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Environmental impact of Holstein Friesian and 3-breed crossbred dairy cows using a Life Cycle Assessment approach applied to individual animals

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ABSTRACT

This study aimed to set up a Life Cycle Assessment (LCA) approach at level of individual animals to assess the effects of a 3-breed crossbreeding program on the environmental impact of cows. It involved 564 cows, 279 purebred Holstein (HO) and 285 crossbreds (CR), originated from a 3-breed crossbreeding program based on the rotational use of Viking Red, Montbèliarde and HO sires and kept in 2 dairy herds of northern Italy (224 and 340 cows/herd, respectively). The reference unit of the LCA model was the lifetime of cows, from the birth to culling or death. Data were collected at different levels: individual animal-based data referred to the whole life (birth, calving, dry, cull or death dates, and milk production); individual test-date collection of body measures and BCS, used to predict body weight and to estimate energy requirements; common farm-based data concerning herd management (diets composition, and materials used). Data were used to compute: dry matter intake, milk and milk components production, gross income (GI) and income over feed costs (IOFC) pertaining to the lifespan of cows. An individual LCA-derived approach was set up to compute global warming potential (GWP), acidification and eutrophication potential (AP and EP, respectively), and land occupation (LO), which have been associated with different functional units (cow in her whole life or per d of life; kg of milk fat plus protein, and € of GI and of IOFC produced in the herd life). Data were analyzed using a generalized linear model including the fixed effects of genetic group (CR vs HO), farm and their interaction (genetic group x farm). Compared with HO, CR cows completed more lactations (+12%), had earlier first calving (−2 weeks), yielded more fat plus protein in milk both in the lifespan (+8%) and per d of life (+4%). Concerning the environmental impact, when compared with HO herd mates, CR cows had nominal greater emissions per cow in the whole life, similar emissions per d of life and nearly 3% lower GWP, AP and EP per

kg of fat plus protein yielded in lifespan. Income over feed costs per unit of emission tended to be nearly 4% greater in CR compared with HO cows. Also the use of land tended to be lower in CR compared with HO in most indicators considered. In conclusion, LCA could be adapted to represent individual animals. Moreover, managing dairy cows according to a 3-breed rotational crossbreeding scheme may be regarded as a strategy that can contribute to mitigate the emissions and to improve the environmental impact of dairy operations. **Key words:** individual cow LCA, crossbreeding, global warming potential, acidification and eutrophication potential

INTRODUCTION

In the last decade, increasing environmental sustainability and decreasing the footprint of agri-food chains have become global challenges. The dairy cattle sector is a notable contributor to the anthropogenic emission of greenhouse gases (GHG; Xu et al., 2021) and other pollutants, such as reactive nitrogen and phosphorus (Steinfeld et al., 2006). Moreover, it contributes to the exploitation of limited resources such as fertile land (Mottet et al., 2017).

Life cycle assessment (LCA; ISO, 2006) has become a standard method for assessing the environmental impact related to a product and has been extensively applied to assess the impacts associated with dairy milk production (Baldini et al., 2017; Mazzetto et al., 2022). The adoption of innovative breeding practices and technologies able to improve the production efficiency of dairy systems may help to reduce emission intensity (Hristov et al., 2013). Several studies have demonstrated that an increase in milk yield can mitigate the impact per unit of milk (Gerber et al., 2011), mainly because of the dilution of the maintenance requirements on a higher milk production (Capper et al., 2009). However, further increases in milk production may not be necessarily associated to further increases in profitability of dairy herds (Moallem, 2016), thus leading to a decrease of the economic sustainability of such a strategy. Also improvements in the functional parameters of dairy

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cows, including reproductive traits, health status, and longevity, may affect the environmental impact associated with dairy production (Grandl et al., 2019; Berton et al., 2023). However, in the last decades, the genetic selection of high production dairy cow breeds, primarily Holstein Friesian (**HO**), has caused a decline in fertility, health, and longevity (Dezetter et al., 2019; Hazel et al., 2020a). Conversely, the crossbreeding technique has shown favorable effects on traits related to reproductive performance, fertility and health (Sørensen et al., 2008; Buckley et al., 2014; Malchiodi et al., 2014a), and has been reported to be more sustainable than HO purebreeding (Magne and Quénon, 2021). For this reason, crossbreeding programs have gained attention among dairy stakeholders in the last decade. In particular, in intensive, indoor milk systems, interest is growing toward a 3-breed rotational program using Viking Red (**VR**), Montbéliarde (**MO**), and HO sires. This program has been reported to positively affect the production and quality of milk (Malchiodi et al., 2014b; Shonka-Martin et al., 2019a; Saha et al., 2020), efficiency (Shonka-Martin et al., 2019b; Piazza et al., 2023b), and the lifetime profitability of cows (Hazel et al., 2021). Thus, rotational crossbreeding appears to be a mating system able to affect both productivity and fitness; however, the effects of this strategy on the environmental impact of dairy systems have still not been quantified.

The assessment of the environmental impact of livestock systems through LCA is generally performed at the farm level, i.e., taking the dairy farm as the reference unit of the procedure, without no consideration for intra-farm variability of animal traits. However, the use of farm average data could be a limitation when the potential mitigation actions would involve aspects for which the individual variability is notable, such as enteric methane production (Garnsworthy et al., 2012; Martínez-Marín et al., 2023a), reproduction performance and health status (Maltz, 2020) or feed efficiency (Evers et al., 2023). Since crossbreeding programs act at the animal rather than at the farm scale, their evaluation as potential mitigation practice requires to consider the variability associated with the individual dairy cow, whereas the effects due to the rearing and management conditions can be overridden by keeping animals of different genetic groups in the same herds and managing and feeding them in the same way. On the basis of these considerations, the use of the individual animal rather than of the farm as the reference unit for LCA modeling has been explored both in dairy (Grandl et al., 2019) and beef (McAuliffe et al., 2018) systems, although with limitations in terms of model settings and animals sample size.

The aim of this study was: i) to adapt the LCA methodology to the animal scale, considering the individual dairy cow as the reference unit and its whole lifespan as the reference period, and ii) to compare the environmental impact of crossbred cows (**CR**) from a 3-breed rotational scheme using VR, MO and HO bulls with that of HO purebred herd mates.

MATERIALS AND METHODS

Samples and data were collected from Italian commercial dairy farms according to procedures compliant with Italian legislation concerning the care and safeguard of animals (DL n. 26, 4 March 2014).

Experimental design and data acquisition

This study, which is part of a larger project on the effects of rotational crossbreeding on specialized dairy herds, was organized according to a 2×2 factorial design based on 2 farms and 2 genetic groups of cows within each farm.

The experimental design and the available data are described in a previous study (Piazza et al., 2023b) involving 791 cows kept in 2 farms located in the Italian Po valley. In that study, the following information was collected once per cow in a single test day: milk yield; milk composition (i.e., fat, protein, and lactose content of milk); body weight (**BW**), predicted on the basis of body size measurements, parity, and days in milk at measurement; and body condition score (**BCS**).

Both farms had been using the 3-breed rotational crossbreeding system known as ProCROSS for more than a decade, according to a mating design described in detail by Saha et al. (2020) which involved HO, VR, and MO as sire breeds. Overall 133 HO bulls, 19 VR bulls and 25 MO bulls were used as sires of the CR and purebred cows in the herds of concern (Saha et al., 2020). In the present study crossbred cows have been taken as a mixture of generations and sire breeds representing the 3-breed rotational system; therefore, the comparison of sire breeds within CR was outside the scope of this research.

The 2 farms produced milk intended for PDO hard cheese production. The herd A was located in the Lombardia region (province of Brescia), and provided milk used for producing the Grana Padano cheese; its average production level was around 11300 kg of milk/cow/year and its farm agricultural area was equal to 105 ha. The herd B was located in the Emilia-Romagna region (province of Modena), and provided milk used for producing the Parmigiano Reggiano hard cheese; its average production level was around 9500 kg of milk/cow/year and its farm agricultural area was equal to 150 ha.

Within each herd, the HO and CR cows were kept in free stalls with cubicles, reared and milked together, and fed the same total mixed rations, compliant with the regulations of their particular cheese consortium (corn and sorghum silages and concentrates for Grana Padano, and dry roughage, mainly alfalfa and meadow hay, and concentrates for Parmigiano Reggiano). The composition of the diets is reported in Table 1.

Data editing and data set preparation

To compute the environmental impact associated with each cow, the starting data set was enriched with additional information as follows. First, data about the major events that occurred during the lifetime of the cows (dates of birth, calving, dry-off and culling or death) were collected from the official Italian milk recording system and from herd databases. Second, data about milk production over the cows' lifetime

(yield of milk, fat and protein) were acquired from the official Italian milk recording system. Data derived from the lifetime events and lifetime milk production were merged with the initial data set using the cow as the common variable. To obtain the final data set we retained the records of all cows that met the following conditions:

- culled or dead by the end of April 2021 (end date for data set acquisition and updating) with at least 36 mo of opportunity of herd life from first calving to the end of data collection;
- having information on their full herd life (production, milk nutrient contents, calving and dry dates).

The final data set consisted of 564 cows: 279 HO (141 in herd A and 138 in herd B) and 285 CR (83 in herd A and 202 in herd B).

Table 1. Ingredients and chemical composition of the diets fed to lactating and dry cows and to heifers in the two farms

	Farm A			Farm B		
	Lactating cows	Dry cows	Heifers	Lactating cows	Dry cows	Heifers
Ingredients, % DM						
Maize silage	20.6	31.4	9.2	—	—	—
Sorghum silage	7.7	—	31.1	—	—	—
Wheat silage	2.6	—	0.4	—	—	—
Maize ears silage	19.4	—	—	—	—	—
Grass silage	2.9	39.4	20.8	—	—	—
Hay	0.4	20.8	—	11.1	78.4	72.1
Alfalfa hay	2.7	—	4.4	28.6	3.9	2.5
Wheat straw	—	—	12.5	—	—	—
Maize grain	5.5	—	0.7	30.6	3.1	12.9
Barley grain	—	—	—	10.8	2.0	0.6
Sunflower meal	—	—	13.5	—	—	—
Molasses	4.6	—	0.6	—	—	—
Compound feed 1	24.4	—	3.7	—	—	—
Compound feed 2	9.2	—	1.1	—	—	—
Compound feed 3	—	8.4	2.0	—	—	—
Compound feed 4	—	—	—	15.5	6.2	11.9
Compound feed 5	—	—	—	2.0	4.8	—
Compound feed 6	—	—	—	1.2	—	—
Compound feed 7	—	—	—	0.1	1.6	—
Chemical composition						
GE, MJ/ kg DM	18.35	16.80	17.60	18.37	17.94	18.36
DE, MJ/ kg DM	14.06	11.56	11.64	13.58	10.20	11.7
NE, MJ/ kg DM	7.10	5.49	5.70	6.73	4.80	5.52
CP, % DM	15.76	10.32	13.04	17.39	11.64	14.59
CF, % DM	12.99	27.40	27.77	15.91	29.04	24.87
EE, % DM	4.54	3.67	3.21	3.20	2.86	3.18
Ash, % DM	3.84	9.01	8.05	6.26	9.17	8.01
NDF, % DM	30.40	49.64	54.88	30.86	59.23	50.5
Starch, % DM	27.59	11.75	4.90	27.27	3.53	13.07
P, % DM	0.33	0.48	0.26	0.36	0.28	0.32
NSC, % DM	45.47	27.35	20.90	42.29	17.10	23.72
OMD, % DM	76.30	66.25	66.77	73.72	59.82	64.34

¹DM: dry matter, GE: gross energy, DE: digestible energy, NE: net energy, CP: crude protein, CF: crude fiber, EE: ether extract, NDF: neutral detergent fiber, P: phosphorous, NSC: non-structural carbohydrates, OMD: digestible organic matter.

Lifetime, herd life, and production of fat, and protein in the herd life

With regard to the lifetime events data, the heifer period (from birth to first calving), herd life (from first calving to culling or death), and lifetime (from birth to culling or death) were calculated for each cow. Moreover, for each lactation, the lactating period was calculated as the dry-off date minus the previous calving date, while the dry period was calculated as the following calving date minus the dry-off date.

With regard to milk production, lifetime milk yield was computed as the sum of the cumulative milk yield of each cow per each lactation. The amount of milk fat and protein yielded per lactation were calculated by multiplying the milk actually yielded in each lactation by the average milk fat and protein content of each lactation. Lifetime milk fat and protein production were computed by summing up the relative productions obtained in each lactation.

The fat and protein yielded per day of life were computed as the amount of each milk component yielded over the lifetime divided by the lifetime duration.

Average BW and BCS during life

In this study, we used the cow in her whole lifetime as the reference unit for our environmental impact assessment. However, predicted BW and BCS were collected once per cow in a specific test-day, and needed to be processed and standardized to refer to the whole cow's lifetime before further analysis. To this purpose, we used a procedure similar to that used for estimating the mature equivalent cow production (Marti and Funk, 1994); procedure is fully described in appendix 1.

Estimation of net energy requirements

The net energy (**NE**) requirements were estimated separately for the different lifetime periods of each cow, namely the heifer, lactating, and dry periods. The total daily NE requirements were computed as the sum of the NE requirements for maintenance, growth, pregnancy, and milk production. All the equations used to estimate NE requirements are reported in Supplement 1. Briefly, the net energy requirements for maintenance were computed for heifers by converting the average BW during the heifer period into metabolic weight and using the coefficient proposed by NASEM (2021) (0.418 MJ/kg/d). The net energy requirements for the maintenance of cows (lactation and dry periods) were computed using the equation based on body protein mass proposed by Piazza et al. (2023b) for CR and purebred HO to take into account the large differences in body

condition scores between HO and CR. The average BW and BCS during the cows' herd life (Appendix 1) were included in the equation of Piazza et al. (2023b) for the estimation of body protein mass. The net energy requirements for growth and pregnancy were computed using IPCC (2019) equations, whereas the NE requirements for milk were based on NASEM (2021).

Estimation of carcass weight and carcass value

The BW of each cow at culling was assumed to be equal to the estimated average BW at the last parity of the cow (i.e., the estimated BW at first, second or third lactation for cows culled during the first, second or third lactation and later, respectively). The dressing percentage (**DP**) per genetic group was derived from a sub-sample of cull cows ($n = 286$, 114 HO and 172 CR) for which the actual carcass weight (**CW**) and carcass value were available (Piazza et al., 2023a). For this subsample, the individual DP was first calculated by dividing the actual CW by the estimated BW at culling; then, the DP values were averaged by genetic group (0.419 and 0.454 for HO and CR, respectively). The average DP within each genetic group (HO and CR) was multiplied by the estimated BW at culling to obtain the individual estimated CW. As internal validation, the estimated CW of the cows from the sub-sample used for calibrating the average DP was compared with the actual measured CW of cull cows at slaughterhouse (Piazza et al., 2023a); the average difference between the estimated and calculated CW was 1.7 ± 58.9 kg. The carcass unit price per genetic group, derived from the study by Piazza et al. (2023a), was equal to 1.89 and 2.09 €/kg CW for HO and CR, respectively, and was used, together with estimated CW, for computing the individual carcass value for the cull cows in this study.

Lifetime gross income and income over feed costs

Lifetime gross income (**GI**) was calculated as the sum of all revenues from cows and included:

- the value of the milk produced in the whole herd life, using the average price of milk aimed to produce Grana Padano from 2010 to 2021 for herd A, equal to 0.375 €/kg, and the average price of milk aimed to produce Parmigiano Reggiano from 2010 to 2021 for herd B, equal to 0.580 €/kg (CLAL, 2022);
- the values of bull calves and female calves exceeding the replacement needs. Both farmers stated that purebred HO and CR calves were paid the

same monetary worth; thus, we used a value of 50 €/calf for all calves sold for meat purposes;

- the revenue for cull cows, determined as carcass value, as detailed above.

Feed costs were determined using the average prices from the database of the Veneto Region Breeders Association (AVA, Padova, Italy) for each ingredient of the rations fed to the different animal categories (Table 1). The cost (reference period: year 2021) was 0.234, 0.211 and 0.187 €/kg dry matter for rations for lactating cows, dry cows, and heifers, respectively, in herd A, and 0.247, 0.223 and 0.221 €/kg dry matter, respectively, in herd B. Feed cost per cow was calculated by multiplying the feed cost (€/kg) by the estimated total dry matter intake (DMI) consumed in the whole lifetime summing up heifer, lactation, and dry periods. As better detailed in the LCA inventory subchapter below, dry matter intake has been estimated at individual level as the ratio between the daily NE requirements and the NE content of the diets. Income over feed cost (IOFC) was last calculated as the revenue from milk and meat production minus feed costs.

Individual LCA goal and scope definition

The computation of environmental impact was based on the ILCD protocol (European Commission, 2010). The goal of the LCA analysis was to test the effect of a 3-breed rotational crossbreeding scheme compared with a HO purebred mating scheme on the environmental impact of individual cows kept in 2 farms during their entire lifetime.

As mentioned above, the reference unit of the LCA model was the individual cow in her whole lifetime (Figure 1). The system boundaries were set to include the impact due to the management of the cows throughout their lifetime (from birth to sale or death), the rearing of the replacement heifers, the handling of their manure, the production of the on- and off-farm feedstuffs consumed by the cows and their replacement heifers, and the production and use of energy sources and bedding materials. Although dairy production is multifunctional, as culled cows and calves exceeding replacement needs are typically aimed for beef production, they can nonetheless be considered as by-products of the dairy milk activity. For this reason, the whole impact of dairy operations was allocated to the production of milk.

The following impact categories were assessed: global warming (GWP, kg CO₂-eq), acidification (AP, g SO₂-eq) and eutrophication (EP, g PO₄-eq) potentials, and land occupation (LO, m²/y). Moreover, 3 different types of functional units (FU, the unit to which the

impact referred) were considered: i) related to the temporal dimension, by referring each impact category to the cow (whole lifetime) and to the day of life (intensity of emissions discounted by the longevity of animals); ii) related to the production dimension, by referring each impact category to the unit of fat plus protein yielded with the milk in the whole lifetime; iii) related to the profitability dimension, by referring each impact category to the unit of gross income and by computing the impact per unit of IOFC (as IOFC may assume positive or negative values, it was necessary to reverse the ratio for quantifying the level of emission).

Life cycle inventory

The inventory for each cow was based on the collection of 2 different types of variables: individual-based (referring to the individual cow) and farm-based (equal for all the cows reared in the same farm) (Figure 1). The computation of inventory with respect to the individual-based variables dealt with the information collected from each cow, whereas the farm-based inventory data were collected through a farm questionnaire and dairy farm inspections. The farm data (assumed common to all cows, regardless of genetic group) dealt with the ingredient composition of the rations fed to the lactating and dry cows and to heifers (Table 1), the production of on-farm feedstuffs, and the amount of materials (energy sources and bedding materials) consumed by the farm (Supplement 2 and 3). Since both farms did not make relevant changes to their structure and management over the last 10 years, the farm-based data were assumed to be representative of the dairy cows' management with respect to their lifespan.

The daily DMI of the heifers and of the lactating and dry cows was computed at individual level as the ratio between the daily NE requirements and the NE content of the rations (MJ/kg of dry matter, DM). The energy content and chemical composition of the rations were computed on the basis of the chemical composition of each feedstuff weighed by its relative inclusion in the ration. The chemical data for the feedstuffs were derived from Sauvant et al. (2004) and INRA (2019), except for the compound feeds, for which data from commercial labels were used. The nitrogen input-output animal flow for each animal category was computed according to the procedure proposed by Ketelaars and Van der Meer (1999) as the difference between N intake (feed intake × dietary crude protein content/6.25) and N retention (the sum of the retentions for the protein content of milk / 6.38, growth – 2.5% and 2.7% of the BW increment for cow and heifer periods, respectively – and pregnancy – 3.5% of the BW of the cow, accord-

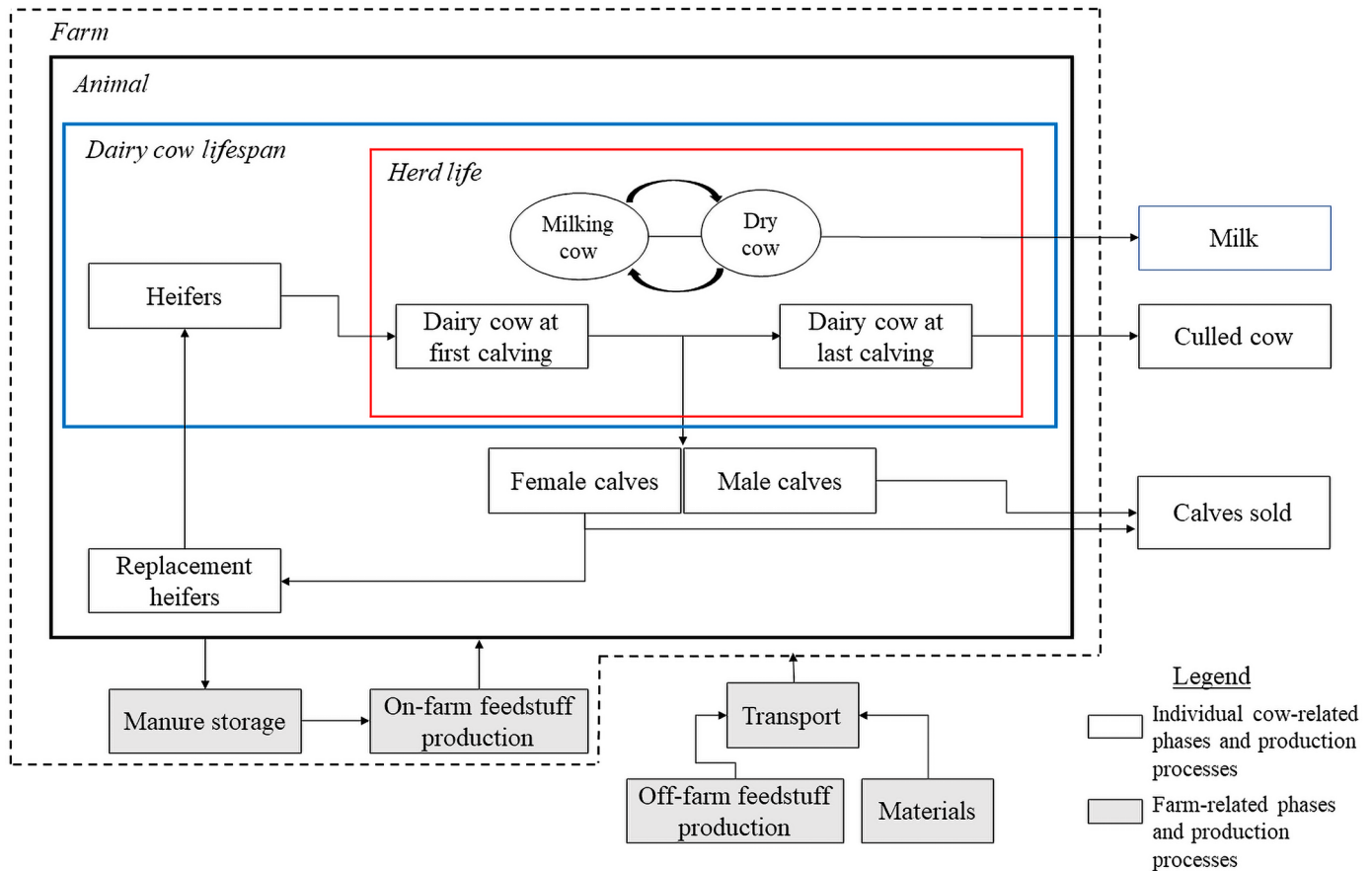


Figure 1. System boundaries for the computation of the environmental impact of crossbred and Holstein Friesian cows along their lifespan ($n = 564$).

ing to the retention coefficients derived from Ketelaars and Van der Meer, 1999).

The computations regarding the different impact categories were performed as follows. The impacts related to the different time periods of the lifespan of each cow (the heifer period and the cow period, comprising one or more parities each including the lactating and the dry phases) were calculated separately. The contribution of the heifer period was increased by an extra 15% to account for mortality and discard. These different contributions were summed up to compute the impact of each cow during her whole life. All equations are in detail in Supplement 4 and 5.

Briefly, the greenhouse gases methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) were included in the computation of GWP. Methane emission due to enteric fermentation was estimated using the equation proposed by Ramin and Huhtanen (2013), based on dry matter intake and chemical composition of the diets. The computation of CH_4 and N_2O emissions occurring during the management of animal manure (housing plus storage) were based on the protocol proposed by

IPCC (2019). In particular, CH_4 estimation was based on the non-digested amount of gross energy intake and on the characteristics of manure storages (for both farms 50% of manure was handled as slurry and 50% as solid); N_2O estimation was based on the N excreted by animals. In addition, the N_2O emissions resulting from fertilizer application in agricultural areas destined to produce on-farm feedstuffs were also accounted for (IPCC, 2019), and considered a direct emission due to N fertilization (emission factor, **EF**: 1% of N applied) and an indirect emission due to N volatilized (EF: 1% of the N volatilized) and N leached into the soil (EF: 1.1% of the N leached).

Acidification potential included the emissions of ammonia (NH_3) and nitrogen oxides, both related to the volatilization of N. The computation of the amount of N volatilized during the manure management phase was derived from European Environmental Agency protocol (EEA, 2019) and based on manure type (slurry and solid) and total ammonia N (60% of the N manure content; EAA, 2019), whereas the calculation of volatilization in the field was based on IPCC

guidelines (2019) (EF: 11% for synthetic fertilizer, 24% for organic ones). Volatilized N compounds, which are deposited on the soil, also contribute to EP together with P-related compounds. Equations and factors derived from Nemecek and Kägi (2007) were applied to calculate P loss during on-farm feedstuffs production, which included the leaching from cropland (equal to 0.07 kg/ha) and the run-off (equal to 0.175 kg/ha), adjusted for the amount of P from organic and chemical sources. Last, the estimation of N loss through leaching was based on IPCC (2019) protocol, which considers an EF equal to 24% of N fertilization rate. The impacts related to purchased inputs (off-farm feedstuffs, energy and bedding resources, and external agricultural inputs used in on-farm feedstuffs production, such as chemical fertilizers, pesticides, and seeds) were computed on the basis of the impact factors derived from the Ecoinvent v3.6 (Wernet et al., 2016) and Agri-footprint v5.0 (Blonk Consultants, 2020) databases implemented in the Simapro v9.3 software (Supplement 6).

Life cycle impact assessment

Each compound emitted was converted into the common measures of the impact categories to which it contributes through the application of characterization factors (e.g., the conversion of CH₄ to CO₂-eq for GWP computation). The characterization factors for GWP (CO₂: 1; CH₄: 28, N₂O = : 265) were derived from Mhyre et al. (2013), whereas those for AP (SO₂: 1, NH₃: 1.6, NO_x: 0.63) and EP (P: 3.06; N-NO₃: 0.43, NH₃: 0.35, NO_x: 0.17) were taken from CML-IA (CML, 2016).

Statistical analysis

Individual life and body attributes, milk component yields, economic traits, and all the environmental impact metrics were analyzed using PROC GLM version 9.4 (SAS Institute Inc., version 9.4, Cary, NC, USA) according to the following linear model:

$$y_{ijk} = \mu + GG_i + Farm_j + (GG \times Farm)_{ij} + e_{ijk},$$

where y_{ijk} is the trait of interest; μ is the overall mean; GG_i is the fixed effect of the i th class of the genetic group of cows ($i = 2$ classes, HO, $n = 279$; CR, $n = 285$), $Farm_j$ is the fixed effect of the j th farm ($j = 2$ classes, herd A, $n = 224$; herd B, $n = 340$), $(GG \times Farm)_{ij}$ is the interaction effect of $GG \times Farm$, and e_{ijk} is the random residual. The residuals were assumed to be normally distributed with a mean of zero and variances of σ^2 . Differences between means of GG were

declared significant at $P \leq 0.05$ and a trend toward significance at $P \leq 0.10$.

RESULTS AND DISCUSSION

Life traits and lifetime milk yield of 3-breed rotational CR and HO cows

Herd effect was mostly significant for life, productive and economic traits, whereas the interaction herd \times genetic group was never significant (data not shown). Herd has been included in the statistical model to adjust LS means of different genetic groups for this effect, but comparisons between herds are outside the scope of this study. Therefore, results of herd effect will not be presented and discussed in this paper.

Least squares means of CR and purebred HO cows for life traits, body attributes, lifetime yield of fat plus protein and economic traits are reported in Table 2. On average, cows in this study had their first calving around 24 mo of age, performed 3.10 lactations, and spent nearly 950 d of their herd life in milk, with an overall lifespan close to 1830 d (data not reported in table). Life-related traits evidenced considerable variation among cows.

Genetic group significantly influenced the age at first calving, the number of lactations performed by cows, and the overall number of days spent in milk and tended to affect the lifespan of the cows. Compared with the HO group, the CR cows calved earlier (-2.2%), performed more lactations ($+11.3\%$), and tended to have a longer lifespan ($+3.9\%$). Moreover, the CR cows spent nearly 8% more of their herd life in milk, while also having nominally more dry days. The greater precocity in the first parity observed for CR cows in this study agrees with the results of Hazel et al. (2020a), who reported that 3-breed CR cows calved for the first time 12 d earlier than their HO herd mates. Similarly, the positive effect of such a crossbreeding scheme on cows' longevity was also reported by Hazel et al. (2021), who found that 3-breed CR cows had a nearly 150-d longer herd life and tended to have lower mortality during the 45 mo after first calving when compared with HO cows. A longer herd life for CR cows compared with purebred HO has also been reported by Heins et al. (2012). Favorable heterosis for herd life and survival may contribute to the greater longevity of CR compared with HO cows (Clasen et al., 2017). Indeed, heterosis for longevity is reputed to stay at a high level even with continued crossing (Sørensen et al., 2008), and in a 3-breed rotational crossing plan the mean heterosis will reach over time 86% of the full heterosis resulting from the first cross between the 2 breeds. Increased herd life may also be supported by

Table 2. Least squares means of 3-breed crossbred (CR) and purebred Holstein (HO) dairy cows for life traits, body attributes, lifetime yield of fat plus protein, and economic traits (285 CR and 279 HO)

Item	CR	HO	CR/HO	SEM	<i>P</i> -value
Life traits:					
Age at first calving, d	714	730	0.978	3.32	<0.01
Number of lactations	3.26	2.93	1.113	0.08	<0.01
Lifetime, d	1866	1796	1.039	29	0.09
Days in milk, d	986	910	1.084	24	0.03
Dry days, d	167	156	1.074	6	>0.1
Body attributes ¹ :					
BW, kg	692	684	1.012	3.68	>0.1
BCS	3.53	3.20	1.103	0.019	<0.01
Fat plus protein yield:					
Lifetime, kg/cow	2258	2084	1.083	62.10	0.05
Per day of life, kg/d	1.15	1.11	1.038	0.02	0.07
Lifetime gross income, €/cow	16127	15227	1.059	459	>0.1
Lifetime IOFC, €/cow	8939	8289	1.078	307	>0.1

¹Average herd life body weight (BW) and body condition score (BCS).

the positive additive effects of the breeds chosen in the mating scheme. In this respect, VR and MO have been reported to provide good results in combination with HO in terms of improving health, fertility and functional traits (Dezetter et al., 2017; Balandraud et al., 2018; Shonka-Martin et al., 2019a).

Crossbreds had similar BW but greater BCS (+10%, $P < 0.01$) than their HO herd mates (Table 2). A comparable BW between the genetic groups involved was also reported by Shonka-Martin et al. (2019a), who did not find any difference in the BW of primiparous and multiparous CR and HO cows from a 3-breed rotational system involving VR, MO, and HO breeds during their first 150 d of lactation. Similarly, Pereira et al. (2022) reported comparable BW for 3-breed CR and HO cows during their first lactation. Conversely, a greater BCS than that seen in purebred HO cows seems to be a common attribute of CR cows from this mating scheme. Indeed, Hazel et al. (2017) reported a 10 to 12% increase in BCS in MO-HO and VR-HO primiparous and multiparous cows when compared with purebred HO, whereas Hazel et al. (2020b) found a nearly 15% increase in the BCS of 3-breed rotational crossbred of VR, MO, and HO breeds compared with purebred HO cows. In addition, Shonka-Martin et al. (2019a) found increases in BCS of nearly 8% and 6% in primiparous and multiparous CR cows from this crossbreeding scheme when compared with purebred HO cows.

Genetic group also affected the milk fat plus protein yield during the lifetime (Table 2), with CR cows evidencing nearly 8% greater values than purebred HO (+174 kg, $P < 0.05$), equal to nearly 4% greater milk fat plus protein per day of life (+ 42 g/d, $P = 0.07$). Several studies have compared the yields of milk and milk solids of such CR cows with those of purebred HO during a part or whole lactation. In general, CR cows are reported to yield a lower volume of milk than HO

cows (Heins and Hansen, 2012; Malchiodi et al., 2014b; Hazel et al., 2020a) but, due to a greater fat and protein content in the milk from CR cows (Shonka-Martin et al., 2019a; Saha et al., 2020), the difference between genetic lines in terms of lactation yield of fat plus protein may decrease. It has been reported to be still lower in CR cows in some studies (Heins and Hansen, 2012; Hazel et al., 2020a) but comparable in others (Hazel et al., 2014; Shonka-Martin et al., 2019a; Pereira et al., 2022). When production refers not to a single lactation but to the whole lifetime of the cows, the opportunity to have more days in milk gives the CR cows the ability to cumulate greater milk solids production. Hazel et al. (2021) reported a 16% increase in lifetime fat plus protein yield for combined 3-breed CR cows compared with HO cows.

With respect to the economic traits, despite a nominal superiority of CR over HO in GI and IOFC (+ 6 and +8%, respectively, Table 2), due to their greater fat plus protein production coupled with a nearly 20% higher cull cows value and a slight reduction in estimated DMI during lactation (data not shown in table), the differences between genetic groups were not significant. Conversely, Hazel et al. (2021) found a 16% greater lifetime revenue for 3-breed CR cows when compared with purebred HO cows; this difference further increased moving from lifetime revenue to lifetime profit, which was nearly 30% greater in CR than HO cows due to their lower feeding, health, and replacement costs. Sørensen et al. (2008) reported that the economic performance of dairy production seems to benefit from systematic crossbreeding, and favorable heterosis for economic merit traits has also been reported for different breed combinations (López-Villalobos et al., 2000; VanRaden and Sanders, 2003).

Environmental impact categories evaluated using LCA at individual animal level

Herd effect significantly affected all the environmental impact categories estimated, whereas the interaction herd x genetic group was mostly not significant (data not shown).

Least squares means of CR and purebred HO cows for the impact categories assessed through LCA performed at individual level are reported in Table 3 (GWP), Table 4 (AP), Table 5 (EP) and Table 6 (LO). The LCA methodology is usually applied to assess the environmental impact of a product at the farm scale (Baldini et al., 2017; Mazzetto et al., 2022). In fact, the farm is the production unit where the data needed for the inventory analysis are primarily collected and stored; farm contributes most to the whole environmental impact of dairy products (Finnegan et al., 2018) and decisions aiming to mitigate the environmental footprint are taken mainly at farm level. However, the farm scale has some limitations as well, among which the impossibility of accounting for the intra-farm variability due to the potential diversity of the animals reared. One main cause of diversity among animals is their genetic framework, which may affect, among other things, their environmental impact (Stranden et al., 2022). Thus, it seems of interest to try to consider individual animals when assessing the effects of different breeds on environmental impact. The use of the individual scale for LCA analysis could be extremely data-demanding, as data have to be collected for each animal instead of referring to the average data at the farm level. This task is challenging because of the number of individual animals present on a single farm, and because variability among animals cannot be considered as a noise to be removed by averaging individual data, but rather the aim of the estimation in the case of comparisons between different genetic lines or feeding or management procedures within herds. Indeed, the few studies that have applied this scale to analyze the carbon footprint of cattle (McAuliffe et al., 2018; Grandl et al., 2019) highlighted that the individual animal scale could give

deeper insights into the assessment of GWP emissions. In particular, McAuliffe et al. (2018) found contrasting results between different farm systems when using individual vs. average data.

In this study, the use of the individual animal scale allowed us to include in the environmental impact assessment the potential differences between the CR and HO dairy cows in terms of the number of lactations, length of herd life, amount of milk solids production, body composition, and economic value of the carcasses. On the other hand, data regarding farm organization, manure management systems, and feedstuff production were obtainable only at the farm scale, and therefore had to be considered as management factors common to all cows in the herd. Our effort of using large-scale data available at the farm level and integrating them with other data collected specifically for the scope of this work, combined with the development of a proper calculation procedure, produced the estimates of several individual environmental indicators for each cow. These data are characterized by variability, thus mirroring the biological variability typical of animal traits. Moreover, the availability of a large number of individual estimates (564 in this study) for every indicator allowed us to analyze the data with the aim of comparing animals of different genetic groups and to test the outcomes of such comparison on a proper line of error.

This combination of individual- and farm-scale data for the construction of the LCA inventory was set to be as accurate as possible with respect to data availability. In this sense, the eventual implementation in dairy farms of precision livestock practices (Pahl et al., 2016) could greatly increase the possibility of monitoring important traits at the individual level in an automated continuative way. This could enhance the power of the individual LCA models to capture the differences between animals and, at a higher level, genetic groups, and help make farm-level decisions intended to decrease the environmental footprint associated with dairy production.

Table 3. Least squares means of 3-breed crossbred (CR) and purebred Holstein (HO) dairy cows for global warming potential (GWP) associated with overall cow lifetime, day of life (D_Life), yield of fat plus protein (FatPrt), gross income, and on income over feed costs per unit of GWP emission (IOFC_GWP) (285 CR and 279 HO)

Item	Unit	CR	HO	CR/HO	SEM	<i>P-value</i>
GWP associated with:						
Lifespan	kg CO ₂ -eq/cow	37329	35868	1.041	753	>0.1
D_life	kg CO ₂ -eq/d	19.65	19.61	1.002	0.10	>0.1
FatPrt	kg CO ₂ -eq/kg	17.93	18.46	0.971	0.21	0.07
Gross income	kg CO ₂ -eq/€	2.56	2.61	0.979	0.03	>0.1
IOFC_GWP	€/kg CO ₂ -eq	0.222	0.214	1.037	0.004	>0.1

Table 4. Least squares means of 3-breed crossbred (CR) and purebred Holstein (HO) dairy cows for acidification potential (AP) associated with overall cow lifetime, day of life (D_Life), yield of fat plus protein (FatPrt), gross income, and on income over feed costs per unit of AP emission (IOFC_AP) (285 CR and 279 HO)

Item	Unit	CR	HO	CR/HO	SEM	<i>P</i> -value
AP associated with:						
Lifespan	kg SO ₂ -eq/cow	755.52	728.16	1.038	16.25	>0.1
D_Life	g SO ₂ -eq/d	396	396	1.000	2.64	>0.1
FatPrt	g SO ₂ -eq/kg	359	370	0.970	3.85	0.04
Gross income	g SO ₂ -eq/€	51	52	0.978	0.49	0.10
IOFC_GWP	€/kg SO ₂ -eq	10.88	10.47	1.039	0.182	0.10

Environmental impact of 3-breed rotational CR and HO cows

The greenhouse gas emissions generated during the lifespan of the HO cows (Table 3) was nearly 35900 kg CO₂-eq, which resulted in nearly 19.6 kg CO₂-eq when equated to one day of life. Considering lifespan production, the mean GWP of HO was around 18.5 kg CO₂-eq per 1 kg fat plus protein yielded. When scaled on economic revenues, GHG emissions of HO was around 2.60 kg CO₂-eq per € of gross income, whereas one kg CO₂-eq emitted was associated with an average IOFC of 0.214 €. Compared with HO cows, CR showed a nominal 4% increased GWP emission in their whole lifespan, nearly identical GWP per day of life, 3% lower GHG emissions per kg of fat plus protein yielded ($P = 0.07$), and nominally generated 3.7% more IOFC per kg of GWP.

Genetic group significantly affected the AP and EP intensity per kg of fat plus protein yielded (Table 4 and

5, respectively), and tended to affect AP when scaled on gross income and IOFC. In particular, compared with HO cows, the CR nominally showed nearly 3.5% greater AP and EP in their lifespan, identical emissions per day of life, but had a significantly ($P < 0.05$) reduced intensity of AP and EP per kg of fat plus protein yielded (- 3.0% and - 2.8%, respectively). Moreover, with respect to the HO group, the CR cows tended to generate 4% more IOFC per kg SO₂-eq emitted and showed a nominal 3.7% greater IOFC per kg of PO₄-eq emitted.

Land occupation resulted on average associated with nearly 25 m²/year when scaled to each day of life of cows (Table 6). The yield of 1 kg of fat plus protein was associated with 22 to 23 m² of LO, whereas one unit of GI required on average nearly 3 m² of land. Last, the occupation of 1 m² of land generated an IOFC of nearly 0.170 €. Genetic group significantly affected LO per kg of fat plus protein yielded ($P = 0.03$) and tended to influence the IOFC generated per unit of LO (P

Table 5. Least squares means of 3-breed crossbred (CR) and purebred Holstein (HO) dairy cows for eutrophication potential (EP) associated with overall cow lifetime, day of life (D_Life), yield of fat plus protein (FatPrt), gross income, and on income over feed costs per unit of EP emission (IOFC_EP) (285 CR and 279 HO)

Item	Unit	CR	HO	CR/HO	SEM	<i>P</i> -value
EP associated with:						
Lifespan	kg PO ₄ -eq/cow	265.53	256.38	1.036	5.93	>0.1
D_Life	g PO ₄ -eq/d	138	138	1.00	0.96	>0.1
FatPrt	g PO ₄ -eq/kg	125	129	0.972	1.35	0.05
Gross income	g PO ₄ -eq/€	17	18	0.981	0.16	>0.1
IOFC_GWP	€/kg PO ₄ -eq	31.03	29.93	1.037	0.498	>0.1

Table 6. Least squares means of 3-breed crossbred (CR) and purebred Holstein (HO) dairy cows for land occupation (LO) associated with overall cow lifetime, day of life (D_Life), yield of fat plus protein (FatPrt), gross income, and on income over feed costs per unit of LO (IOFC_LO) (285 CR and 279 HO)

Item	Unit	CR	HO	CR/HO	SEM	<i>P</i> -value
LO associated with:						
Lifespan	m ²	47157	45435	1.038	1.064	>0.1
D_Life	m ² /d	24.59	24.56	1.00	0.18	>0.1
FatPrt	m ² /kg	22.25	22.89	0.972	0.22	0.03
Gross income	m ² /€	3.10	3.16	0.981	0.03	>0.1
IOFC_GWP	€/m ²	0.174	0.168	1.037	0.003	0.10

= 0.10). The directions of the trends when comparing CR and HO cows were similar to those observed for the emission categories described previously, where CR cows had a 2.8% lower LO per kg of fat plus protein yielded and generated nearly 3.7% more IOFC per unit of LO.

Considering the differences in allocation procedure and FU choice, the impact values obtained in this study for the yield of fat plus protein were comparable to those found in the literature (Baldini et al., 2017; Mazzetto et al., 2022). Studies comparing the environmental footprint of cows of different genetic groups are scarce and mainly focused to the enteric methane emission of cows belonging to different breeds (Lassen et al., 2012; Vanlierde et al., 2021), or crossbreds (Xue et al., 2011; Hynes et al., 2016; Martínez-Marín et al., 2023b). As far as the authors are aware, there are no studies on cows from 3-breed rotational crossbreeding schemes that employ the LCA methodology.

The results obtained evidenced that CR may contribute to reduce the environmental burden associated with the lifespan yield of fat and protein with milk, which is among the major objectives of the milk industry, especially when the milk is destined for cheese-making. This result may be related to the combination of a greater fat plus protein content of milk, a longer herd life and lower maintenance requirements (Piazza et al., 2023b) of CR compared with HO cows.

The mitigating effect of increasing output yield per animal has been well established in the literature (Gerber et al., 2011; Lorenz et al., 2019). However, strategies aimed to further increase the levels of intensification in high-input dairy production systems seem not sustainable (Brito et al., 2021) and may not be necessarily profitable (Moallem, 2016). Consequently, the longer herd life observed for CR cows compared with HO dairy cows is of interest. The potentiality of increasing herd life duration to mitigate the environmental impact of milk production has already been explored (Grandl et al., 2019), although involving small dairy samples and only the GWP category. The effect of a longer herd life on environmental impact can be observed at different scale. The first effect is related to the decrease in the unproductive part of life associated with an increase in herd life, i.e., days when animals consume, generating impacts, but do not produce. In these terms, the results were in accordance with the mitigating effect of diluting the animal maintenance requirements (Hristov et al., 2013) associated with unproductive life. The second effect is related to the decreased need to rear replacement heifers, as fewer cows would be culled over a reference period of time. A decrease in replacement rate has been proven to have mitigating effects on the environmental impact of dairy production, as young animals consume

feed resources, that must be produced, emit enteric methane, and excrete nutrients (Hristov et al., 2013; Knapp et al., 2014; Berton et al., 2023).

The inclusion of different impact categories related to different issues (emissions and resources) strengthens the outcomes obtained from the LCA analyses of livestock systems (McClelland et al., 2018) and contributes, together with other indicators, to the development of properly innovative strategies for improving the overall sustainability of livestock systems (Harrison et al., 2021).

The effects of genetic group on the environmental metrics were different according to the different FU taken into account. In fact, outcomes for all the impact categories considered were nominally higher for CR cows over their whole lifespan, mainly because of their longer life. When expressed per day of life, the differences disappeared and were reversed when expressed per kg of fat plus protein. From an economic point of view, the CR cows presented more favorable indicators both in terms of GI and IOFC, even though differences between genetic groups were mostly nominal.

The use of different FUs has been reported to increase the robustness of LCA results by considering different aspects of farm outputs (Salou et al., 2017). If product-based FUs such as fat plus protein are associated with the farm function of providing food, economic-based FUs such as those concerning GI and IOFC are related to the underlying function of providing economic income to farmers. Some other studies have included the economic function in their FU definition (Rice et al., 2019; Chen et al., 2020) or assessed dairy cows' GHG emissions together with IOFC (Pulina et al., 2020). The inclusion in the present study of economic-based FUs together with product-based ones allowed us to evaluate the effects of the use of the crossbreeding strategy from different points of view, including also issues related to economic revenues and profitability, that are of great relevance when mitigation options are assessed for the implementation in a farm (Vellinga et al., 2011).

CONCLUSIONS

In this study we compared for the first time the environmental impact of CR cows from a 3-breed rotational scheme with that of HO herd mates, reared together according to the same feeding and management conditions. The LCA-derived approach developed allowed us to estimate impact indicators for individual animals, thus accounting for the diversity in production ability, feed efficiency, and longevity and the value of products from a lifespan perspective. The CR evidenced a decrease in emissions of GHGs and nutrients such as

N and P per unit of fat and protein yielded in lifespan compared with HO. Consequently, they tended to generate a greater IOFC per unit of emission. Moreover, the land occupation tended to be lower in CR than HO for most indicators considered. A longer lifespan, a greater fat plus protein yield during herd life, and a decrease in replacement needs of CR may contribute to explain the results observed. Although they should be strengthened using a larger sample of cows and herds, our estimates were consistent among the different indicators considered and coherent with expectations. Therefore, the use of crossbreeding schemes such as the one described in this study may contribute to mitigating emissions and improving the environmental footprint of dairy herds.

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APPENDIX 1

Procedures of standardization for BW and BCS

The average BW was estimated during the cows' herd life, from the first calving to the culling or death date (BW_{COW}) and during the heifer period (BW_{HEI} , from birth to the first calving). The BW_{COW} and BW_{HEI} were required to estimate dry-matter intake (DMI), as an individual direct measure of feed intake was not available.

The estimation of BW_{COW} was performed as follows: as the test-day BW value was collected once from dairy cows with different (and cow-specific) DIM and parity at the date of collection, test-day BW was first adjusted to the same DIM class. This adjustment was based on the coefficients obtained from a GLM that included the fixed effect of the combined factor farm (2 classes) \times genetic group (2 groups: CR and HO) \times parity (3 classes: 1, 2 and > 2 parity) \times DIM (3 classes: 1 to 100, 101 to 200 and > 200 d in milk); this model had a coefficient of determination of 0.44. The DIM class 101 to 200 d was taken as the reference (adjustment coefficient equal to 1). Within each class of farm, genetic group, and parity, the adjustment coefficients for measures taken from cows belonging to ≤ 100 DIM and > 200 DIM classes were calculated as the ratio between the LSM of the 2 classes and the LSM of the reference class (Supplement 7). Then, the test-day BW actually measured for each cow was multiplied by the corresponding adjustment coefficient to obtain BW adjusted at 100–200 DIM ($BW_{100-200d}$) for all cows in the study. Thereafter, a similar procedure was used to standardize $BW_{100-200d}$ to the different orders of parity spent by each cow during her whole life. Consequently, $BW_{100-200d}$ was analyzed with a second GLM that included

the fixed combined effect of farm \times genetic group \times parity class; this model had a coefficient of determination of 0.45. The projection coefficients, within each farm and genetic group were calculated by dividing the LSM of BW100–200d of each class of parity by the LSM of the BW100–200d of all the other parity classes, to obtain a set of projection coefficients useful to estimate an average weight adjusted for DIM for all the lactations performed by each cow (Supplement 8). Thus, if a cow had just one lactation in her herd life, her test-day body weight was adjusted just for DIM class, if necessary (for cows actually weighed outside the DIM interval 100 to 200). If a cow had 2 lactations and was actually weighed during her second lactation, the average weight of the cow in the second lactation was her test-day BW100–200d, and this weight was multiplied by the coefficient of projection from parity 2 to parity 1 to predict the average BW100–200d in lactation 1, etc. We assumed that cows reached their mature weight at 3rd parity; hence, if a cow had more than 3 lactations, the average weight for lactation > 3 was considered equal to the average weight in lactation 3. Lastly, the average BW_{COW} was calculated as the sum of average body weights of different lactations performed by each cow divided by the total number of lactations.

The average BW_{HEI} was computed for each cow averaging the 1st parity BW adjusted to the DIM class ≤ 100 (the DIM class closest to 1st calving), and the BW at birth, computed according to NASEM (2021).

The average BCS during the cows' herd life was computed using the same procedures adopted to obtain BW_{COW}, with coefficients of determination of 0.36 for the model run for adjusting test-day BCS to the reference 100 to 200 DIM class (BCS100–200d) and of 0.34 for the model run for projecting BCS100–200d to the lactations actually performed by each cow.