbona I, Lammoglia-Villagómez MÁ, Marini PR and Hernández-Carbajal GR (2022) Indicadores productivos y reproductivos de vacas Holstein y Holstein x Jersey durante la primera lactancia en sistemas a pastoreo. *Revista Biológico Agropecuaria Tuxpan* **10**, 155–170.
- Buckley F, Lopez-Villalobos N and Heins JB (2014) Crossbreeding: implications for dairy cow fertility and survival. *Animal: An International Journal of Animal Bioscience* **8**, 122–133.
- Caballero A, Villanueva B and Druet T (2020) On the estimation of inbreeding depression using different measures of inbreeding from molecular markers. *Evolutionary Applications* **14**, 416–428.
- Caballero A, Fernández A, Villanueva B and Toro MA (2022) A comparison of marker-based estimators of inbreeding and inbreeding depression. *Genetics Selection Evolution* **54**, 82.
- Cámara Argentina de Biotecnología de la Reproducción e Inseminación Artificial (2021) Estadísticas movimiento anual de dosis de semen bovino. Buenos Aires: CABIA. Available at https://lookerstudio.google.com/u/0/reporting/894b22bc-618a-4586-b021-e240e94111ee/page/p_2x3l28qwtc (accessed 29 June 2023).
- Carignano H, Roldan DL, Beribe MJ, Raschia MA, Amadio AF, Nani JP, Gutiérrez G, Alvarez IM, Trono KG, Poli MA and Miretti M (2018) Genome-wide scan for common SNPs affecting bovine leukemia virus infection level in dairy cattle. *BMC Genomics* **19**, 142.
- Cassell B, Adamec V and Pearson R (2003) Effect of incomplete pedigrees on estimates of inbreeding and inbreeding depression for days to first service and summit milk yield in Holsteins and Jerseys. *Journal of Dairy Science* **86**, 2967–2976.
- Clasen JB, Fikse WF, Kargo M, Rydhmer L, Strandberg E and Østergaard S (2020) Economic consequences of dairy crossbreeding in conventional and organic herds in Sweden. *Journal of Dairy Science* **103**, 514–528.
- Coffey EL, Horan B, Evans RD and Berry DP (2016) Milk production and fertility performance of Holstein, Friesian, and Jersey purebred cows and their respective crosses in seasonal-calving commercial farms. *Journal of Dairy Science* **99**, 5681–5689.
- Curik I, Ferencaković M and Sölkner J (2014) Inbreeding and runs of homozygosity: a possible solution to an old problem. *Livestock Science* **166**, 26–34.
- Dadousis C, Ablondi M, Cipolat-Gotet C, van Kaam JT, Marusi M, Cassandro M, Sabbioni A and Summer A (2022) Genomic inbreeding coefficients using imputed genotypes: assessing different estimators in Holstein–Friesian dairy cows. *Journal of Dairy Science* **105**, 5926–5945.
- Dezetter C, Leclerc H, Mattalia S, Barbat A, Boichard D and Ducrocq V (2015) Inbreeding and crossbreeding parameters for production and fertility traits in Holstein, Montbéliarde, and Normande cows. *Journal of Dairy Science* **98**, 4904–4913.
- Doekes HP, Veerkamp RF, Bijma P, de Jong G, Hiemstra SJ and Windig JJ (2019) Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. *Genetics Selection Evolution* **51**, 1–16.
- Dutour Pérez EJ and Melucci LM (2011) Relationship between productive and reproductive parameters of dairy cows in different production systems. *Latin American Archives of Animal Production* **18**, 133–147.
- Falconer DS and Mackay TFC (1996) *Introduction to Quantitative Genetics*. Harlow, England: Prentice Hall.
- FCA (2016) Conformación del ganado lechero. Córdoba: Facultad de Ciencias Agrarias Universidad Nacional de Córdoba. Available at <http://www.agro.unc.edu.ar/~wpweb/pleche/wp-content/uploads/sites/8/2016/06/Conformacion-de-holando-y-razas-2016.pdf> (accessed 29 June 2023).
- Ferencaković M, Hamzić E, Gredler B, Solberg TR, Klemetsdal G, Curik I and Sölkner J (2013) Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *Journal of Animal Breeding and Genetics* **130**, 286–293.
- Fuerst C and Sölkner J (1994) Additive and nonadditive genetic variances for milk yield, fertility and lifetime performance traits of dairy cattle. *Journal of Dairy Science* **77**, 1114–1125.
- Gastaldi L, Litwin G, Maekawa M, Moretto M, Marino M, Engler P, Cuatrin A, Centeno A and Galetto A (2020) Encuesta Sectorial Lechera del INTA. Resultado del ejercicio productivo 2018/2019. Buenos Aires, Argentina: INTA. Available at <https://repositorio.inta.gob.ar/handle/20.500.12123/9564> (accessed 26 June 2023).
- Gutiérrez-Reinoso MA, Aponte PM and García-Herreros M (2022) A review of inbreeding depression in dairy cattle: current status, emerging control strategies, and future prospects. *Journal of Dairy Research* **89**, 3–12.
- Hodges J, Tannen L, McGillivray BJ, Hiley PG and Ellis S (1979) Inbreeding levels and their effect on milk, fat and calving interval in Holstein–Friesian cows. *Canadian Journal Animal Science* **59**, 153–158.
- Hofmannová M, Přibyl J, Krupa E and Pešek P (2019) Estimation of inbreeding effect on conception in Czech Holstein. *Czech Journal Animal Science* **64**, 309–316.

- Howrigan DP, Simonson MA and Keller MC** (2011) Detecting autozygosity through runs of homozygosity: a comparison of three autozygosity detection algorithms. *BMC Genomics* **12**, 460.
- Iversen MW, Nordbø Ø, Gjerlaug-Enger E, Grindflek E, Lopes MS and Meuwissen T** (2019) Effects of heterozygosity on performance of purebred and crossbred pigs. *Genetics Selection Evolution* **51**, 8.
- Keller MC, Visscher PM and Goddard ME** (2011) Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics* **189**, 237–249.
- Lazzarini B, Baudracco J, Tuñón G, Gastaldi L, Lyons N, Quattrochi H and Lopez-Villalobos N** (2019) Review: Milk production from dairy cows in Argentina: current state and perspectives for the future. *Applied Animal Science* **35**, 426–432.
- Lembeye F, Lopez-Villalobos N, Burke JL and Davis SR** (2016) Estimation of genetic parameters for milk traits in cows milked once- or twice-daily in New Zealand. *Livestock Science* **185**, 142–147.
- Leutenegger AL, Prum B, Génin E, Verny C, Lemainque A, Clerget-Darpoux F and Thompson EA** (2003) Estimation of the inbreeding coefficient through use of genomic data. *American Journal of Human Genetics* **73**, 516–523.
- Lopez-Villalobos N, Garrick DJ, Holmes CW, Blair HT and Spelman RJ** (2000) Profitabilities of some mating systems for dairy herds in New Zealand. *Journal of Dairy Science* **83**, 144–153.
- Lozada-Soto EA, Tiezzi F, Jiang J, Cole JB, VanRaden PM and Maltecca C** (2022) Genomic characterization of autozygosity and recent inbreeding trends in all major breeds of US dairy cattle. *Journal Dairy Science* **105**, 8956–8971.
- Maiwashe A, Nephawe K and Theron H** (2008) Estimates of genetic parameters and effect of inbreeding on milk yield and composition in South African Jersey cows. *South African Journal of Animal Science* **38**, 119–125.
- Malécot G** (1969) *The Mathematics of Heredity*. Freeman, San Francisco. [Translated by D. M. Yermanos, Ed.].
- Mancuso WA and Marini PR** (2012) Comportamiento de vacas lecheras primíparas y sus cruza en un sistema a pastoreo de Entre Ríos (Argentina). *Revista Veterinaria* **23**, 138–143.
- Marras G, Gaspa G, Sorbolini S, Dimauro C, Ajmone-Marsan P, Valentini A, Williams JL and Macciotta NP** (2015) Analysis of runs of homozygosity and their relationship with inbreeding in five cattle breeds farmed in Italy. *Animal Genetics* **46**, 110–121.
- McParland S, Kearney JF, Rath M and Berry DP** (2007) Inbreeding effects on milk production, calving performance, fertility, and conformation in Irish Holstein-Friesians. *Journal of Dairy Science* **90**, 4411–4419.
- Mullen KAE, Dings EHA, Kearns RR and Washburn SP** (2015) Case study: A comparison of production, reproduction, and animal health for pastured dairy cows managed either conventionally or with use of organic principles. *The Professional Animal Scientist* **31**, 167–174.
- Nietlisbach P, Keller LF, Camenisch G, Guillaume F, Arcese P, Reid JM and Postma E** (2017) Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proceedings of the Royal Society, B: Biological Science* **284**, 20162763.
- Peripolli E, Munari DP, Silva MV, Lima AL, Irgang R and Baldi FS** (2017) Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics* **48**, 255–271.
- Prendiville R, Shaloo L, Pierce KM and Buckley F** (2011) Comparative performance and economic appraisal of Holstein-Friesian, Jersey and Jersey×Holstein-Friesian cows under seasonal pasture-based management. *Irish Journal of Agricultural and Food Research* **50**, 123–140.
- Pryce JE, Haile-Mariam M, Goddard ME and Hayes BJ** (2014) Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. *Genetics Selection Evolution* **46**, 1–14.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ and Sham PC** (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* **81**, 559–575.
- Purfield DC, Berry DP, McParland S and Bradley DG** (2012) Runs of homozygosity and population history in cattle. *BMC Genetics* **13**, 70.
- Purfield DC, McParland S, Wall E and Berry DP** (2017) The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PLoS ONE* **12**, 5.
- Ritland K** (1996) Estimators for pairwise relatedness and inbreeding coefficients. *Genetics Research* **67**, 175–185.
- Rokouei M, Torshizi RV, Shahrabak MM, Sargolzaei M and Sørensen A** (2010) Monitoring inbreeding trends and inbreeding depression for economically important traits of Holstein cattle in Iran. *Journal of Dairy Science* **93**, 3294–3302.
- SAS® Institute Inc.** (2013) *Base SAS 9.4 Procedures Guide*. Cary, NC: Statistical Analysis System Institute Inc.
- Smith LA, Cassell B and Pearson R** (1998) The effects of inbreeding on the lifetime performance of dairy cattle. *Journal of Dairy Science* **81**, 2729–2737.
- VanRaden PM** (2008) Efficient methods to compute genomic predictions. *Journal of Dairy Science* **91**, 4414–4423.
- Villanueva B, Fernández A, Saura M, Caballero A, Fernández J, Morales-González TM and Pong-Wong R** (2021) The value of genomic relationship matrices to estimate levels of inbreeding. *Genetics Selection Evolution* **53**, 42.
- Wright S** (1922) Coefficients of inbreeding and relationship. *The American Naturalist* **56**, 330–338.
- Yang J, Lee SH, Goddard ME and Visscher PM** (2011) GCTA: a tool for genome-wide complex trait analysis. *American Journal of Human Genetics* **88**, 76–82.
- Yengo L, Zhu Z, Wray NR, Weir BS, Yang J, Robinson MR and Visscher PM** (2017) Detection and quantification of inbreeding depression for complex traits from SNP data. *Proceedings of the National Academy of Sciences* **114**, 8602–8607.
- Yoshida GM, Cáceres P, Marín-Nahuelpi R, Koop BF and Yáñez JM** (2020) Estimates of autozygosity through runs of homozygosity in farmed coho salmon. *Genes* **11**, 490.