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CICLO XXXV

MILK AND CULL COWS TRAITS, PRODUCTIVITY AND ENVIRONMENTAL FOOTPRINT OF PUREBRED HOLSTEIN AND 3-BREED ROTATIONAL CROSSBREEDING COWS FROM VIKING RED, MONTEBELIARDE AND HOLSTEIN SIRES

Coordinatore: Ch.ma Prof.ssa Angela Trocino

Supervisore: Ch.mo Prof. Luigi Gallo

Studente: Martina Piazza

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ABSTRACT

In the last decade, the interest of the research in crossbreeding systems has grown due to the beneficial effects in improving functional traits, such as fertility, longevity and health related traits. Among crossbreeding programs, a scheme involving Viking Red, Montebeliarde and Holstein (HO) sires, marketed as ProCROSS, had risen a growing interest from the dairy sector. Therefore, this thesis aimed at comparing the performances of purebred HO and crossbred cows originated within this crossbreeding scheme in terms of mineral composition of milk, carcass attributes and value of cull cows, body traits and production efficiency and individual environmental footprint of cows reared in two different dairy farming systems.

In the first chapter, the detailed mineral profiles of milk from purebred HO and four generations of crossbred (CR) cows were compared. Individual milk samples were collected from 120 multiparous cows and analyzed for macrominerals (Na, Mg, P, S, K, Ca), essential (Cr, Mn, Fe, Cu, Zn, Mo), and environmental microminerals (Li, B, Ba). This study has evidenced that the milk mineral profile of CR was comparable to that of milk from HO cows, while variations in the macro- and micromineral profiles were greater among different CR generations than between purebreds and crossbreds.

The second chapter aimed at comparing carcass attributes and value of cull cows derived from the ProCROSS program compared to that of purebred HO cows. Moreover, the risk of urgent culling was assessed between CR and HO cows and within number of parity and stage of lactation. Data, collected on 1814 cull cows kept in one herd and slaughtered in four slaughterhouses, cconcerned the weights, fleshiness and fatness scores, the overall value and the price of carcasses. The study revealed that the carcasses of the crossbred cull cows were heavier, better graded for fleshiness and fatness, reached higher prices, and had a total value nearly €100 greater than the carcasses of their purebred HO herd-mates.

The third chapter dealt with the comparison between purebred HO and CR cows with respect to a set of body measurements and body condition score (BCS), used as predictors of the net energy requirements for maintenance (NEm), and to production traits based on milk and milk nutrients yields used together with NEm to predict metrics of cows' production efficiency. Data were collected on 791 cows and used to compute NEm based on metabolic weight (NEm_{MW}) and on body protein mass (NEm_{PM}). This study evidenced that differences in milk production between the CR and purebred cows were no longer significant when referred to body composition of cows, suggesting that the crossbreds and purebreds have the same productive ability and efficiency per unit of body protein mass.

The fourth chapter explored the effects of the ProCROSS crossbreeding program on the individual environmental footprint of cows using an individual Life Cycle Assessment (LCA) derived approach. Data on 564 cows concerned herd life and lifetime production traits, body weights and measures, used to estimate feed intake and energy requirements; thse data were used together with collective herd data regarding farm management traits to compute the following impact categories: global warming potential (CO_2-eq), acidification (SO_2-eq) and eutrophication (PO_4-eq) potentials and land occupation (m^2). Compared to purebred HO cows, CR had longer herd life and productive days, greater production of fat plus protein in life and per day of life, greater BCS and carcass value (+20%). Hence, emissions of CR cows resulted nominally greater for lifetime but lower per day of life and per kg of fat plus protein yielded in the whole career. Moreover, CR cows had lower emissions per unit of gross income and tended to have greater income over feed costs per unit of impact.

The results evidenced that, compared to HO, crossbred cows had improved milk composition qualities, carcass attributes and health condition at culling, different body composition but comparable production efficiency, and lower emissions per fat plus protein and per unit of gross income.

GENERAL INTRODUCTION

The inbreeding depression contest

The genetic selection in breeding programs have been widely implemented over the last decades, leading to a continuous increasing in yields of milk and milk components of dairy breeds. In particular, the genetic selection programs for the Holstein breed focused mainly on milk and nutrients production, and Holstein has been extremely successful in achieving those goals (Miglior et al., 2005). However, although such selection programs had positive effects on productive attributes, the increased inbreeding rate within breed led to a deterioration of functional traits of animals, such as fertility, survival and health status (Sewalem et al., 2006). In the Holstein breed efforts have been made to increase selection for these traits, and the use of the genomic selection has helped in their improvement, in spite of their low hereditability (Ducrocq, 2010). Anyway, the genetic improvement in these traits has still been very slow, and in the last years inbreeding depression achieved an average inbreeding coefficient of 8.02 % for Holstein females (ProCROSS, 2019). This fact brought to a reconsideration of the genetic selection strategies adopted.

Heterosis, which is a measure of the improved performance of crossbred animals compared with the average of the purebred parental populations, can be interpreted as inbreeding depression in reverse (Lynch and Walsh, 1998). The heterosis, or hybrid vigor, can be achieved through crossbreeding, which consists in the mating of individuals less related to each other than the average population; in the practice, this often means mating animals of different breeds or genetic lines (Swan et Kinghorn, 1992). Crossbreeding is a system able to solve the problems occurred with the inbreeding depression, providing improvements on traits related to survival and fertility. Moreover, it allows to exploit the benefits of the genetic selection within breeds for traits of interest and to make use of complementarity when using two or more breeds (Buckley et al., 2014). Crossbreeding has been adopted by several animal production industries, such as poultry, turkeys, and pigs, since more than 40 years, and has more recently gained the attention also of the dairy sector.

Heterosis in crossbreeding programs

The aim of a successful crossbreeding program is to maintain the heterosis across generations. The effect of heterosis is defined as the difference between the average performances of the crossbred offsprings and those of the parental populations (Bidanel, 1992). The first generation of a cross between two different breeds (F1) displays full heterosis. However, it should be clear that heterosis effects are not heritable additions accompanying the combined additive effects of a cross, but it conversely tends to decrease with the advancing of the generations of crosses (Freyer et al., 2008). Therefore, a crossing system should lead to a constant genetic composition over generations (Hill, 1971).

Heterosis is not entirely transmit to the next generations, anyway it is not completely gone. The strategy adopted after the first cross has an important role in the amount of which heterosis will be expressed (Buckely et al., 2014). Several crossbreeding schemes have been studied to maximize the expression of the hybrid vigor across generations (Cunningham and Syrstad, 1987), but the most common programs studied for dairy cattle involve the use of 2 or 3 breeds.

The two breeds crossbreeding consists in mating animals of two different breeds, having in the first generation (F1) a full heterosis; then F1 cow is mate to a high genetic merit sire belonging to one of the parental breeds used initially, obtaining only half of the F1 heterosis. If the crossing continues after generations, heterosis will settle at 67% of the F1 one (Sorensen, 2008). This program is mostly used to rapidly improve functional traits of cows but maintaining a quite high production and quality of milk (Dezetter et al., 2019).

The three, or more, breeds rotational crossbreeding program uses high genetic merit sires of three breeds. If F1 cows are mated to a third breed, the heterosis of F2 remains 100%, but when the sire of a purebred parental breed is reintroduced, heterosis of subsequent generations decreases to get a steady level of 86% after n generations. The more breeds are used, the more hybrid vigor is kept high: for instance, a 4 way crossbreeding program assures in future generations an heterosis of 93% (Hansen, 2006). However, it might be hard for dairy producers to find four unrelated breeds whose crosses result competitive in terms of production.

Heterosis is more marked for functional traits, such as fertility, longevity and health related attributes, and much less for production ones (Kristensen and Sørensen, 2005; Sørensen et al., 2008). Recently, Dezetter at al. (2019) reported that heterosis ranged between 5% and 6% for traits related to milk production, while passed over 10% for functional traits, but heterosis values are much variable and this affects greatly the performances of crossbred cows, which result sometimes positive and in other case negative, dependently from the breeds and the trait observed.

Several authors reported positive results for fertility and health-related traits (Auldist et al., 2007; Malchiodi et al., 2014; Rinell and Heringstad, 2018), and for longevity and survivability (Heins et al., 2006a; Sørensen et al., 2008; Hazel et al., 2014) of crossbred cows from different crossbreeding schemese, different breeds and different level of production compared to purebred Holsteins.

Regarding milk production attributes, generally purebred Holstein cows have higher milk yield compared to crossbred cows, but this difference ranges from 130 kg to more than 1500 kg of milk yielded in 305 days lactation (Dezetter et al., 2019). On the other hand, crossbred cows have generally greater fat plus protein content in milk compared to purebred Holsteins (Walsh et a., 2007; Freyer et al., 2008; Vance et al., 2013; Hazel et al., 2017b). However, other studies (Heins et al., 2006b; Buckley et al., 2007; Blottner et al., 2011) found no differences in milk volume production and fat and protein yields in milk.

The expression of heterosis in improved performances is a function of the genotype and the environment contest. Some studies investigated the effect of different level of production, and found that the environmental level of production affected heterosis expression and cows' performances. Penasa et al. (2010) found that the effect of heterosis is highest in herds with lowest production level, on the contrary, Bryant et al. (2007) and Kargo et al. (2012) showed that heterosis was smallest in the low-producing herds, rejecting the idea that heterosis expression for milk production decreases with increasing herd management level.

Therefore, it is important to keep in mind that the right choice of the breeds is a focal point for dairy producers who need to select properly breeds and breeding programs according to their production and herd management level.

The ProCROSS crossbreeding program

Among crossbreeding programs proposed and tested, a three breeds rotational crossbreeding scheme involving Viking Red, Montbéliarde and Holstein sires, which is marketed internationally as ProCROSS by Coopex Montbéliarde (Roulans, France) and Viking Genetics (Randers, Denmark), has recently gained attention within the dairy sector, especially in intensive dairy farming systems. The project aimed at seeking a solution for the decreasing fertility, health and longevity of Holstein herds of some Californian dairy farmers, and it currently is currently implemented by several dairy farms in different countries across USA, Europe and South America. The choice of the breeds for this crossbreeding program was crucial: some breeds, such as Jersey and Brown Swiss, were initially considered but then excluded because the crossbred offspring did not reach the expected standards of production, health or general vitality. Finally, two breeds seemed to achieve the required standards when crossed to Holstein dams:

- Montbéliarde, which is the second most popular breed in France, selected for the high environmental adaptability, udder health and milk solids content, which make its milk particularly suitable for cheese making (Coopex Montbéliarde, Roulans, France);
- Viking Red, which is the result of the breeding program that combine the Nordic Red dairy breeds (Danish Red, Swedish Red and Finnish Aryshire), and is known to have easy calving, long life and good health (Viking Genetics, Randers, Denmark).

Generaly this program starts with the Holstein cow mated with Montbéliarde sire, obtaining the F1 heifer which will be inseminated with a Viking Red bull. Then resulting second generation (F2) heifer will be inseminated with a Holstein sire to produce the third (F3). From here, the F3 will be mated again with Montbeliarde semen and continue with this mating plan to maintain a high heterosis, that in fourth generation will reach the 86%.

During the last years, different research teams have been involved in studying the effect of this crossbreeding program on several traits of many generations of crossbred cows, supported by the fact that more farmers have joined the program and then more data were available to be used for research.

Since 2017 several evidences have been published about ProCROSS cows' performances. Two studies from Hazel et al. (2017a,b) on the same population were conducted on production and calving traits, and fertility, survival and conformation traits during the first lactation of F1 crossbred compared to purebred Holstein cows. They found both MO × HO and VR × HO crossbreds had greater production of milk solids but similar SCS compared with their HO herdmates. and purebred and VR-sired crossbreds had similar calving difficulty, but compared to purebred HO calves, the stillbirths rate of crossbred calves was halved. Moreover, in the study of Hazel et al. (2017b), they analyzed different body traits related to stature, body depth and udder attributes of cows together with body condition score (BCS) of F1 crossbred and purebred HO cows, finding that a shorter stature, shallower body depth and higher BCS may have provided positive feedbacks on health and production efficiency. Shonka-Martin et al. (2019a,b) investigated feed efficiency traits, such as dry matter intake and residual intake, body traits and income over feed cost in the first 150 days of the first three lactations of F1 ProCROSS cows compared to HO. They found crossbred cows had lower dry matter intake than HO cows, had comparable body weight but higher body condition score than HO cows. Consequently, the combination of a lower DMI and a similar body weight resulted in a greater feed efficiency of crossbred cows and a higher mean income over feed costs than HO cows. At the same time, other studies were conducted on milk technological properties and cheese yield by Saha et al. (2020) on four generations of ProCROSS crossbred cows to study differences within generations and between purebred HO and crossbred cows. In this study crossbred cows had slightly better milk coagulation properties than the milk of purebred Ho, but cheese yield did not differ between purebred and crossbreds. This research added knowledge about the long term effects of this program on traits scarcely investigated, but important in a dairy farming system based on cheese production.

Recent studies from Hazel et al. (2020b; 2021) aimed at evaluating the lifetime profitability and the health treatment costs associated to the rearing of ProCROSS cows compared to purebred HO, providing evidences that crossbred cows derived from this 3-breed rotational crossbreeding scheme are a viable alternative to purebred Holstein cows.

The intensity of the research on the long term consequences of this breeding strategy is growing, and the investigations on this topic are deepening. This thesis adds further contribution to the existing knowledge on this topic, proposing new methodologies and aspects to be considered when assessing the effectiveness of this crossbreeding program and evaluating traits rarely investigated until now.

Thesis structure and journal contribution

This dissertation is composed by four contributions divided in four chapters:

In the first chapter, the detailed mineral profiles of milk from purebred Holstein (HO) and four generations of crossbred (CR) cows derived from the ProCROSS 3-breed rotational crossbreeding system were compared. Individual milk samples were collected from 120 multiparous cows (40 HO and 20 for each generation of CR from F1 to F4) and analyzed for macrominerals (Na, Mg, P, S, K, Ca), essential (Cr, Mn, Fe, Cu, Zn, Mo), and environmental microminerals (Li, B, Ba). This study added knowledge about traits scarcely investigated in literature within crossbreeding effects, and found the milk mineral profile of CR was comparable to that of milk from HO cows, while variations in the macro- and micromineral profiles were greater among CR generations than between purebreds and crossbreds.

The second chapter aims at comparing carcass attributes and value of cull cows derived from the ProCROSS program compared to that of purebred HO cows. Moreover, the risk of urgent culling was assessed between CR and HO cows and within number of parity and stage of lactation. Data were collected on 1814 cows from one herd and four slaughterhouses of carcass weights, fleshiness and fatness scores, overall value and price. The study revealed that the carcasses of the crossbred cull cows were heavier, better graded for fleshiness and fatness, reached higher prices, and had a total value nearly €100 greater than the carcasses of their purebred HO herd-mates. Moroever, CR had a 37% lower risk of being urgently removed from the herd compared to HO. Since cull cows represent a supplementary source of income for dairy farmers, this contribution evidenced that the greater overall value of crossbred cull cows should be taken into account in evaluating the economic effectiveness of crossbreeding schemes.

The third chapter dealt with the comparison between purebred HO and CR cows with respect to a set of body measurements and body condition score (BCS), used as predictors of the net energy requirements for maintenance (NEm), and to production traits based on milk and milk nutrients yields used together with NEm to predict metrics of cows' production efficiency. Data were collected on 791 cows and used to compute NEm based on metabolic weight (NEm_{MW}) and on body protein mass (NEm_{PM}), as the CR cows were supposed to have different body composition compared to HO cows. Then milk production data were scale on the two NEm. This study evidenced that the differences between the CR and purebred cows were no longer significant when the production traits were scaled on NEm_{PM}, suggesting that the crossbreds and purebreds have the same productive ability and efficiency per unit of body protein mass. We concluded that combining cows' production capability with traits related to body composition and energy cost of production seemed to be more effective criteria for comparing CR and purebred cows than just milk and milk nutrient yields.

The fourth chapter explored the effects of the ProCROSS crossbreeding program on the individual environmental footprint of cows using a Life Cycle Assessment (LCA) derived approach. Individual data on 564 cows concerned herd life and lifetime production traits, body weights and measures, feed intake and requirements traits. Collective herd data regarding farm managing (manure storage, materials, feeds, fuels) were also taken and used together with individual data in order to compute impact categories, namely global warming potential, acidification and eutrophication potentials, and land occupation, which were then associated to different functional units, such as lifetime, day of life, milk, fat plus protein and cheese yields, gross income and income over feed costs. In this contribution emerged that, compared to purebred HO cows, CR had longer lifetime and productive days, greater production of fat plus protein in life and per day of life, greater BCS and carcass value (+20%). Hence, emissions of CR cows resulted greater for lifetime but lower per day of life and per kg of fat plus protein, whereas per kg of milk resulted comparable to HO cows emissions. Moreover, CR cows had lower emissions per unit of gross income and tended to have greater income over feed costs per unit of impact. We concluded that the crossbreeding scheme considered can be regarded as a strategy to mitigate emissions of GHGs and other pollutants.

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AIMS OF THE THESIS

The main objective of this dissertation was to compare purebred Holstein and crossbred cows derived from a 3-breed rotational crossbreeding mating system in terms of:

- detailed mineral profiles of milk, considering four generations of ProCROSS crossbreds and purebred Holstein cows kept in two farm representatives of the two main Italian systems of hard cheese production (Parmigiano Reggiano and Grana Padano);
- cull cows carcass attributes and overall carcass value, and the comparison of the risk of culling between ProCROSS crossbred cows and their purebred Holstein herdmates;
- body measurements, BCS, used to predict net energy requirements based on metabolic weight or predicted body protein mass, and production metrics in terms of milk and milk nutrient yields used to compute production metrics in order to assess production efficiency;
- environmental footprint of cows derived from ProCROSS scheme compared to their purebred Holstein herdmates using a Life Cycle Assessment (LCA) derived methodology applied on each cow.

CHAPTER 1

Macro- and micromineral composition of milk from purebred Holsteins and four generations of three-breed rotational crossbred cows from Viking Red, Montbéliarde and Holstein sires

S. Saha[1], M. Piazza[2], G. Bittante[3], and L. Gallo[4]

[1]Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[2]Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy

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ABSTRACT

This study compared the detailed mineral profiles of milk from purebred Holstein (HO) and four generations (F1 to F4) of crossbred cows (CR) derived from a 3-breed rotational crossbreeding system involving Viking Red (VR) and Montbéliarde (MO) sires. Purebred and CR were kept and fed together in the same herd. Individual milk samples were collected once per cow from 120 multiparous cows (40 HO and 20 for each generation of CR from F1 to F4). The milk samples were analyzed for macrominerals (Na, Mg, P, S, K, Ca), essential (Cr, Mn, Fe, Cu, Zn, Mo), and environmental microminerals (Li, B, Ba) using inductively coupled plasma-optical emission spectrometry. The coefficients of variation ranged from 11 to 28% for macrominerals and from 17 to 40% for microminerals. The mineral composition of milk from purebred HO and CR were mostly comparable. Conversely, it differed in different breed combinations of CR: VR-sired cows had the greatest macromineral contents, MO-sired cows the lowest, while HO-sired CR was intermediate. The milk from MO-sired crossbreds had lower contents of some microminerals (Mo, Zn, and Ba), while the milk from HO-sired crossbreds had greater contents (Fe and Mo). We conclude that the mineral profile of milk of CR derived from different combinations of VR, MO and HO breeds was comparable to that of milk from HO cows kept in the same herd, and that the variations in the macro- and micromineral profiles were greater among CR than between purebreds and crossbreds.

Keywords: dairy cow; crossbreeding; macrominerals; trace-elements

INTRODUCTION

Although the mineral content of milk is generally lower than 10 g/L (Gaucheron 2005), the mineral profile is characterized by many elements present in several different chemical configurations, such as inorganic ions and salts, and constituents of organic molecules (Cashman 2011a). As a result, milk and milk products are important sources of minerals for human nutrition (Cashman 2006; Zamberlin et al. 2012). Aside from this role, minerals are implicated in the structure and stability of casein micelles (Gaucheron 2005). They also influence the renneting ability of milk, thus playing a role in cheese manufacturing (Gustavsson et al. 2014; Malacarne et al. 2014), and can be used in the diagnosis of specific diseases in dairy cows, such as mastitis (Hamann and Krömker, 1997; Summer et al., 2009).

The mineral content of milk depends on several environmental factors, such as diet and feeding practices, and it varies across and throughout lactations (Gaignon et al. 2018; Denholm et al. 2019; Stocco et al. 2019). Genetic factors also affect the variation of several minerals, both within and across breeds. Significant heritability estimates have been reported for some macro- and microminerals (van Hulzen et al. 2009; Buitenhuis et al. 2015; Denholm et al. 2019). Stocco et al. (2019) reported that breed was a major source of variation for most essential minerals in milk. Comparative effects on the mineral profiles of milk have been investigated in some specialized and dual-purpose breeds (Hermansen et al. 2005; Barlowska et al. 2006; Buitenhuis et al. 2015; Stocco et al. 2019), yet the effect of crossbreeding on the mineral content of milk has so far not been dealt with. Crossbreeding is of increasing interest due to the declining fertility, health and longevity of modern specialized dairy cows (Malchiodi et al. 2014a; Hazel et al. 2017) and is now extending to many countries (Hazel et al. 2017; Clasen et al. 2019). A 3-breed rotational crossbreeding system, in particular, involving Montbèliarde (**MO**), Viking Red (**VR**) and Holstein (**HO**) breeds has raised interest in the dairy sector (Shonka-Martin et al. 2019). This study aimed to compare the detailed mineral profiles of milk produced by purebred HO and four generations of crossbred cows (**CR**) derived from a 3-breed rotational crossbreeding system using VR, MO, and HO sires.

MATERIALS AND METHODS

This study is part of a wider project evaluating 3-breed rotational crossbreeding (Saha et al. 2020). It was carried out on a dairy herd located in northern Italy, managed according the rules of protected denomination of origin Parmigiano Reggiano cheese. The cows were fed a total mixed ration based on dry roughage, mainly alfalfa and meadow hay, and concentrates. This farm was managed under the Procross 3-breed rotational crossbreeding program, which is jointly marketed by Coopex Montbéliarde (Roulans, France) and Viking Genetics (Randers, Denmark). A total of 120 cows (40 purebred Holstein and 80 CR) were randomly selected from a group of multiparous cows at lactation stages between 100 and 300 DIM (average parity 2.8 ± 0.9, average lactation stage 165 ± 49 DIM). The following sire-breed sequence was used for crossbreeding the cows (20 cows for each genetic combination): VR semen on HO cows to produce F1 (VR×HO); MO semen on F1 cows to produce F2 (MO×(VR×HO)); and HO semen on F2 cows to produce F3 (HO×(MO×(VR×HO))). F3 cows were inseminated using again VR semen to obtain F4 cows (VR×(HO×(MO×(VR×HO)))). Purebred HO and CR were raised and milked together, and were fed the same diets.

Individual milk samples (2.0 L/cow) were collected once per cow during the evening milking in six different sampling sessions (20 cows per session), in each of which purebreds and CR of all breed combinations were sampled. After collection, milk samples (without preservative) were refrigerated (4° C) and analyzed within 24 h of collection. Test day milk yield were recorded for each cow at the day of the sampling. Milk samples were analyzed for somatic cell count (SCC, Fossomatic Minor, Electric A/S, Hillerød, Denmark) and the values log transformed to somatic cell score (SCS). Protein and fat contents were measured with a Milkoscan FT2 infrared analyzer (Foss Electric A/S, Hillerød, Denmark) calibrated according to the reference methods (ISO 1211/IDF for fat, ISO-IDF, 2010; ISO 8968-2/IDF 20-2 for protein, ISO-IDF, 2014).

The mineral composition of the milk samples was determined with a Spectro Arcos EOP ICP-OES (Spectro A.I. GmbH, Kleve, Germany) according to the procedure detailed by Stocco et al.

(2019). Although a total of 32 minerals were measured, only 15 were analyzed in this study, as the other 17 were below the instrumental limits of detection in some or all of the samples.

Instrumental parameters were optimized as detailed in Stocco et al. (2019). Subsamples of 2 g of milk were taken from each sample and placed in a TFM vessel with 2 mL of 30% hydrogen peroxide and 7 mL of concentrated (65%) nitric acid, both Suprapur quality (Merck Chemicals GmbH, Darmstadt, Germany), and subjected to microwave closed-vessel digestion (Ethos 1600 Milestone S.r.l., Sorisole, BG, Italy). The dissolved samples were then diluted with ultrapure water (resistivity 18.2 Ω cm at 25 °C) to a final volume of 20 mL. Calibration standards were prepared using multi-element and single-element standards solutions (Inorganic Ventures Inc., Christiansburg, VA, USA) in 10% Suprapur nitric acid (Merck Chemicals GmbH, Darmstadt, Germany) to obtain similar matrices to the samples. The calibration solutions were in concentrations of 0, 0.002, 0.005, 0.02, 0.5 and 2 mg/L of the analytes for all minerals. Further concentrations of 5, 20, 50 and 200 mg/L were prepared for Ca, K, Mg, Na, P and S. The accuracy and precision of the analytical procedures were tested by analyzing the following control solutions: a blank; a low- and a medium-level solution with recovery limits ± 30% and ± 10%, respectively; and the international standard reference material BCR–063R "Skim milk powder" (Institute for Reference Materials and Measurements, Geel, Belgium). The measured and the certified values were in excellent agreement for all the minerals measured.

Before analysis, all data were classified for parity (**PAR**, 2nd parity and >2nd parity, 53 and 67 cows, respectively), days in milk (**DIM**, class 1: <150d; class 2: 151 to 210 d; class 3: >210 d, 50, 46 and 24 cows, respectively) and breed combinations (purebred HO, and F1, F2, F3 and F4 CR). Data on the contents of 6 macrominerals (Na, Mg, P, S, K, Ca), 6 essential microminerals (Cr, Mn, Fe, Cu, Zn, Mo) and 3 environmental microminerals (Li, B, Ba) were analyzed using a mixed model procedure in SAS 9.4 (SAS Institute, 2013), which included the random effects of sampling date and the fixed effects of PAR, DIM and breed combination.

Orthogonal contrasts (*P* <0.05) were used to investigate the effects of crossbreeding (purebred HO vs CR) and the effects of the breed of the terminal sire/generation in the CR: VR sire: (F1+F4) vs (F2+F3); MO sire: F2 vs (F1+F3+F4); and HO sire: F3 vs (F1+F2+F4).

RESULTS AND DISCUSSION

On average, the cows yielded 32.0 kg/d of milk containing 4.30% fat and 3.95% protein (Table 1), with a nearly 10% greater milk yield and nearly 5% lower protein content in HO compared to CR cows (data not in table). A total of 32 minerals were measured, but 17 were below the limit of detection in some or all of the milk samples and were not included in the analysis.

Average macromineral concentrations ranged from 96 mg/kg for Mg to 1,477 mg/kg for K, with coefficients of variation from 11 to 14% for all the macrominerals except Na, where it was 28%. The average contents of the different macrominerals in milk and their variations found in the present study are generally in good agreement with the values obtained using the same or similar methods by Cashman (2011a), Stocco et al. (2019) and Denholm et al. (2019). Average essential micromineral concentrations ranged from 3 µg/kg for Cr to 3,876 µg/kg for Zn, with coefficients of variation from 20 to 40%. The means of the essential micromineral contents of milk are also in agreement with the values reported by Cashman (2011b), with the exception of Mn, which was lower in our study. For most of the essential trace elements, the variability in the contents in this study (average coefficient of variation: 25%) was nearly half (on average 45%) that reported by Stocco et al. (2019); however, they sampled milk from cows kept in 27 multi-breed herds. The average contents of the environmental microminerals ranged from 5 (Li) to nearly 400 µg/kg (B), and their variability was comparable to that of the essential microelements.

Least squares means for the mineral contents of milk across breed combinations are reported in Table 2. The breed combination significantly affected the concentrations of all macrominerals and 6 of the 9 microminerals. Also Stocco et al. (2019), when comparing milk from different purebred cows, found that breed affected the content of all macrominerals and 7 of the 9 microminerals.

The differences between the purebred HO and the CR were never significant, with the exception of Mn and Ba (Table 2). On the other hand, large differences were observed within CR according to their sire-breed/generation. Namely, milk from crossbred daughters of VR sires (F1 cows and F4 cows) had greater (from 3.7 to 9.5%) P, S, K and Ca contents, and a 10% lower content of Na compared with the crossbred daughters of MO and HO sires. In addition, the milk from MO-sired cows (F2) had lower (-4.7 to -7.5%) Mg, P, S, K and Ca contents, and a 19% greater content of Na compared with the other CR, as well as lower levels of some essential microelements (Cr, Fe, Zn, and Mo, from -8 to -12%) and one environmental microelement (Ba, -11%). Lastly, the mineral content of milk from HO-sired CR (F3) did not differ from that of the average of the other 2 sire lines, except for the greater content of Fe and Mo in the former.

Very little is to be found in the literature regarding the detailed mineral profiles of milk from different breeds, and none on the different crossbreed combinations, particularly when the search is restricted to data obtained using certified primary laboratory analytical methods. When compared with other breeds, Holstein Friesian milk has generally been found to have a lower mineral content. This has been reported by Barlowska et al. (2006), who compared local Polish breeds and HO for 8 macro- and microminerals, and by Hermansen et al. (2005) and Buitenhuis et al. (2015), who compared Jersey and HO for several macro- and microminerals. Similarly, Gaucheron (2005) reported that the milk from Normandy cows had a higher mineral content than the milk from HO. Stocco et al. (2019) analyzed 15 macro- and microelements in milk from 6 different breeds and they reported that milk samples from HO cows had the lowest amounts of almost all the essential minerals. Glantz et al. (2009) found no differences in the Ca, P, K, Mg and Zn contents of milk from herds rearing HO and Swedish Red in summer, but in winter the Swedish Red milk had lower Ca and P contents than the HO milk. In the present study, milk samples from purebred HO and CR were comparable for all macrominerals and the majority of microminerals analyzed. Purebred HO and 2- and 3-breed crosses from the HO, MO, and VR breeds have been generally reported to differ in milk volume (higher in HO), and in fat, protein and casein concentrations (higher in CR) (Malchiodi et al. 2014b; Shonka-

Martin et al. 2019; Saha et al. 2020), and this trend has been confirmed also in this study (data not provided). Therefore, it seems that differences in milk yield and milk nutrients contents reported between the different breed combinations are not linked to differences in the concentrations of the minerals in milk. We are not aware of any previous research comparing the mineral profiles of purebred and CR, and we are therefore unable to corroborate our findings with the literature.

We found that the mineral profiles of milk from the CR were not homogeneous, but rather varied significantly across the different genetic lines: the milk samples from daughters of VR sires generally had greater levels of macrominerals, while that from daughters of MO sires had lower levels of macrominerals and several essential microminerals.

The minerals in milk are important not only as sources of bio-available nutrients for human nutrition, but also because along with protein fractions, protein genetic variants and acidity they help define the technological properties of milk during cheese-making (Bittante et al. 2012). Scandinavian breeds are known for having higher frequencies of milk protein alleles (especially A and E alleles of the CSN3 gene codifying for k-casein), which have unfavorable effects on milk coagulation and curd firming (Poulsen et al. 2013). The greater contents of some favorable macrominerals in the milk from VR-sired CR found here could in some way compensate for the higher frequencies of some unfavorable genetic variants, whereas the opposite situation is true for MO-sired CR. The proportions of different protein fractions, and particularly k-casein, also follow a similar pattern (Maurmayr et al. 2018). This could explain the modest differences in milk technological properties between the two types of CR, and also their advantage over the purebred HO cows (Malchiodi et al. 2014b; Saha et al. 2020).

CONCLUSIONS

This study furthers our knowledge of the effects of crossbreeding on milk composition by analyzing traits not previously investigated. The results reported here show that the mineral profiles of milk from CR obtained from different combinations of VR, MO and HO breeds within a 3-breed rotational crossbreeding program are greatly affected by the breed combination, and this is particularly apparent when comparing VR- with MO-sired cows. However, the average mineral profile of milk produced by the different crossbred types/generations was comparable to that of the purebred HO cows kept in the same herd.

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DISCLOSURE STATEMENT

None of the authors has a financial or personal relationship with other people or organisations that could inappropriately influence this publication.

ETHICAL APPROVAL

All authors declare that this study follows the principles of the Declaration of Helsinki.

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TABLES

Table 1. Descriptive statistics of yield, composition and mineral concentration of milk (n = 120)

Trait	Mean	SD	Min	Max
Milk yield, kg/d	32.0	8.1	10.0	48.0
Milk composition:				
Fat, %	4.30	1.12	2.09	7.63
Protein, %	3.95	0.36	3.22	5.08
SCS[1]	2.83	1.61	-0.64	6.73
Macro-minerals, mg/kg				
Na	401	114	210	907
Mg	96	13	66	126
P	960	119	618	1,296
S	351	39	256	464
K	1,477	206	995	2,092
Ca	1,224	130	912	1,575
Essential micro-minerals, µg/kg				
Cr	3	1	2	5
Mn	19	5	10	35
Fe	285	64	158	522
Cu	81	33	28	190
Zn	3,876	775	1,805	5,676
Mo	52	10	33	84
Environmental micro-minerals, µg/kg				
Li	5	2	2	10
B	393	68	264	565
Ba	48	11	28	84

[1] SCS = 3 + log2 (SCC/100,000).

Table 2. Least squares means and standard error of the means (SEM) for mineral concentration of milk across breed combinations (HO = Holstein; VR = Viking Red; MO = Montbéliarde)

	Breed[1]						Contrasts (*P-value*)			
Trait	HO	F1 VR ×	F2 MO ×	F3 HO ×	F4 VR ×	SEM	HO vs Cross (F1+F2+F3+F4)	VR sire (F1+F4) vs (F2+F3)	MO sire F2 vs (F1+F3 +F4)	HO sire F3 vs (F1+F 2+F4)
Macro-minerals, mg/kg										
Na	395	363	469	400	418	26	-	0.05	<0.01	-
Mg	97	97	91	98	99	3	-	0.09	<0.01	-
P	969	981	900	923	1 15	32	-	<0.01	<0.01	-
S	348	366	343	344	370	10	-	<0.01	0.05	0.07
K	1,447	1 32	1 88	1 20	1 12	70	-	<0.01	0.01	-
Ca	1,228	1 83	1 67	1 97	1 47	34	-	<0.01	0.02	-
Essential micro-minerals, µg/kg										
Cr	3	3	3	3	3	0.3	-	-	0.04	-
Mn	18	19	20	21	21	1	0.02	-	-	-
Fe	280	277	272	316	300	17	-	-	0.10	0.04
Cu	86	64	72	86	85	7	-	-	-	-
Zn	4,005	3 15	3 14	4 30	4 49	194	-	-	0.01	-
Mo	53	51	47	56	54	3	-	-	<0.01	0.04
Environmental micro-minerals, µg/kg										
Li	5	5	5	5	4	0.4	-	-	-	-
B	391	388	389	395	388	24	-	-	-	-
Ba	51	52	43	47	46	2	0.04	-	0.05	-

[1] F1 = VR×HO; F2 = MO×(VR×HO); F3 = HO×[Mox(VR×HO)]; F4 = VR×{HO×[MO×(VR×HO)]}

CHAPTER 2

Cull cow carcass traits and risk of culling of Holstein cows and 3-breed rotational crossbred cows from Viking Red, Montbéliarde, and Holstein bulls

M. Piazza, M. Berton, N. Amalfitano, G. Bittante, and L. Gallo

Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy

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ABSTRACT

Culled dairy cows represent a significant source of meat production, but their carcasses may vary greatly in quality because of the wide variation in the age, stage of lactation, breed, body condition, and other characteristics of the cows at slaughter. However, the effect of crossbreeding on the value of culled cows has so far received little investigation. The aim of this observational study was to compare a range of carcass attributes of cull cows from 3-breed rotational crossbreeding using Viking Red (**VR**), Montbéliarde (**MO**) and Holstein (**HO**) bulls with those of HO purebred cows. Data on 1814 dairy cows were collected. Cows were reared together in one herd and slaughtered in four slaughterhouses. The weight (**CW**), fleshiness (**FLESH**) and fatness scores (**FAT**), the total value (**VAL**) and the price (**PRI**, €/kg) of each cow carcass were recorded. The culling of a few cows in the sample (n = 86) was classified by the farm manager as "urgent" following a diagnosis of injury or sickness, and this information was recorded. Carcass traits were analyzed with a mixed model which included the fixed effects of parity, days in milk, genetic group (purebred HO, 787 cows, and crossbred cows, classified according to the breed of sire within crossbreds, with 309, 428, and 290 cows sired by VR, MO, and HO bulls, respectively), and interactions, and the random effects of month x year of the date of slaughter, and slaughterhouse. Logistic regression was used to investigate the association of parity, days in milk and purebred or crossbred origin with unplanned, "urgent" culling compared with regular culling. Average CW across genetic groups was 297 ± 65 kg, average PRI €2.03 ± 0.53/kg, and average VAL €631 ± 269. Compared to HO, crossbred carcasses were 7 to 12% heavier depending on the breed of sire, were graded + 0.12 to + 0.28 units higher for FLESH and + 0.26 to + 0.30 units higher for fat FAT, and fetched a 8 to 11% higher PRI. As a consequence, compared to purebred HO, carcasses from crossbreds had 15 to 24% higher VAL (+ €84 to + €133/cow), with crossbred cows sired by MO showing the greatest values. Moreover, compared to the HO cows, the crossbred cows had a 37% lower risk of being urgently removed from the herd, which raises welfare concerns and may reduce the salvage value of cull cows. Since cull cows represent a supplementary source of income for dairy farmers, the greater overall value of crossbred cull cows should be taken into account in evaluating the economic effectiveness of crossbreeding schemes.

Keywords: dairy beef, slaughter cows, carcass traits, crossbreeding, Holstein Friesian

INTRODUCTION

Culling is important in the management of dairy herds, as less-productive, old, infertile or unhealthy cows need to be continually replaced with younger and genetically superior heifers in order to maximize profitability. Although there is considerable variation, the average culling rate in the dairy sector is around 30% (Stojkov et al., 2018), which means that culling provides a consistent number of animals per year for meat production. Reproduction, mastitis, low production and injury have been reported as predominant reasons for culling (Moreira et al., 2021a), although often the causes for disposal cows are several, distant and not always fully evident (Fetrow et a., 2006). Dairy cows accounted for nearly 10% of commercial beef production in the United States in 2019 (Moreira et al., 2021b). Likewise, over 30% of bovine meat in the European Union came from cull cows (Eurostat, 2017), mostly of dairy breeds, whereas about 19% of the cattle slaughtered in Italy in 2020 were from dairy herds (ISTAT, 2021). However, despite their significant contribution to the beef industry, the importance of cull dairy cows and the factors affecting their value have received little attention in research.

The economic value of cull cows largely depends on their body weight and several other characteristics of the cow, such as age and stage of lactation at culling, muscle conformation and fattening grade, as well as potential visual defects, which directly affect the market price (Moreira et al., 2021a). As cull cows vary greatly in these characteristics (Vestergaard et al., 2007), wide variation in their value should also be expected.

Breed is another of the factors directly related to the cow that influences the carcass price and value of dairy cull cows. Bazzoli et al. (2014) compared the carcass attributes of cull cows of different dairy and dual-purpose breeds and reported that breed strongly affected carcass weight, price and value, with relevant differences not only between, but also within the dairy and dual-purpose categories. Gallo et al. (2017) also observed significant differences in the characteristics and value of cull cow carcasses of different dairy and dual-purpose breeds.

There has, however, been little research on the carcass traits and value of crossbred dairy cull cows. Crossbreeding programs have gained ground in recent years because of the need to improve the functional traits of high-yielding dairy breeds, mainly Holsteins. Crossbreeding has been found to have positive effects on milk quality, and on the fertility and udder health of cows, without detrimental effects on the daily yield of milk nutrients (Malchiodi et al., 2014; Shonka-Martin et al., 2019; Saha et al., 2020), and is therefore considered beneficial for the sustainability of dairy cattle farms (Buckley et al., 2014). Of the various crossbreeding programs, interest is growing in the 3-breed rotational system using Holstein (**HO**), Montbéliarde (**MO**) and Viking Red (**VR**) breeds, which is marketed internationally as ProCROSS by Coopex Montbéliarde (Roulans, France) and Viking Genetics (Randers, Denmark). Several studies have investigated the effects of this crossbreeding program on various production, functional and body traits (Malchiodi et al., 2014; Shonka-Martin et al., 2019; Saha et al., 2020), and shown it to be a profitable alternative to the HO pure-breeding system (Hazel et al., 2021). However, the value of culled cows originated from this crossbreeding scheme has been only scarcely investigated (Hazel et al., 2021).

Therefore, this observational study aimed to compare some carcass attributes and the overall carcass value of dairy cull cows originated from a 3-breed rotational scheme using VR, MO and HO bulls with those obtained by HO purebred (**PU_HO**) cows.

MATERIALS AND METHODS

Animals enrolled in the study

The study involved 1814 dairy cull cows slaughtered from 2015 to 2020 in 4 different commercial slaughterhouses, labeled A, B, C, D; around 73% of the cows in the study went to A, and around 17%, 6% and 4% went to B, C and D, respectively. Ethical approval was not sought for the present study because research did not involve direct manipulation of animals by authors. Moreover, the study was carried out following the recommendations of the ARRIVE guidelines. The cows came from one dairy farm located in the Emilia-Romagna region of Italy (province of Modena), in the Parmigiano Reggiano Protected Designation of Origin hard cheese production area. The farm had been using for more than a decade the 3-breed rotational crossbreeding system known as ProCROSS, according to a mating design descripted in details by Saha et al. (2020) and Hazel et al. (2021). Purebred and crossbred cows were reared together and managed as one group. The animals were kept in freestalls with cubicle and were milked twice per day. Cows were fed the same TMR, based on dry roughage, mainly alfalfa and meadow hay, and concentrates, and with no silage, in accordance with the regulations governing the production of Parmigiano Reggiano hard cheese.

Two to four shipments of culled cows, each consisting on average of 9 ± 5 cows, were delivered each month to the slaughterhouses, situated between 32 and 37 km from the farm. An average of 26 ± 10 cows were culled monthly, and both PU_HO and crossbreds were always present in each monthly delivery. In addition to the scheduled culling of cows, there was also some unscheduled culling of cows (n = 86) due to injury or sickness, classified by the farm manager as "urgent" and requiring prompt removal upon diagnosis. These were frequently delivered individually to the slaughterhouse. As cows sent for salvage slaughter still have a financial value, they were retained for the analyses, whereas euthanized or dead cows and those without carcass value were not enrolled in the study (n = 15).

Overall, the study involved 787 PU_HO, and 1027 crossbred cull cows. Crossbreds were classified according to the breed of sire as follows: those sired by VR (**VR_CR,** 309 cows), including the following breeds combinations: VR(HO), VR(MOHO), VR(HOMOVRHO), VR(MOHOVRMOHO); those sired by MO (**MO_CR**, 428 cows), including the following breeds combinations: MO(HO), MO(VRHO), MO(HOVRMOHO), MO(VRHOMOVRHO); and those sired by HO (**HO_CR**, 290 cows), including the following breeds combinations: HO(VRMOHO), HO(MOVRHO).

Data collection and variables definition

The data collected on farm comprised the following information for each cow: the genetic group (PU_HO or breed combinations of crossbreds), the parity order, the date of the last calving, the date of culling, and the culling type (scheduled or urgent, as defined above). The reasons for culling were not recorded regularly on the farm, and were therefore unavailable for this study. Similarly, body weight of dairy cows was not available because cows were not regularly weighed on the farm.

The following information was collected at the slaughterhouse for each cow (experimental unit): carcass weight (**CW**, kg), carcass value (**VAL**, €), and fleshiness (**FLESH**) and fatness (**FAT**) scores. The grading system based on the classes developed by the European Parliament and Council Regulation (EU) No. 1308/2013 has been used for the assessment of beef quality of carcasses, with FLESH evaluated according to the SEUROP grading system from S (superior) to P (poor) fleshiness, and FAT evaluated from 1 (low) to 5 (very high) fat cover (DG AGRI, 2011). Fleshiness and FAT were recorded only from 2016 onwards, and for 1421 cows.

Data editing and statistical analysis

Before analysis, the difference in days between the date of culling and the date of the last calving (**DIM**) have been calculated, whereas the carcass price (**PRI**, €/kg) has been computed as the ratio of VAL to CW (€/kg). Parity was classified into 5 classes (1st parity, n = 304 cows; 2nd parity, n = 514 cows; 3rd parity, n = 432 cows; 4th parity, n = 310 cows; ≥5th parity, n = 254 cows), DIM was classified into 5 classes of 100 days each (≤ 100, n = 284 cows; 101 to 200, n = 291 cows; 201 to 300, n = 420 cows; 301 to 400, n = 478 cows; > 400, n = 341 cows), and the cows' genetic group was classified into four classes (PU_HO, VR_CR, MO_CR, HO_CR). The fleshiness scores were converted into numeric variables ranging from 1 (P) to 6 (S).

After a preliminary analysis performed to check assumptions required for model fitting and hypothesis testing, carcass traits (CW, PRI, VAL, FLESH and FAT) were analyzed using PROC MIXED version 9.4 (SAS Institute Inc., version 9.4, Cary, NC, USA) and the following linear mixed model:

$$y_{ijklmn} = \mu + PAR_i + DIM_j + (PAR\ x\ DIM)_{ij}\ + M\_Y_k + SLH_l + GG_m$$
$$+ (GG\ x\ PAR)_{im}\ + (GG\ x\ DIM)_{jm}\ +\ e_{ijklmn}$$

where y_{ijklmn} is the trait of interest; μ is the overall mean; PAR_i is the fixed effect of the i^{th} parity (i = 5 classes); DIM_j is the fixed effect of the j^{th} class of days from calving (j = 5 classes); $(PAR \times DIM)_{ij}$ is the interaction effect between parity and days from calving; GG_m is the fixed effect of the m^{th} class of the genetic group of cows (m = 4 classes); $(GG \times PAR)_{im}$ is the interaction effect between the parity and the genetic group of cows; $(GG \times DIM)_{jm}$ is the interaction effect between the parity and the genetic group of cows; M_Y_k is the random effect of the month-year group of culling (k = 67); SLH_l is the random effect of slaughterhouse (l = 4); and e_{ijklm} is the random residual. Month-year group of culling, SLH and the residuals were assumed to be normally distributed with a mean of zero and variances of σ_k^2, σ_l^2 and σ_e^2, respectively.

Least square means between genetic groups were contrasted using a Bonferroni correction for multiple testing. Moreover, an orthogonal contrast [PU_HO vs (VR_CR + MO_CR + HO_CR)] was estimated to investigate the differences between purebreds and crossbreds taken as a mixture of generations and sire breeds representing the 3-breed rotational system.

Logistic regression was used to investigate the association of a set of explanatory variables with unscheduled "urgent" culls (compared with scheduled culls) through estimates and confidence intervals of the odds ratios (**OR**), a multiplicative measure of probability that ranges from 0 to infinity. Odds ratio was used to evaluate differences in the risk of being urgently culled among the effects considered. Odds ratio values of >1 or <1 indicate a greater or lower probability of a cow being urgently culled, compared with a reference condition expressed by the intercept of the logistic regression model. The 95% confidence interval represents the range within which the true OR of the population is expected to fall; if the 95% confidence interval included the value 1.00, the group of concern is assumed to be not significantly different from the reference. The LOGISTIC procedure of SAS was run with a model in which first parity PU_HO cows culled in the first 100 DIM were considered the "reference" animals, and the fixed effects of parity, DIM, and PU_HO or crossbred origin were included as categorical explanatory variables.

In all the models tested, a given effect (or interaction) was declared significant at $P < 0.05$.

RESULTS

Descriptive statistics of carcass traits

Descriptive statistics of carcass characteristics are reported in Table 1. In this study, cows that were culled had on average 2.9 ± 1.4 lactations and were 274 ± 150 DIM (data not shown in the table). The average CW was 297 kg and most cows had the lowest muscularity class (P = score 1.0), so the average FLESH score was 1.3 points. The average FAT score was higher (2.2 points), as was its variability (SD = ± 0.9 scores). The average total value of a cull cow carcass was close to €630, and the variation in VAL was nearly twice the variation in CW. Derived from VAL and CW, the average carcass PRI was €2.03/kg, with a coefficient of variation close to 26% and rather constant across years.

Sources of variation in carcass traits

The results for the carcass traits are given in Table 2. Both parity and, to a greater extent, DIM significantly influenced all carcass traits considered, with the exception of FAT, which was similar in cows of different parities. Moreover, a significant interaction between parity and DIM was observed for all carcass traits. In multiparous cows (Figure 1), CW increased almost linearly with increasing DIM class, with comparable trend in cows of different parity. Carcass weight also increased with increasing parity within DIM class in multiparous cows. Conversely, primiparous cows showed a different pattern of variation, because CW increased at increasing DIM until 200 DIM and from 400 DIM thereafter, whereas the increase in CW between 200 and 400 DIM was negligible in cows culled during their first parity. Primiparous and multiparous cows showed also a different trend of variation of FLESH and FAT at increasing DIM at culling (Figure 2). Indeed, both carcass attributes linearly increased with the advancing of DIM in multiparous cows, and FLESH tended also to increase at increasing parity. Conversely, in primiparous cows both FLESH and FAT nominally decreased at increasing DIM at culling until around 300 DIM, and increased thereafter, although no significnt difference was detected comparing least squares means of FLESH and FAT of primiparous cows culled at different DIM. As a consequence, PRI and VAL (Figure 3) increased with increasing DIM class in multiparous cows, whereas their increase in primiparous cows was substantial only for cows culled from 300 DIM thereafter. Carcass price and VAL also increased with increasing parity order within DIM class, but the greatest differences concerned primiparous cows culled between 200 and 400 DIM compared to multiparous ones.

Genetic group significantly ($P < 0.01$) affected all carcass traits considered (Table 1). Compared to PU_HO carcasses, carcasses from crossbreds were 7 to 12% heavier, depending on the breed of sire, were graded + 0.12 to + 0.28 units higher for FLESH and + 0.26 to + 0.30 units higher for FAT, and fetched 8 to 11% higher PRI (Table 3). As a consequence, compared to PU_HO, carcasses from crossbreds had 15 to 24% higher VAL (+ €84 to + €133/cow), with MO _CR cows showing the greatest values.

Relative risk of "urgent" culling

The estimated OR of parity and DIM class, and of crossbreds for unscheduled urgent culling are presented in Table 4. Eighty-six cows were culled outside the scheduled replacement plan, i.e. 4.7% of the sample.

Parity order did not appear associated to the the risk of being urgently culled ($P > 0.05$). However, cows with DIM of 200 d or more had a significantly lower risk of being urgently culled than those with DIM of ≤ 100 d, and OR progressively decreased with the increasing length of time since calving.

The origin of the cows (PU_HO or crossbreds) also significantly affected unscheduled urgent culls ($P < 0.04$), and crossbred cows were at lower risk of being urgently removed from the herd than PU_HO cows (OR = 0.63).

DISCUSSION

Variation in carcass traits and effects of parity and lactation stage at culling

Parity order at culling in this study averaged 2.9, and 69% of cull cows were in their first three lactations, which is consistent with data reported by Moreira et al. (2021a) for US dairy operations. Similarly, the average DIM at culling of around 274 d is comparable with the average calving to culling interval found in a large sample of dairy and dual-purpose cull cows reared in northern Italy (Gallo et al, 2017).

Carcass traits and the market value of cull cows are expected to be characterized by large variations, because cows no longer suitable for milk production may be removed at any parity and at any calving to cull interval, resulting in substantial differences in age, stage of lactation and body condition at slaughter (Shemeis et al., 1994; Vestergaard et al., 2007). The average CW in this study was almost 300 kg, greater than the average CW of dairy and dual-purpose cull cows reported by Gallo et al. (2017), Minchin et al. (2009), and Berry et al. (2021) (257, 277 and 289 kg, respectively), comparable to the average CW of dairy cull cows reported by Harris et al. (2018) in the National Beef Quality Audit - 2016 (303 kg), and lower than the average CW reported by Moreira et al. (2021b) (325 kg). The coefficient of variation of CW in this study was close to 22%, consistent with the variation in CW reported by Moreira et al. (2021b) and Harris et al. (2018), which ranged from 21 to 28%. Lastly, the average FLESH and FAT scores found in this study were slightly higher than those reported by Minchin et al. (2009), Gallo et al. (2017), and Berry et al. (2021) for dairy cull cows.

Both parity and, with a greater extent, DIM significantly affected CW and carcass attributes, but the pattern of variation of carcass traits at increasing DIM was partly different in primiparous and multiparous cows. Carcass weight increased with increasing parity for all class of DIM at culling, whereas the increase in FLESH, FAT and PRI with age at culling was slight but consistent in multiparous cows and followed an inconsistent pattern of variation within class of DIM at culling in primiparous cows. Similar associations between the lactation order or the age of cows at culling and CW have been reported by Seegers et al. (1998) and Gallo et al. (2017). The relationships between age at culling and carcass quality seem less consistent: Moreira et al. (2021b) found that dairy cows removed from the herd in later lactations fetched a lower PRI than those culled in the first 2 lactations; Gallo et al. (2017) found only slight associations between the FLESH and FAT of carcasses of dairy and dual-purpose cull cows and the age of the cows at culling, while the PRI was unaffected. Shemeis et al. (1994) also reported that age had no significant effect on scores related to carcass conformation and fatness. However, none of these studies considered an interaction between the lactation number and the DIM at culling in their analyses.

In general, CW increased with the increasing interval between calving and culling, although this trend was more consistent in multiparous than in primiparous cows. Also FLESH and FAT consistently increased at increasing DIM in multiparous cows, whereas they were nominally lower, although not significantly different, in primiparous cows culled between 200 and 400 DIM compared to those culled in early or very late lactation. It is well known that body reserves are mobilized in early lactation to support milk production, mainly as body fat and, to a certain extent, as body protein, leading to a reduction in weight and condition scores (Gallo et al., 1996; Phillips et al., 2003) which then recover from the 2nd - 3rd month of lactation onwards. Moreover, culling due to injury and poor health status is more frequent during early lactation, and this may contribute to reduce the value of cows culled early after calving (Pinedo et al., 2010; Moreira et al., 2021a). The increase in carcass quality traits with increasing DIM after the lactation peak was therefore expected, and the findings that cows culled later in lactation fetch higher PRI and have a greater VAL due to better weight and carcass composition are consistent with previous studies (Seegers et al., 1998; Gallo et al., 2017). The different trend of variation of carcass traits at increasing DIM at culling observed for primiparous cows in comparison to multiparous ones may be due to differences in lactation curves (Macciotta et al., 2011) and in pattern of the body reserve mobilization (Gallo et al. 1996), which reflect also the different nutritional competition between milk production and body growth. Moreover, as the cull of a cow at its first lactation may have a particular negative economic impact (Rilanto et al., 2020), it is possible that farmers adopt different criteria for cows of first parity compared to multiparous when taking culling decisions.

Effects of Viking Red - Montbeliarde - and Holstein rotational crossbreeding on carcass traits

In the present study, the carcasses of crossbred cows were found to be superior to those of PU_HO cows for all the attributes considered, resulting in a 20% greater total VAL. Montbeliarde sired cows showed the greatest VAL and PRI and the highest FLESH within crossbreds, but all the breed combinations considered were substantially superior to PU_HO for the traits investigated.

Breed is known to be one of the main sources of variation of carcasses from cull cows. Bazzoli et al. (2014) compared cull cows of different dairy and dual-purpose breeds, and they found that HO cows yielded lighter carcasses, fetched a lower PRI and had a lower VAL than Brown Swiss, Simmental and other dual-purpose cull cows. Likewise, Gallo et al. (2017) reported that the weight, fatness, conformation and price of carcasses of cull cows varied significantly according to breed, with Simmental always superior to HO. Zanon et al. (2020) also observed a clear effect of breed on the auction price of cull cows, with HO fetching the lowest price compared with other dairy cattle breeds reared in the Alpine region.

Higher revenue from cull cows from this 3-breed crossbreeding system compared with their PU_HO herd-mates was also reported by Hazel et al. (2021) in a study investigating the lifetime profit of PU_HO and crossbred cows kept in high-performance commercial herds in Minnesota. In that study, compared to PU_HO, the cull cow revenue was nearly 16% higher for the combined 3-breed crossbreds, with a slight greater value for MO_CR than for VR_CR cows.

The higher overall value of carcasses of crossbred cull cows compared with carcasses of PU_HO was due almost equally to their greater CW (+ 9%) and PRI (+ 11%). The higher PRI may be attributed to the greater FLESH and FAT of carcasses from crossbred compared with those from PU_HO. Literature investigating the carcass attributes of purebred and crossbred dairy cull cows is scarce, and mainly referred to breed combinations different from those of the present study (Berry et al., 2018; Coyne et al., 2019). However, some useful indications can be gathered from studies dealing with the body traits of cows belonging to these genetic groups. Crossbred cows have generally been found to have higher BCS compared with their PU_HO herd-mates. Several authors have reported this to be the case with this 3-breed rotational crossbreeding scheme (Saha et al., 2018; Shonka-Martin et al., 2019; Hazel et al., 2020), while greater backfat thickness have also been found in crossbred cows originated from crossbreeding programs involving Brown Swiss (Blöttner et al., 2011). The VAL of cull dairy cows depends on their body composition in terms of the proportions of muscle and fat (Moreira et al., 2021a), and BCS significantly affects the carcass conformation, PRI and VAL of cull dairy cows (Ahola et al., 2011; Shemeis et al., 1994).

Among breeds used as sire in the crossbreeding scheme, MO_CR provided the best performance in term of FLESH, with VR_CR just nominally better than HO_CR. Similar conformation score between HO and Danish Red young bulls, a subpopulation included in the Viking Red (Shonka-Martin et al., 2018), has been reported by Alberti et al. (2008) in a study comparing carcass characteristics of young bulls of fifteen European breeds. On the other hand, the MO, as dual purpose breed, has been actively selected also for improving muscularity scores, and it is known to provide carcasses heavier and characterized by greater FLESH, PRI and VAL than HO (Balandraud et al., 2018; Cabaraux et al., 2005). Indeed, an increase in the proportion of MO compared to HO breed has been associated to an increase in the selling price of crossbred cows at livestock marts in Ireland (McHugh et al., 2010), and greater condition score, CW, PRI and VAL have been reported for MO cull cows compared to HO (Evans et al., 2004).

Unscheduled urgent culling

Voluntary culling occurs when a cow is considered no longer of value to the herd, and the producer decides to remove it and replace it with a younger animal with greater productivity potential, whereas involuntary culling occurs when the producer needs to remove a cow due to infertility, illness, or injury (Moreira et al. 2021a). Nonetheless, both voluntary and involuntary removal are generally planned decisions and result in scheduled, non-urgent cull events. However, in some extreme circumstances, ill health or injuries not only impair the cow's productivity, but also cause significant suffering (Cockram, 2021), necessitating its removal outside the ordinary schedule, often urgently. Emergency culling typically follows a diagnosis of milk fever, downer cow syndrome, left displaced abomasum, severe teat problems, or foot and leg problems (Orpin and Esslemont, 2010).

In this study, urgent culling represented just under 5% of all removals from the herd, and it occurred irrespective of the parity order of the cows. It is known that the overall culling risk is increasing with lactation number (Hadley et al.. 2006), and this is caused by both voluntary and involuntary culling. What is not well known is the effect of parity on that part of involuntary culling that lead to urgent culling (Beaudeau et al., 1993). The risk of urgent removal decreased with increasing stage of lactation, and was much lower from 200 DIM onwards than in the previous phases. Cows in their first 100 DIM had only a nominally greater risk of urgent removal compared to those in mid lactation (100 to 200 DIM). In general, the highest risk of culling occurs shortly after calving, it then drops but increases again in the later stages of lactation (Fetrow et al., 2006). Nevertheless, culling due to injury and sickness, which is likely to be urgent and unscheduled, occurs more frequently in early lactation (Pinedo et al., 2010; Langford and Stott, 2012).

Crossbred cows were at a significantly lower risk of urgent unscheduled culling than purebred HO. Although the reason for urgent culling was only sporadically recorded, and hence was not included in this study, it is fair to assume it was mainly injury and sickness (where it was recorded, the cause was mainly given as "legs"). Aside from welfare concerns, culling due to extremely poor health status may drastically reduce the salvage value of cull cows (Stojkov et al., 2018; Moreira et al., 2021a). Indeed, in this study the average value fetched by urgently culled cows was around €160 ± 95, compared with the €654 ± 253 paid for regularly culled cows. Crossbreeding in dairy cattle has been associated with improved immunity (Cartwright et al., 2012), lower total health costs, fewer health disorders (Blöttner et al., 2011; Hazel et al., 2020), and greater robustness (Sørensen et al., 2008). This greater general robustness may also help explain the finding in this study that crossbred cows are at a lower risk of urgent unscheduled culling than PU_HO cows.

CONCLUSIONS

The data collected on Hostein cows and cows originated from a 3-breed rotational crossbreeding scheme reveal that the carcasses of the crossbred cull cows were heavier, better graded for fleshiness and fatness, fetched higher prices, and had a total value nearly €100 greater than the carcasses of their PU_HO herd-mates. Furthermore, crossbred cows had a lower risk than PU_HO of urgent unscheduled culling, which raises welfare concerns and results in a drastic reduction in the salvage value of cull cows. Since cull cows represent a supplementary source of income for dairy farms, the superiority of crossbred cows in terms of the total value of cull cows should be taken into account when evaluating the effectiveness of crossbreeding schemes.

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TABLES AND FIGURES

Table 1. Descriptive statistics of weight, fleshiness and fatness score, price, and value of carcasses.

Trait	Weight kg	Fleshiness[1]	Fatness[2]	Price €/kg	Value €/cow
Cows, n	1814	1421	1421	1814	1814
Mean	297	1.32	2.19	2.03	631
SD	65	0.5	0.92	0.53	269
Minimum	92	1	1	0.3	46
Maximum	482	3	4	3.5	1401

[1] On a six-point scale (the greater the greater fleshiness)

[2] On a five-point scale (the greater the fatter)

Table 2. Results from the mixed model for weight, fleshiness and fatness score, price, and value of carcasses: F-values and significance (* P < 0.05; ** P < 0.01) of parity and days in milk classes and of genetic group of the cows; percentage of variance explained by the random effects of month-year (M_Y) and slaughterhouse (SLH).

	Weight kg	Fleshiness	Fatness	Price €/kg	Value €/cow
Parity (PAR)	20.11**	2.74*	1.95	3.72**	9.66**
Days in milk (DIM)	135.83**	23.30**	36.76**	76.09**	104.52**
PAR x DIM	1.66*	2.39*	2.45**	3.16**	2.67**
Genetic group (GG)	33.87**	60.99**	8.66**	26.68**	31.33**
GG x PAR	0.75	1.64	1.10	0.97	0.66
GG x DIM	1.37	2.80**	1.19	1.61	1.57
M_Y, %	1.05	2.18	0.50	12.39	4.37
SLH, %	0	0	0.89	3.77	0
RMSE	51.91	0.46	0.84	0.44	220

Table 3. Least squares means and SEM for weight, fleshiness and fatness score, price, and value of carcasses from the 3-breed rotational crossbred cows classified according to the sire breed (Viking Red, VR, n. = 309 cows; Montbeliarde, MO, n. = 428 cows, and Holstein, HO, n. = 290 cows) or combined (CR, n. = 1027 cows) compared with those from purebred Holstein cull cows (n. = 787 cows).

	Weight kg	Fleshiness	Fatness	Price €/kg	Value €/cow
Purebred HO:					
- mean	278.4	1.17	2.02	1.89	550
- SEM	2.19	0.02	0.06	0.06	10.5
Crossbreds sired by VR:					
- mean	298.2**	1.36**	2.32**	2.11**	645**
- SEM	3.79	0.06	0.08	0.06	15.4
Crossbreds sired by MO:					
- mean	311.4**	1.46**	2.27**	2.13**	683**
- SEM	2.81	0.03	0.07	0.06	12.9
Crossbreds sired by HO:					
- mean	298.2**	1.30**	2.28**	2.05**	634**
- SEM	3.79	0.04	0.08	0.06	16.8
Combined CR:					
- mean	303.9**	1.38**	2.28**	2.10**	659**
- SEM	1.90	0.02	0.06	0.05	9.7

** Significant difference (P<0.01) from purebred Holstein

Table 4. Estimated odds ratio and 95% confidence interval of parity and days in milk class, and genetic group for unscheduled urgent culling (n = 1814 cows).

	Odds ratio[1]			
	Point estimate	95% confidence interval		*P* value
Parity class:				
1st (reference)	1	-	-	-
2nd	0.733	0.380	1.414	> 0.05
3rd	0.674	0.340	1.334	> 0.05
4th	0.976	0.480	1.949	> 0.05
≥ 5th	0.469	0.189	1.160	> 0.05
Days in milk class				
≤ 100 (reference)	1	-	-	-
101 - 200	0.675	0.404	1.991	> 0.05
201 - 300	0.304	0.160	0.575	< 0.001
301 - 400	0.193	0.095	0.391	< 0.001
> 400	0.113	0.043	0.294	< 0.001
Genetic group:				
Holstein (reference)	1	-	-	-
Crossbred	0.633	0.404	0.991	0.04

[1] OR > 1 (OR < 1) means a higher (lower) risk of being urgently culled unscheduled than the reference class.

Figure 1. Least square means of interaction between parity and lactation stage (DIM) of the weight of carcasses obtained from purebred Holstein and 3-breed rotational crossbred cull cows.

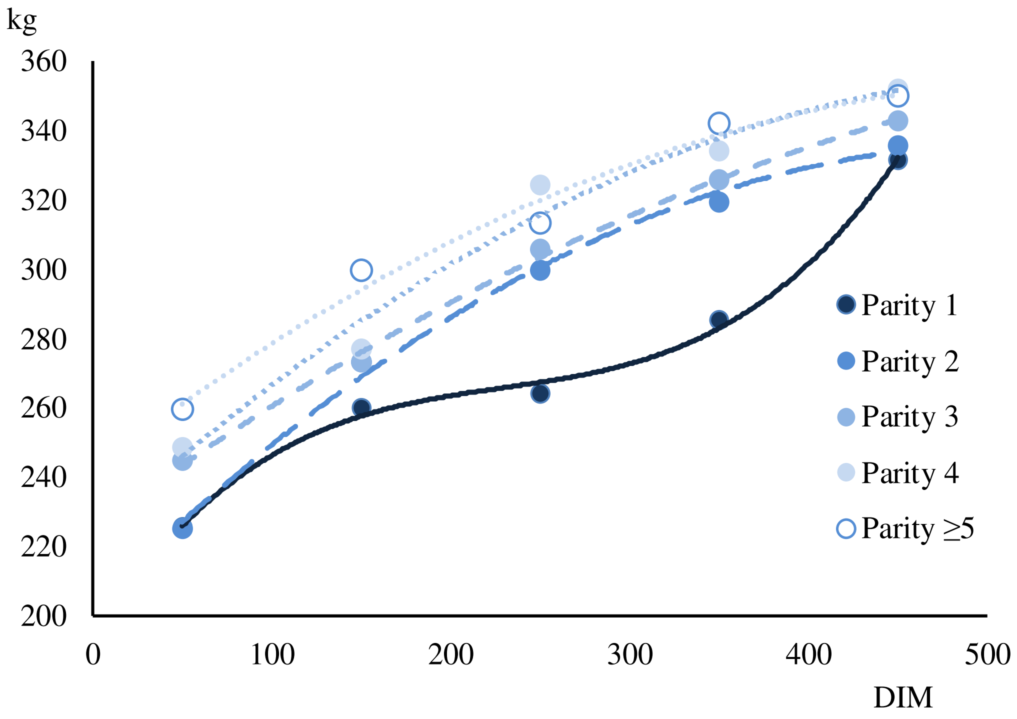
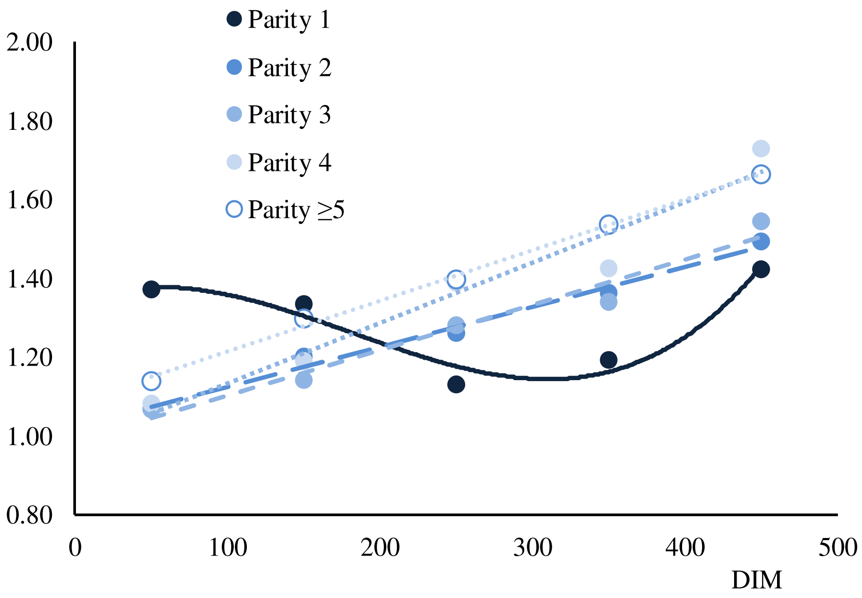


Figure 2. Least square means of interaction between parity and lactation stage (DIM) of a) fleshiness score (1 to 6) and b) fatness score (1 to 5) of carcasses obtained from purebred Holstein and 3-breed rotational crossbred cull cows.

a) carcass fleshiness score



b) carcass fatness score

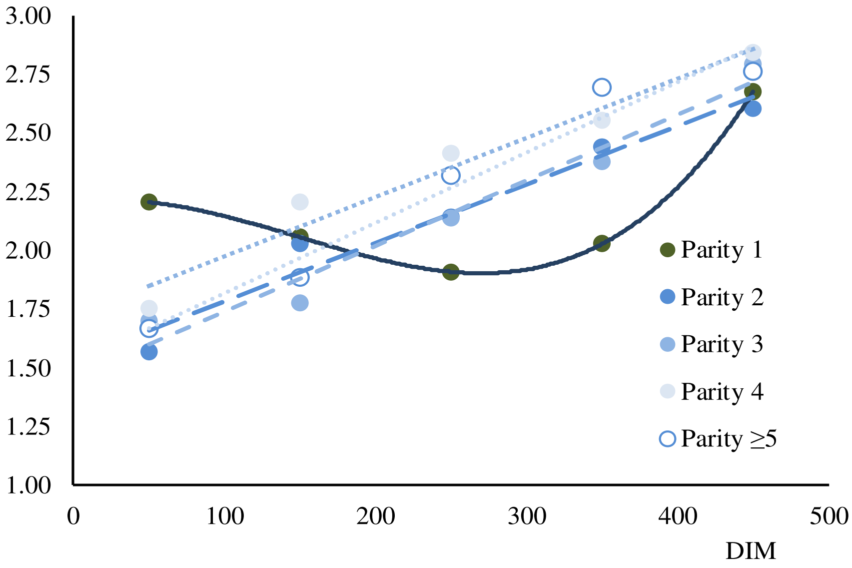
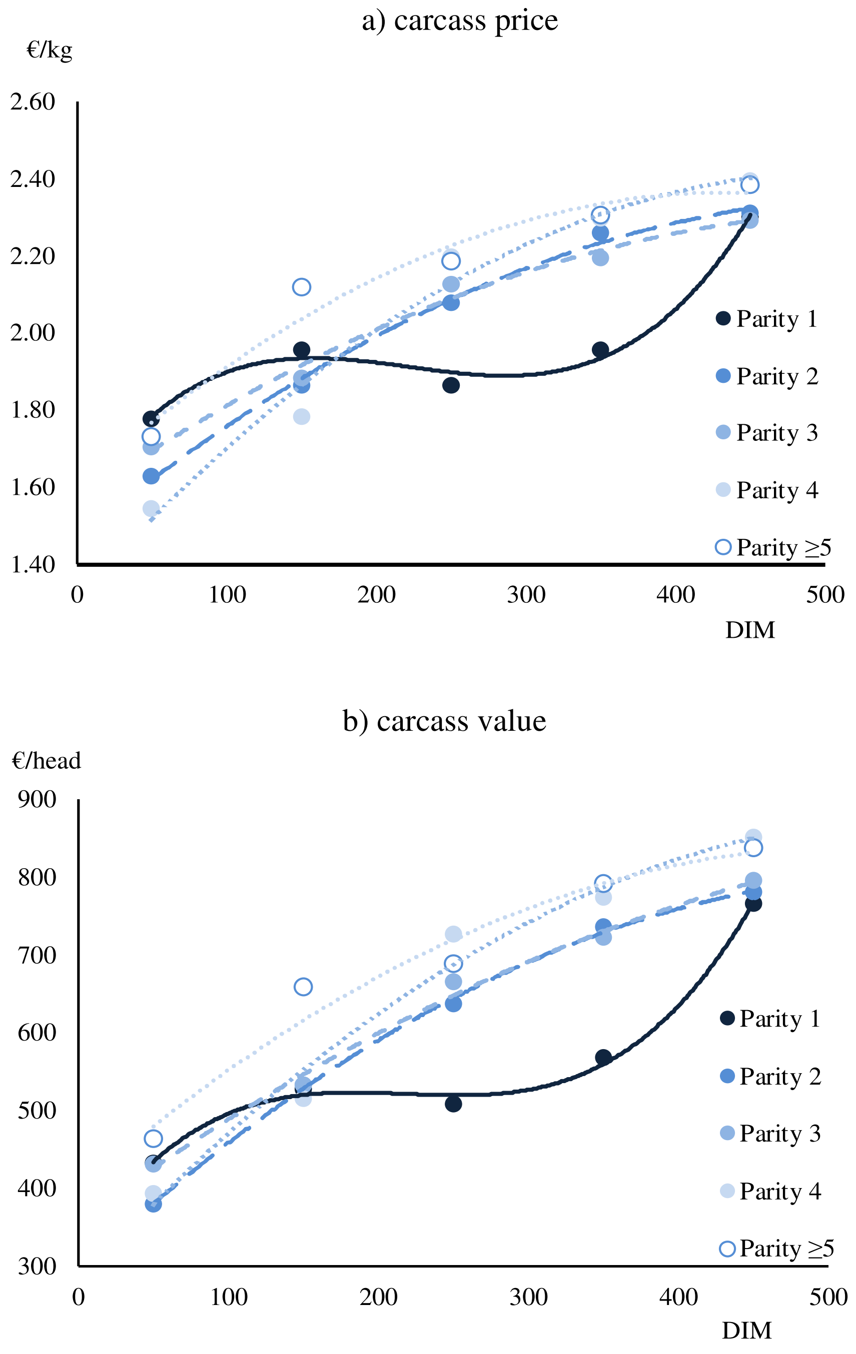


Figure 3. Least square means of interaction between parity and lactation stage (DIM) of a) price, and b) total value of carcasses obtained from purebred Holstein and 3-breed rotational crossbred cull cows.



Body and milk production traits as indicators of energy requirements and efficiency of purebred Holstein and 3-breed rotational crossbred cows from Viking Red, Montbéliarde, and Holstein sires

M. Piazza[1], S. Schiavon[1], S. Saha[2,3], M. Berton[1], G. Bittante[1], and L. Gallo[1]

[1]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro (PD), Italy

[2]Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[3]Laboratory of Animal Food Function, Graduate School of Agricultural Science, Tohoku University, Sendai 980-8572, Japan

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ABSTRACT

This study compared crossbred (**CB**) cows, obtained from a 3-breed rotational mating system involving Viking Red, Montbéliarde, and Holstein (**HO**) breeds, with purebred HO cows with respect to a range of body measurements and body condition score (**BCS**), used as predictors of the net energy requirements for maintenance (**NEm**), and to production traits based on milk and milk nutrients yields used together with NEm to predict metrics of the cows' production efficiency. The study involved 791 cows (351 purebreds and 440 crossbreds), kept in two herds managed according to different farming systems. The heart girth, height at withers, and body length of each cow were measured once, and BCS was determined. The body weights (**BW**) of 225 cows were taken to develop an equation to predict BW from body size traits, parity and days in milk (external validation: $R^2 = 0.94$, average bias of -4.95 ± 36.81 kg), which was then used to estimate the BW of all the cows. Equations from the literature were used to estimate body protein and lipid contents using BW and BCS. The net energy requirements for maintenance were computed on the basis of the metabolic weight (**NEm_$_{\text{MW}}$**: 0.418MJ/kg BW0.75) and the estimated body protein mass according to a coefficient (**NEm_$_{\text{PM}}$**: 0.631 MJ/kg body protein mass) computed on the subset comprising the purebred HO cows. The individual milk yield of all cows was measured, and the milk was sampled for protein and fat analysis, and to determine fresh cheese yield. Measures of net energy requirements for maintenance were used to scale the production traits. Statistical analyses of the body size measurements, body condition scores, estimated NEm and production metrics took into account the fixed effects of herd, days in milk, parity, and genetic group (purebred HO and CB), and the herd x genetic group interaction. The CB cows had similar BW and NEm_$_{\text{MW}}$, but, as they had an 11% greater BCS and a different estimated body composition, their NEm_$_{\text{PM}}$ was 3.8% lower than that of the HO cows. The CB cows yielded 4.8% less milk, and 3.4% less milk energy than the purebred HO cows. However, the differences between the CB and purebred cows were no longer significant when the production traits were scaled on NEm_$_{\text{PM}}$, suggesting that the crossbreds and purebreds have the same productive ability and efficiency per unit of body protein mass. In conclusion, measures of productivity and efficiency that combine the cows' production capability with traits related to body composition and the energy cost of production seem to be more effective criteria for comparing CB and purebred cows than just milk and milk nutrient yields.

Key words: crossbreeding; body size; body condition score; milk yield traits; Holstein Friesian

INTRODUCTION

Holstein (**HO**) has over time become the predominant dairy breed globally, due to a huge increase in milk yield resulting from extremely effective selection for production (Magne and Quenon, 2021; Hazel et al., 2021). However, increases in milk production have been accompanied by a phenotypic decline in female fertility, health and longevity (Oltenacu and Broom, 2010; Ma et al., 2019), although some improvements in the genetic trends for these traits have been recently reported (Brito et al., 2021). There has therefore been increasing interest in crossbreeding programs in order to exploit breed complementarity to incorporate the favorable traits of different breeds into the crossbred cows, to reduce the negative influence of inbreeding, and to capture the benefits of heterosis, especially with regard to fertility, reproductive performance, and robustness (Sørensen et al., 2008; Buckley et al., 2014; Hazel et al., 2020). Nevertheless, many farmers seem reluctant to adopt crossbreeding as a systematic mating program, mainly because HO purebreds have higher milk yields per cow than crossbreds (**CB**) (Buckley et al., 2014; Magne and Quenon, 2021). However, specific rotational 2- or 3-way crossbreeding schemes using a range of modern breeds may exert favorable effects on animal performance in both pasture-based and high-input confinement production environments by improving the fertility and longevity of dairy herds (Malchiodi et al., 2014a; Buckley et al., 2014) and enhancing profitability (Sørensen et al., 2008; Clasen et al., 2020). In intensive dairy farming systems, interest is growing in the 3-breed rotational system (ProCROSS) using sires of Viking Red (**VR**), Montbéliarde (**MO**), and HO breeds (Shonka-Martin et al., 2019b).

Comparisons of purebred and CB dairy cows are frequently based on lactation yield as the main indicator, and the vast majority of studies confirm the superiority of purebred HO cows over 2- and 3-breed crosses obtained from VR, MO, and HO sires in terms of milk volume yield (Malchiodi et al., 2014; Hazel et al., 2017b; Shonka-Martin et al., 2019b; Saha et al., 2020). However, the milk produced by CB cows is generally richer in nutrients (Malchiodi et al., 2014; Shonka-Martin et al., 2019b; Saha et al., 2020), which reduces the superiority of HO purebreds over CB cows in terms of the lactation yield of fat plus protein (Shonka-Martin et al., 2019b).

Milk and milk nutrient yields are indicators of production, not of efficiency, which is expressed with ratio-based indicators (e.g. the ratio of milk output to feed inputs or traits correlated to feed inputs, such as body weight and metabolic weight), and with residual-based traits (Berry and McCarthy, 2021; Connor, 2015; Köck et al., 2019). In terms of efficiency of production, differences in body weight (**BW**) are of paramount importance when HO cows are compared with smaller crossbred cows, such as those obtained from crossbreeding schemes involving the Jersey breed, often used in low-input, pasture-based systems (Prendiville et al., 2009; Evers et al., 2021). In the case of the ProCROSS system, on the other hand, the CB cows have been found to have a similar BW, and consequently a similar metabolic weight (**MW**), to purebred Holsteins (Hazel et al., 2017a; Shonka-Martin et al., 2019b; Hazel et al., 2020). As body weight measurements have been used as scaling factors of lactation yield to define efficiency indicators (Macdonald et al., 2008; Lembeye et al., 2016; O'Sullivan et al., 2019), such kinds of efficiency metrics are expected to be similar in purebred HO and CB cows or better in HO. However, the few studies monitoring dry matter intake at the individual cow level seem to indicate the greater efficiency of CB cows (Shonka-Martin et al., 2019a; Pereira et al., 2022).

We hypothesized that this apparent contradiction could be explained, at least in part, by potential differences in the body compositions of CB and HO cows, which may lead to over-estimation of the maintenance energy requirements of CB when they are based on MW. Indeed, in using MW, which scales BW by a power ($MW=BW^{0.75}$), it is assumed that cows with similar BW also have similar body compositions, and therefore similar daily requirements of net energy for maintenance (**NEm**). So, the use of MW for estimating the NEm requirements of purebred HO and CB cows rests on the assumption that the cows of these two genetic types at the same BW have about the same body composition, and consequently the same proportions of fat, protein and water. However, several studies have shown that the body condition scores (**BCS**) of CB cows were greater than those of their HO herd mates (Hazel et al., 2017a; Shonka-Martin et al., 2019b; Hazel et al., 2020). Differences in BCS reflect differences in body lipid and protein mass (Fox et al., 1999; NRC, 2016; NRC, 2021), and increasing evidence suggests that maintenance requirements are more closely related to body protein mass than to MW (Yang et al., 2020; Agnew and Yan, 2000; NRC, 2021). In the case of beef cattle, comparing animals characterized by a very different body composition such as UK breed steers and double muscled young bulls , Schiavon and Bittante (2012) have shown the bias inherent in using MW to predict NEm, and demonstrated the need to take into account body composition .

Our aim in the present study was therefore to compare CB cows originated from a 3-breed rotational mating system using VR, MO and HO sires with purebred HO cows for a range of body measurements, BCS, and production metrics in terms of milk and milk nutrient yields and predictors of NEm based on MW or on predicted body protein mass.

MATERIALS AND METHODS

Experimental design, herds and cows

In order to account for possible major confounding effects, we adopted an experimental design using two herds representing two different farming systems, each of which kept a group of purebred Holsteins, and a group of CB cows obtained from four generations of 3-breed rotational crossbreeding. Details of the experimental design are given in previous papers from the same project (Saha et al. 2020, 2021). The present study involved 791 dairy cows kept on 2 specialized dairy farms (232 and 559 cows, respectively) located in northern Italy. Both farms produced milk for the production of protected denomination of origin (PDO) hard cheeses (Parmigiano Reggiano and Grana Padano, respectively). The cows were kept in freestalls with cubicles, were milked twice a day, and fed total mixed rations in compliance with the regulations of their particular cheese consortium: dry roughage, mainly alfalfa and meadow hay, for Parmigiano Reggiano; corn and sorghum silages and concentrates for Grana Padano. The herds of both dairy farms comprised purebred HO cows and CB cows obtained from the 3-breed rotational crossbreeding system known as ProCROSS, according to the mating design described in detail by Saha et al. (2020) and Hazel et al. (2021). There were a total of 351 purebred HO cows (147 and 204 in the two herds, respectively), and 440 CB cows (85 and

355 in the two herds). As both farms were gradually increasing the number of CB cows produced from purebred HO, new CB cows of first generation were obtained every year, so there were cows of every generation and parity on the farms at the same time. Within each herd, purebred HO and CB cows were reared and milked together, fed the same diets, and managed as one group.

Milk sampling, milk composition and cheese-making procedure

Milk samples (100 mL) were collected once from each cow during one evening milking per farm. One aliquot of the milk was transferred to the laboratory of the breeders' association for analysis of fat and protein contents as part of the official Italian milk recording system (Milkoscan FT6000 infrared analyzer, Foss A/S, Hillerød, Denmark). This recording system also provided individual milk yields on the day of milk sampling. A second aliquot of each milk sample was stored without preservative in a refrigerator at -20 ºC and transferred to the Milk Laboratory of DAFNAE (Department of Agronomy, Food, Natural Resources, Animals and the Environment), where the lactose content was measured with a Milkoscan FT2 infrared analyzer (Foss A/S, Hillerød, Denmark), and individual curd yields (**CYCURD**) were measured using the 9-MilCA method (Cipolat-Gotet et al., 2016) according to a procedure which is comprehensively described for these samples in Saha et al. (2020). Briefly, after heating each 9 mL sample of milk to 35 °C, 0.2 mL of 1.2% diluted (wt/vol) rennet solution (Hansen Standard 215, with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG) was added, and the temperature maintained at 35 °C for 30 min. After a first manual cut, the samples were heated to 55 °C for other 30 min, and in the middle of this cooking phase were manually cut again. The curd was then separated from the whey for 30 min at room temperature, and gently pressed to expel the whey. The resulting curd was weighed using precision scales to determine CYCURD, expressed as a percentage of the milk processed.

Body traits measurements and BCS

The heart girth (**HG**, around the cow behind the shoulder), height at withers (**HW**, from the floor to the top of the back in a line up the middle of the shoulder), and body length (**BL**, from front tip of shoulder to edge of pin bone) of all the cows in the study were measured by the same operator. Body condition scores were assigned to each cow independently of their genetic group by the same skilled operator according to Edmonson et al. (1989), from 1 (lean) to 5 (fat) in increments of 0.25. In addition, on the same day the body traits were measured a subsample of 227 cows from one herd were weighed on an electronic weighing scale after the morning milking but prior to feeding, in order to develop reliable estimation equations for predicting body weight.

Estimation of body composition and net energy requirements for maintenance

The cows' body compositions were estimated using the equations proposed for dairy cows by Fox et al. (1999), cited by NRC (2016). As these equations refer to empty body composition and BCS on a scale of 1 to 9, we modified them to reflect BW (assuming empty BW = 0.82 BW; NASEM, 2021), and BCS on a scale of 1 to 5. The resulting equations were used to estimate the total fat and protein masses (kg) of each cow as follows:

$$\text{Body fat mass (kg)} = (0.06171 \times \text{BCS} - 0.0308706) \times \text{BW};$$

$$\text{Body protein mass (kg)} = (-0.01287 \times \text{BCS} + 0.170174) \times \text{BW};$$

$$\text{Body water and ash mass (kg)} = (-0.05076 \times \text{BCS} + 0.680697) \times \text{BW}$$

The total body energy content of each cow was estimated assuming energy values of 38.49 MJ/kg for body fat, and 23.22 MJ/kg for body protein (NASEM, 2021).

To account for the differences in body composition between purebred and CB cows, the NEm of the cows was calculated using two different methods. The first one uses the NASEM (2021) equation, in which NEm is based on MW (**NEm-$_{\mathrm{MW}}$** = 0.418 × BW0.75, MJ/d). The other was developed assuming the cow's average daily NEm to be primarily due to the lean tissues, and then to body protein mass (Agnew and Yan 2000, NASEM, 2021). Given this, we first computed the daily NEm-$_{\mathrm{MW}}$ using the NASEM (2021) equation for the subset of HO cows; we then computed the coefficient to calculate the NEm based on protein mass (**NEm$_{\mathrm{-PM}}$**) by dividing the NEm-$_{\mathrm{MW}}$ by the body protein mass of each cow for the subset of HO cows; lastly, the average value of this coefficient (0.631 MJ/kg body protein) was multiplied by the protein mass of each cow (HO and CB) to calculate individual predicted NEm$_{\mathrm{-PM}}$ values.

Production metrics

Based on the traits derived from the milk analysis, we computed the net energy content of the milk (NEl) according to the following equation (NRC, 2021):

$$\text{NEl (MJ/kg)} = 0.3887 \times \text{fat} + 0.2301 \times \text{protein} + 0.1653 \times \text{lactose;}$$

where fat, protein and lactose are the percentages of fat, protein and lactose in one kg of milk.

Six individual daily yield indicators - daily milk yield, the yields of fat, protein and fat + protein, milk energy, and fresh curd - were computed for each cow by multiplying the daily milk yield by the corresponding traits derived from the milk analysis and the cheese-making procedure.

Lastly, to obtain production efficiency metrics, 12 productivity indicators per cow were computed by scaling the 6 above-mentioned daily yield indicators by the estimated NEm$_{\mathrm{-MW}}$ and NEm$_{\mathrm{-PM}}$.

Editing and statistical analysis

Body weight prediction. Preliminary editing step aimed at handling extreme values resulted in a final dataset of 225 cows (70 HO and 155 CB), which were grouped into 3 parity (**PAR**) classes (PAR 1, 2, ≥ 3; 77, 71 and 77 cows, respectively), and 5 days in milk (**DIM**) classes of 60 days each (from ≤ 60 to > 240 d; number of cows per class ranging from 41 to 53). These categorical data were coded as dummy variables (0 or 1). The dataset was split into two subsets: two-thirds (n = 150) were used to develop a calibration equation for predicting BW, and one-third (n = 75) to validate the prediction equation. Pearson correlations were computed to assess multicollinearity among traits treated as predictors in subsequent analyses. A multiple regression model was applied to the calibration dataset using the PROC REG function of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) with a stepwise procedure that included the following independent variables: HG, HW and BL as continuous variables, and DIM and PAR as dummy variables. The best prediction equation was as follows:

$$BW = -700.67 + 18.72 \times PAR_2 + 25.06 \times PAR_3 + 18.85 \times DIM_2 + 6.98 \times DIM_3 + 9.58 \times DIM_4 + 15.59 \times DIM_5 + (-3.35 \times HW) + (6.70 \times HG) + (2.59 \times BL)$$

where PAR_2 and PAR_3 were the cows in the second and third parities, respectively, and DIM_2, DIM_3, DIM_4, and DIM_5 were the cows of 61 to 120, 121 to 180, 181 to 240, and > 240 DIM, respectively.

To test its performance, the equation was used to obtain predicted values for the validation dataset and the residuals for evaluation. Regression of the residuals obtained from the equation revealed uniform residual patterns, indicating no bias for either genetic group. Therefore, the equation was applied to predict the BW of all 791 cows in the study.

Statistical analysis. All records were classified for PAR (3 classes: 1, 2 and ≥ 3, 304, 241, and 246 cows, respectively), DIM (5 classes of 60 d each, from ≤60 to >240, the number of cows per class ranging from 127 to 203 d), herd of origin (2 classes), and genetic group (2 classes: HO and CB). After a preliminary exploratory data analysis to identify outliers and the assumptions required for model fitting and hypothesis testing, the milk and body traits, and productivity indicators were treated as dependent variables and analyzed using the following linear model in SAS PROC GLM (version 9.4; SAS Institute Inc., Cary, USA):

$$\mathrm{y_{ijklm}} = \mu + \mathrm{DIM}_i + \mathrm{PAR}_j + \mathrm{GG}_k + \mathrm{HD}_l + (\mathrm{GG} \times \mathrm{HD})_{kl} + \mathrm{e_{ijklm}}$$

where $\mathrm{y_{ijklm}}$ is the observed trait (i.e. body, milk, productivity traits); μ is the overall mean; DIM_i is the fixed effect of the i^{th} class of days in milk (i = 5); parity_j is the fixed effect of the j^{th} parity (j = 3); GG_k is the fixed effect of the k^{th} class of the genetic group (k = 2); HD_l is the fixed effect of the l^{th} herd (l = 2); $(\mathrm{GG} \times \mathrm{HD})_{kl}$ is two-way interaction between GG_k and HD_l; $\mathrm{e_{ijklm}}$ is the random residual, assumed to be normally distributed with a mean of zero and a variance of σ^2_e. A given effect was declared significant at $P < 0.05$, and tendential at $P > 0.05$ but ≤0.10.

RESULTS

Milk composition, biometric body traits, and estimated NEm

The equation predicting BW from body size measurements, PAR and DIM had a coefficient of determination of 0.81 in calibration, and a residual standard error of 36.7 kg. External validation, achieved by regressing the observed and the predicted values on an independent validation dataset, yielded a coefficient of correlation of 0.94 (Figure 1), and an average bias (predicted BW - observed BW) of -4.95 ± 36.81 kg.

Descriptive statistics and the results of the ANOVA for milk composition, curd yield, body traits and NEm are given in Table 1. The average percentage contents of fat and protein were 3.75 and 3.64%, respectively, and the variation for fat content was nearly twice the variation for protein content. Average body size measures were HW 140 cm, BL 164 cm, and HG 209 cm, which gave an average predicted BW of about 680 kg.

As expected, herd, PAR and DIM had significant effects on the vast majority of traits, with a few exceptions, whereas the effect of the herd x genetic group interaction was never significant. Genetic group influenced the milk protein content, which was nearly 2% greater ($P = 0.004$) in the milk produced by the CB cows compared with the HO purebreds (Figure 2). Similarly, the fresh curd yield of the CB cows was about 2.2% greater ($P = 0.045$) than that of their purebred herd mates.

The biometric measures of the two genetic groups also differed ($P < 0.001$): the BL, HW and HG of the crossbred cows were 1.2, 2.7 and 0.9% smaller, respectively, than those of the HO cows, whereas predicted BW was not affected by genetic group (Figure 2).

The average BCS of the CB cows was 10.2% higher ($P < 0.001$) than that of the HO cows, which resulted in the CB cows having an 11% greater estimated body fat mass ($P < 0.001$), a 6.4% greater estimated body energy content ($P < 0.001$), and a 3.8% lower estimated body protein mass ($P < 0.001$) compared with the purebred cows (Figure 2).

Maintenance energy requirements were similar for the CB and HO cows when computed from MW, but 3.8% lower for CB cows when computed from body protein mass ($P < 0.001$).

Production metrics of crossbred and purebred cows

Descriptive statistics and the results of the ANOVA for the production metrics are given in Table 2, while the differences between the CB and the purebred cows are illustrated in Figure 3. In this study, the cows yielded on average nearly 33.9 kg of milk, equivalent to 5.7 kg/d of fresh curd and 2.5 kg/d of fat plus protein, which, when scaled to NEm based either on metabolic weight or on protein mass, were around 610-620 g/MJ, 102-104 g/MJ and 44-45 g/MJ, respectively. The coefficient of variation ranged from 25 to 30% for all production metrics.

As expected, herd, parity and DIM significantly affected all the various production traits (P <0.01); again, no significant herd x genetic group interaction was detected. Genetic group significantly influenced milk yield when expressed per cow and per day of lactation (P <0.01), with CB cows having a 4.8% lower daily milk yield than purebred HO cows (Figure 3). The CB cows' daily milk yield was also significantly lower than that of purebred HO when the output was measured as milk energy (-3.4%, P <0.05), whereas the differences were only tendential when the output was measured as fat plus protein yield (- 2.9%, P = 0.08), and non-significant when it was fresh curd yield.

The differences between CB and HO cows slightly decreased when the daily yield was scaled on NEm-$_{MW}$, but remained significant for raw milk (-4.4%, P <0.05) and tendential for milk energy (-3%, P = 0.08). When the daily yields of outputs were scaled on NEm-$_{PM}$, CB and purebred HO did not significantly differ for any production trait, and the difference between the two genetic groups ranged from -1 to +1% according to production metric.

DISCUSSION

Crossbreeding is a management strategy that uses genetic resources with the aims of reducing the negative influence of inbreeding, and of exploiting heterosis, mainly for fertility and functional traits (Sørensen et al., 2008). Individually, CB cows are often less productive in terms of daily milk yields compared with their purebred herd mates, although their milk has higher fat and protein contents (Malchiodi et al., 2014; Heins and Hansen, 2012; Shonka-Martin et al., 2019b). More complicated farm management, and the lower individual milk yield of CB cows are the major reasons why crossbreeding is still not often used by farmers (Magne and Quenon, 2021). However, some studies suggest that when the entire life of the cow is taken into consideration, the lower productivity of crossbreds is frequently counterbalanced by improved milk nutrient contents, longer herd life, a lower replacement rate and lower health costs, making CB cows even more profitable compared with their purebred counterparts, usually the HO breed (Buckley et al, 2014; Hazel et al., 2021). Moreover, lower dry matter intake, potentially better rates of feed conversion efficiency, and higher income over feed costs have been reported for CB cows compared with HO (Shonka-Martin et al., 2019a; Shonka-Martin et al., 2019b).

The outcomes of crossbreeding are greatly influenced by the specific breed combinations and mating programs (Buckley et al, 2014). A successful crossbreeding program is one that considers the complementarity of the breeds in a given productive context (Sørensen et al., 2008). A specific 3-breed rotational breeding system using VR, MO, and HO breeds from different selection programs has been recommended for use in high-input productive systems with limited opportunity for grazing (Dechow and Hansen, 2017). The VR breed is a combination of different populations of Danish Red, Swedish Red and Finnish Ayrshire, whose milk has higher milk fat and protein contents, and, as a consequence of selection indices adopted in Scandinavian countries, better fertility and health than HO cows (Shonka-Martin et al., 2019b). The MO breed is less productive, but has better fertility, health traits and milk quality than the HO breed, and, as a dual purpose breed, has also been actively selected for improving beef traits (Balandraud et al., 2018). Heterosis in crossbred cows may further improve all these traits, especially fertility and longevity, compared with purebreds.

In this study, we compared the body traits and various production metrics of purebred HO and CB cows taken as a mixture of generations and sire breeds representing the 3-breed rotational system. Comparison of the sire breeds within CB was therefore outside the scope of the study.

Body weight prediction

The body weight of cows may be important information for proper management, nutritional practices and reproductive choices (Yan et al., 2009), and has been proposed as a useful scaling factor to gauge feed efficiency when measuring individual dry matter intake is unfeasible (Berry and McCarthy, 2021). However, BW is rarely available, due to the cost of weighing scales, the time required to weigh the animals, and the risk to them of trauma (Heinrichs et al., 2017). Body conformation measures, such as HG, HW and BL, are related to the body size of animals, and are considered possible predictors of BW in the absence of weighing scales (Heinrichs et al., 1992;

Costigan et al., 2021). In the current study, the best equation for estimating BW was obtained using these three body size measures together with PAR and DIM class as independent variables. Variance inflation factors were <2.5 for all the parameters retained in the equation, and we can therefore assume that the traits treated as predictors did not exhibit any appreciable multicollinearity (Johnston et al., 2018). The performance of the proposed equation is comparable to other equations obtained in previous similar studies (Heinrichs et al., 2007, 2017; Yan et al., 2009; Banos and Coffey, 2012; Piazza et al., 2022), and it was used to predict BW in the whole dataset of this study. It is worth noting that the BW of an individual cow can also change considerably over a short space of time (within a day or days), mainly due to the effects of eating, drinking, defecating, urinating and milking. The residual standard deviation of the difference between the predicted and measured BW (± 36.81 kg) is probably not much larger than the combined effects of these sources of variation. Clearly, the predictors used for estimating BW mainly reflect the cow's skeletal development, and, in the case of heart girth, the cow's fatness and muscularity, and as these factors do not change appreciably during the day or from one day to another, we expect predicted BW to have greater repeatability than measured BW, and to be more useful for estimating the cow's frame/size, and particularly its empty body weight (which should not be affected by the content of the gastrointestinal tract and the udder).

Body condition and estimated body protein and lipid masses

The results of the present research suggest that CB cows are significantly more compact than their HO herd mates, as they have smaller BL, HG and HW, but comparable BW. This is due to the different sign of the regression coefficients of the three measures on BW (positive for HG and BL, negative for HW). Therefore, with the same HG and BL, cows that are shorter in stature are heavier than cows that are taller in stature, possibly because of a more "beefy" conformation. Given the same circumference, the cross section of the trunk of short cows is expected to be more circular, and that of tall cows to be more narrow. So the greater reduction in stature of the CB cows compared with HO cows compensates for the smaller reduction in HG and BL.

Moreover, CB cows had higher BCS (+11%, equal to 0.32 units of BCS). Body condition scoring is a rapid, noninvasive, low-cost, subjective method for estimating the cows' degree of fatness (Waltner et al., 1994), and is a valuable tool for dairy farmers to monitor fat and energy changes in the cows during lactation (Edmonson et al., 1989; Gallo et al., 1996). Higher BCS, and greater differences in body conformations and measures have also been reported elsewhere for cows originated from this rotational crossbreeding scheme compared with purebred HO cows (Hazel et al., 2017a; Shonka-Martin et al., 2019b; Hazel et al., 2020). Similarly, comparable measured BW values of these two genetic types have also been reported by Shonka-Martin et al. (2019b). The higher BCS has been called upon to explain some of the CB cows' advantages in fertility over purebred HO cows (Hazel et al., 2017a).

In this study, we used body weight and BCS together to predict the body chemical composition of cows. Equations to estimate fat, protein, water and ash contents of the body were developed from empty body chemical composition data and the BCS of 106 mature cows of different breed types, mature weights, and BCS (NRC, 1996). Fox et al. (1999) proposed modifying these equations to extend their use to dairy cattle. They found that with these equations, the body fat of Holstein cows at a particular BCS was predicted with an R^2 of 0.95 and a bias of 1.6%, which is considered good. However, in general, the precision and the accuracy of this method has not been widely studied, mainly because of the scarcity of body composition data, and should therefore be investigated further, given the subjective nature of the method, differences in the anatomical presence and distribution of fat depots and muscles between breeds and individuals, differences in physiological stages within individuals, and differences in the proportion of BW attributable to digesta and milk (Gibb and Ivings, 1993; Gregory et al., 1998). Differences in BW of about 40 kg due to variations in non-body components may generate errors of about 12 kg in the estimation of body fat (Gibb and Ivings, 1993). As discussed previously, the use of BW predicted on the basis of body measures probably reduces the variations due to non-body components (gastro-intestinal, bladder and udder contents). Because of this uncertainty, estimates obtained using this method should be treated with caution, and used mainly for comparative purposes under the same productive circumstances, as in the present study. In any case, quantitative estimation of protein and lipid contents in live cows is key to predicting maintenance energy requirements accurately (Yan et al., 2009). The average body protein and lipid masses obtained here using the above-described equations (15.7% and 21% of empty body weight, respectively) were in the range observed for lactating Holstein cows (Andrew et al., 1994; NRC, 2016; Agnew et al., 2005). Despite similar BW (CB 686 kg vs. HO 691 kg), the crossbreds had higher BCS scores, and were therefore estimated to have, on average, 3.9% less body protein (86.1 vs 89.4 kg), 10.0% greater body fat mass (126.2 vs 113.6 kg), and 6% greater body energy content (6885 vs. 6471 MJ) than the HO cows.

Energy requirement for maintenance and production metrics

Maximizing milk yield has been one of the dairy industry's main focuses, as an increase in the yield per cow means that maintenance expenses can be distributed over more units of milk, thereby increasing the profitability of dairy operations (VandeHaar and St-Pierre, 2006). The yields per cow of milk, fat plus protein, and energy produced with the milk are therefore typical production indicators used for comparing the production potential of dairy cows. However, possible decline of the dilution of maintenance with further increases in milk yield (Bach et al., 2020), and pressing demands from society to reduce the negative effects on the environment of dairy production has gradually shifted the focus to production efficiency, generally expressed in terms of saleable output per unit input, each appropriately weighted by their relative economic importance (Berry and Crowley, 2012).

Among the different components of production efficiency, feed efficiency has drawn particular attention, given that feed typically accounts for 50 to 60% of the operating expenses on a dairy farm (Connor, 2015). Irrespective of how it is defined, feed efficiency in dairy cows requires a measure of feed intake, which is hardly feasible in commercial dairy farms. Other metrics, although less accurate and less specific, have therefore been suggested (Berry and McCarthy, 2021). Traits related to the animals' body size or to certain predictors of their energy requirements for maintenance have frequently been used as scaling factors for milk production, while milk yield per kg of cow BW (or metabolic BW) has been used as a metric of gross efficiency in several studies (Prendiville et al., 2009; Köck et al., 2019; Berry and McCarthy, 2021).

Maintenance accounts for a substantial proportion of the energy costs in dairy cows, and, unlike all the other energy costs (milk yield, pregnancy, and body growth), it is not related to a saleable output. For this reason, the maintenance requirement is considered a "fixed" cost of production, and measures of efficiency depend on the ratio of outputs (measured as product mass, energy or monetary value) per unit (BW, MW, NEm, or feed cost) of the maintained cow.

In most energy systems, maintenance energy expenditure is considered to be related to the unit of MW (NRC, 2021). The recent edition of NASEM (2021) suggests computing NEm (MJ/d) as $0.418 \times BW^{0.75}$, which is substantially higher than the previous 0.335 $MJ/kg^{0.75}$ (NRC, 2001), given that "modern cows have metabolic rates for maintenance greater than they were 50 years ago". In the modern specialized dairy cow, this is probably due to the higher proportion of empty BW represented by tissues with high energy demands for activity (almost all the organs and glands) compared to those with moderate (muscles, bones and tendons) or low (adipose tissues) requirements. The metabolic weight function assumes that the proportion of energy-requiring tissues decreases in favor of moderate and, especially, low energy-requiring tissues with increasing BW. It is reasonable to assume that, within a given breed, the metabolic activity per unit of weight of young, light, lean animals will be higher than that of mature, heavy, fat animals. There are, therefore, doubts over the adequacy of MW as a scaling factor for defining NEm, particularly when comparing cows of different breeds, production potentials or physiological statuses. In regard to MW, cows of the same BW are expected to have the same body composition and maintenance requirements, but these traits could instead be very different if the cows are of different breeds, yield potentials, body conditions, pregnancy statuses, etc. Indeed, the NASEM (2021) committee held that the NEm of lactating cows could be greater than that of non-lactating cows, due to their greater masses of liver and internal organs, which produce more heat per mass unit of muscle; by the same token, high-producing cows would have greater energy requirements for maintenance than low-producing cows Moreover, the need of adjustment factors for maintenance have been hypothesized to account for breed differences imn beef cattle (NRC, 2016). Oldham and Emmans (1990) consider it physiologically unreasonable to consider NEm as related to metabolic weight given that body composition varies in terms of protein and fat content, and the energy required to maintain 1 kg of protein is much greater than the energy required to maintain 1 kg of fat (DiCostanzo et al., 1990). The idea of scaling NEm to body protein mass is therefore gaining credit (Agnew and Yan, 2000), although it is acknowledged that further research is needed (NASEM, 2021). In this study, we calculated an NEm requirement of 0.631 MJ/kg body protein, which allowed us to differentiate between the NEm of purebred HO and CB cows, which had similar BW but different estimated body protein masses. The results suggest that the CB cows' daily NEm would be in the order of 2 MJ/d lower than the NEm expenditure of the HO purebreds (-3.9%), a small but statistically significant difference.

In the present study, the CB cows yielded a significantly lower amount of milk (-4.8%) than purebred HO cows in terms of absolute production. Looking instead at the nutrient content of milk, the differences between the CB and purebred HO cows still remained significantly lower in terms of the energy produced with the milk (-3.5%), but were only tendential in terms of the fat plus protein produced (-2.9%), although once again in favor of the purebred HO. In this crossbreeding scheme, HO cows were found to be consistently superior to their CB herd mates in terms of the volume of milk yielded (Heins and Hansen, 2012; Malchiodi et al., 2014; Hazel et al., 2020). Conversely, the fat plus protein yield was found to be greater in purebred HO in some studies (Heins and Hansen, 2012; Hazel, 2020), but comparable to CB cows in others (Hazel et al., 2014; Shonka-Martin et al., 2019b). The differences between purebred HO and CB cows were only slightly smaller when yield traits were scaled on NEm_{-MW}, because the two genetic groups had similar BW. Interestingly, when yield traits were scaled on NEm_{-PM}, all the differences between the purebred HO and CB disappeared, and instead, the CB cows exhibited a slight, although non-significant, superiority in terms of fat plus protein and fresh curd yielded per MJ of NEm-$_{\text{PM}}$. Therefore, when NEm is computed taking into account the differences in body composition between different genetic groups, purebred HO and the CB cows of this mating scheme did not appear to differ in gross feed efficiency expressed in terms of yield per unit of NEm. Shonka-Martin et al. (2019a) reported that rotational 3-breed crossbred cows of this mating scheme had in their first 150 d of lactation greater fat plus protein yields per unit of dry matter intake, lower dry matter intake per unit of BW, and lower residual feed intake compared to their purebred HO herd mates, suggesting that CB could have different energy requirements due to different amounts of muscle and fat compared with purebred HO, and greater overall feed efficiency.

CONCLUSIONS

The results of this study showed that crossbred cows had a similar body weight, but greater BCS, and lower estimated body protein mass compared with their purebred HO herd mates, suggesting that crossbred cows could also have lower energy requirements for maintenance. Crossbred cows tend to have lower daily milk yields, milk nutrient contents, milk energy and fresh curd. However, when production metrics are scaled on estimations of energy requirements for maintenance taking into account differences in body composition, the production performances of crossbreds and purebred HO are entirely comparable. Therefore, measures of efficiency that combine the cows' production ability with traits related to the cost of production seem to be more effective measures than simply milk and milk nutrient yields for comparing the outcomes of the crossbreeding mating scheme studied here and purebred HO cows. Further research is needed to develop more precise and accurate methods for estimating the body composition of the cows, and for evaluating the relationship between body protein mass and the energy expenditure for maintenance.

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TABLES AND FIGURES

Table 1. Raw means, standard deviation and P-values from ANOVA for milk composition, fresh curd yield, body traits and estimated net energy requirements for maintenance based on metabolic weight (NEm-MW) and on body protein mass (NEm-PM)[1].

			P-values[2]					
Trait	Raw mean	SD	Herd	Parity	DIM	GG	Herd × GG	RMSE
Milk composition and cheese yield								
Fat content, %	3.75	0.75	0.006	0.10	<0.001	0.24	0.78	0.72
Protein content, %	3.64	0.35	<0.001	0.07	<0.001	0.004	0.14	0.29
Energy content, MJ/kg	3.13	0.34	0.124	0.013	<0.001	0.13	0.51	0.32
Fresh curd yield, %	16.84	2.25	0.016	0.54	<0.001	0.045	0.73	2.20
Body traits:								
Body condition score (1 to 5 scores)	3.26	0.37	<0.001	<0.001	<0.001	<0.001	0.06	0.32
Body length, cm	164	7.33	<0.001	<0.001	<0.001	<0.001	0.22	5.96
Hearth girth, cm	209	9.45	<0.001	<0.001	<0.001	0.004	0.63	7.93
Height at withers, cm	140	5.19	<0.001	<0.001	0.012	<0.001	0.25	4.29
Predicted body weight, kg	677	71.8	<0.001	<0.001	<0.001	0.30	0.69	53.8
Body composition:								
Predicted body fat mass, kg	116	23.0	<0.001	<0.001	<0.001	<0.001	0.16	20.30
Predicted body protein mass, kg	87	8.7	0.018	<0.001	<0.001	<0.001	0.82	5.83
Predicted body energy content, MJ	6492	1003	<0.001	<0.001	<0.001	<0.001	0.21	866
Maintenance requirement:								
NEm-MW, MJ/d	55.39	4.42	<0.001	<0.001	<0.001	0.31	0.55	3.31
NEm-PM, MJ/d	54.68	5.50	0.018	<0.001	<0.001	<0.001	0.82	3.68

[1] N = 791 cows, 351 purebred Holstein and 440 crossbreds.

[2] Parity= 1st, 2nd and ≥3rd lactation; DIM= days in milk classes: ≤ 60 days, 61 to 120; 121 to 180 days; 181 to 240 days; ≥ 241 days; GG= Genetic group: Holstein and crossbred cows.

Table 2. Raw means, standard deviation and P-values from ANOVA for yield of milk, milk components, milk energy, and fresh curd in absolute value (daily yield) or scaled on net energy requirement for maintenance based on metabolic weight (NEm-$_{MW}$) and on body protein mass (NEm-$_{PM}$)[1].

			P-values[2]					
Trait	Raw means	SD	Herd	Parity	DIM	GG	Herd × GG	RMSE
Milk production:								
Daily yield, kg/d	33.85	10.11	<0.001	<0.001	<0.001	0.007	0.49	7.74
Daily yield/ NEm-$_{MW}$, g/MJ	613	182	<0.001	<0.001	<0.001	0.018	0.66	147
Daily yield/ NEm-$_{PM}$, g/MJ	621	178	<0.001	<0.001	<0.001	0.58	0.44	146
Fat production:								
Daily yield, g/d	1240	371	<0.001	<0.001	<0.001	0.14	0.31	306
Daily yield/ NEm-$_{MW}$, g/MJ	22.5	6.61	<0.001	0.001	<0.001	0.21	0.48	5.67
Daily yield/ NEm-$_{PM}$, g/MJ	22.8	6.59	<0.001	0.040	<0.001	0.63	0.29	5.67
Protein production:								
Daily yield, g/d	1214	316	<0.001	<0.001	<0.001	0.079	0.11	258
Daily yield/ NEm-$_{MW}$, g/MJ	22.0	5.59	0.003	<0.001	<0.001	0.15	0.19	4.87
Daily yield/ NEm-$_{PM}$, g/MJ	22.2	5.49	<0.001	<0.001	<0.001	0.61	0.10	4.87
Fat plus protein production:								
Daily yield, g/d	2454	647	<0.001	<0.001	<0.001	0.081	0.16	521
Daily yield/ NEm-$_{MW}$, g/MJ	44.4	11.49	<0.001	<0.001	<0.001	0.15	0.29	9.79
Daily yield/ NEm-$_{PM}$, g/MJ	45.0	11.35	<0.001	<0.001	<0.001	0.59	0.15	9.79
Milk energy production:								
Daily yield, MJ/d	104.6	28.4	<0.001	<0.001	<0.001	0.036	0.22	22.2
Daily yield/ NEm-$_{MW}$, MJ/MJ	1.89	0.51	<0.001	<0.001	<0.001	0.077	0.37	0.42
Daily yield/ NEm-$_{PM}$, MJ/MJ	1.92	0.50	<0.001	0.003	<0.001	0.85	0.20	0.42
Fresh curd production:								
Daily yield, kg/d	5.65	1.70	<0.001	<0.001	<0.001	0.14	0.52	1.41
Daily yield/ NEm-$_{MW}$, g/MJ	102	30.41	<0.001	<0.001	<0.001	0.22	0.65	26.4
Daily yield/ NEm-$_{PM}$, g/MJ	104	30.14	<0.001	0.005	<0.001	0.64	0.45	26.4

[1] N = 791 cows, 351 purebred Holstein and 440 crossbreds)

[2] Parity= 1st, 2nd and ≥3rd lactation; DIM= days in milk classes: ≤ 60 days, 61 to 120; 121 to 180 days; 181 to 240 days; ≥ 241 days; GG= Genetic group: Holstein and crossbred cows.

Figure 1. Relationship between body weight (BW) measured using an electronic weighing scale (observed BW) and BW predicted on an independent validation data se using different body measures (heart girth, wither height and body length), parity and days in milk classes (n = 76, R^2 = 0.876).

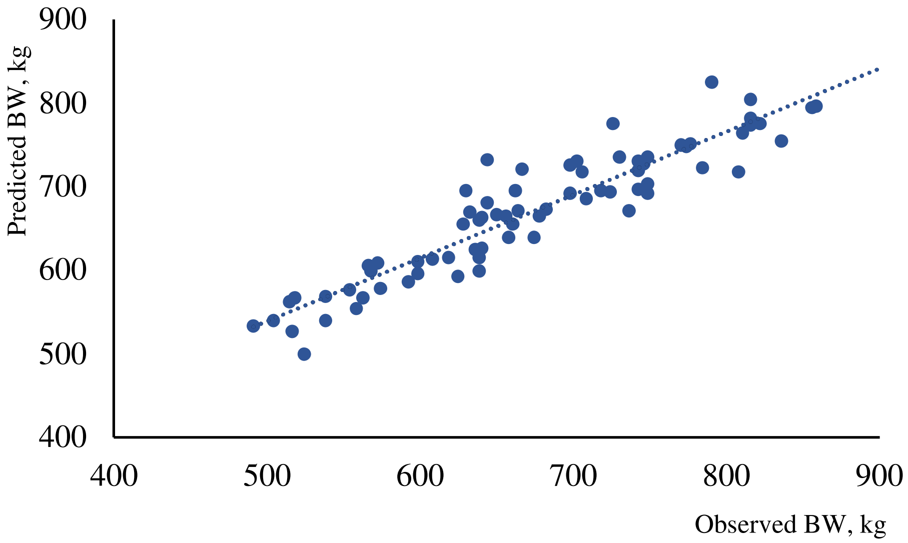


Figure 2. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for milk nutrients and energy content, curd yield, body condition score (BCS), body measures, predicted body weight (pBW), fat, protein and energy content of the body, net energy requirement for maintenance based on metabolic weight (NEm-$_{MW}$) or on body protein mass (NEm-$_{PM}$). Asterisks refer to the level of significance of differences between crossbred and purebred HO cows (** P<0.01, * P<0.05).

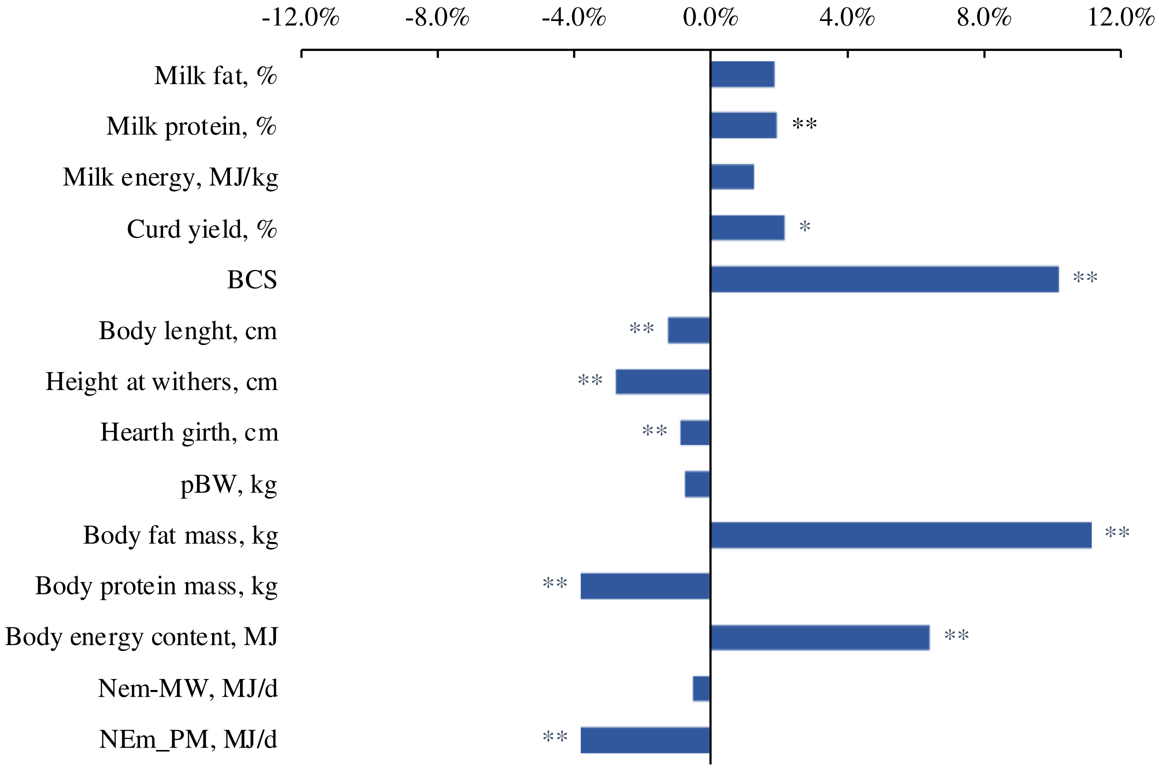
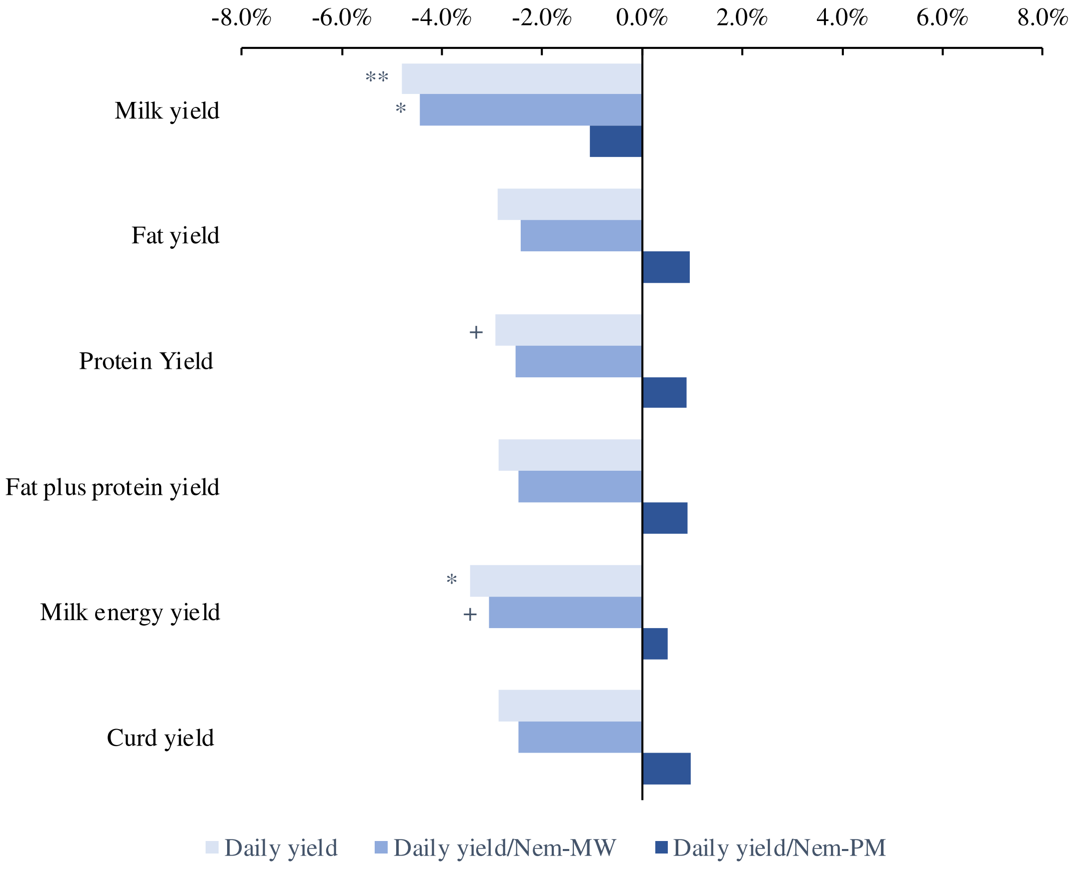


Figure 3. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for yield of milk, milk components, milk energy, and fresh curd in absolute value (Daily yield) or scaled on net energy requirement for maintenance based on metabolic weight (NEm-MW) or on body protein mass (NEm-PM). Asterisks refer to the level of significance of differences between crossbred and purebred HO cows (** P<0.01, * P<0.05, + P<0.10).



CHAPTER 4

Environmental footprint of Holstein Friesian and crossbred dairy cows using an individual Life Cycle Assessment approach

M. Piazza, M. Berton, S. Schiavon, G. Bittante, and L. Gallo

[1]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro (PD), Italy

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ABSTRACT

This study aimed at assessing the effects of a 3 breed crossbreeding program on the individual environmental footprint of cows using a LCA-derived approach. Data concerned 564 cows, 279 purebred Holstein (HO) and 285 crossbreds (CR), originated from a 3-breed crossbreeding program (PROCROSS) based on the rotational use of Viking Red, Montebèliarde and HO sires and kept in two dairy herds of northern Italy (224 and 340 cows/herd, respectively). The reference unit of the LCA model was the whole career of cows, from the birth to the farm gate. Data were collected at different levels: individual animal-based data referred to the whole life (birth, calving, dry and cull dates, 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, materials used). Data were used to compute or estimate dry matter intake, milk and milk components production, gross income (GI) and income over feed costs (IOFC) pertaining to the whole career of cows. An individual LCA-derived approach was used to compute global warming potential (GWP) and other emissions in the whole life of cows (acidification and eutrophication potential, AP and EP, respectively), and land occupation (LO) which have been associated to different functional units (cow in her whole life; d of life; kg of milk; kg of fat + protein; € of gross income and IOFC). Data were analyzed using GLM including the fixed effects of farm, genetic line (CR vs HO) and the interaction. Compared to HO, CR completed more lactations (+ 12%), had earlier first calving (-2 weeks), produced less milk per day of production (- 3%), but more fat plus protein per d of life (+ 4%), and tended to provide a greater IOFC (+ 7%). Taking into account the environmental footprint of cows, when compared to HO herdmates, CR tended to have greater emissions per cow in the whole life, similar emissions per d of life and from 4 to 5 % lower GWP, AP and EP per kg of fat plus protein produced in the whole life ($P \leq 0.05$). Also emissions per € GI tended to be lower in CR compared to HO cows. Also LO followed the same trend, and the use of land tended to be lower in CR compared to HO in most indicators considered. In conclusion, managing dairy cows within the crossbreeding scheme taken into account may be regarded as a strategy able to mitigate the emissions and to impriove the eìnvironmental footprint of dairy operations.

Key words: dairy cows; crossbreeding; environmental footprint; greenhouse gases

INTRODUCTION

In the last decade, increasing environmental sustainability and decreasing the footprint of the agri-food sector have become a global challenge. The livestock sector has been defined as a notable contributor to the anthropogenic emissions of greenhouse gases (GHG; Gerber et al., 2013; IPCC, 2022) and other pollutants, such as reactive nitrogen and phosphorus (Steinfeld et al., 2006; Bittman et al., 2014), as well as to the exploitation of limited resources such as fertile land (Mottet et al., 2017). In particular, cattle systems are the main contributor to the total livestock sector's GHG emissions, and the dairy sector contributed in 2015 with 1,7 million tonnes of CO_2 equivalent to the total emissions (FAO, 2019), that is almost 20% of livestock sector's GHG emissions (Gerber et al., 2013). To assess the environmental footprint related to a product or a service, the Life cycle assessment (**LCA**; ISO, 2006) has become a standard method (Sala et al., 2021) and it was extensively applied to evaluate the impacts associated with the dairy milk production (Baldini et al., 2017). The adoption of innovative breeding practices and technologies to improve production efficiency of dairy systems may help to reduce emission intensity (FAO, 2019). Several studies demonstrated that the increase in milk yield can mitigate the impact per unit of milk (Gerber et al., 2011; Cohn et al., 2014), mainly because of the dilution of the maintenance requirements on a greater amount of milk (Capper et al. 2009). However, further increments in the milk yield through breeding selection and improved husbandry practice could be difficult to obtain and not always profitable (Moallem, 2016). In addition, also improvements in functional parameters of dairy cows, such as reproductive traits, health status, and longevity, may affect the environmental impact associated with dairy production (Place and Mitloehner, 2010; Hristov et al., 2013; Llorch et al., 2017; Grandl et al., 2019). However, in the last 20 years, the genetic selection of high production dairy cow breeds, and firstly of Holstein Friesian (HO), has caused a decline in fertility, health and longevity (Dezetter et al., 2019; Hazel et al., 2020a). Within the breeding strategies, crossbreeding technique have been reported to benefit traits related to reproductive performances, fertility and health (Sørensen et al., 2008; Buckley et al., 2014; Hazel et al., 2020a), and it has the potential to be a more sustainable technique than HO purebreeding (Magne and Quénon, 2021). For this reason, during the last decade crossbreeding programs have gained attention among dairy stakeholders. In particular, within the intensive dairy farming systems, interest is growing in the 3-breed rotational system, named ProCROSS, using Viking Red (**VR**), Montbéliarde (**MO**), and HO breeds sires. This program has been reported to positively affect not only milk production traits, such as fat and protein content, technological properties and cheese making ability of milk (Malchiodi et al., 2014; Shonka-Martin et al., 2019a; Saha et al., 2020), but also feed efficiency parameters (Shonka-Martin et al., 2019b), short- and long-term profitability (Hazel et al., 2020b; Piazza et al., 2022b) and production efficiency-related parameters (Piazza et al., 2022a). So, crossbreeding seems to be a mating system able to affect both productivity and several animal rearing parameters, but effects of this strategy of utilization of genetic resources of animals on the environmental impacts of dairy systems have still never been quantified.

The assessment of the environmental footprint of livestock systems is generally performed at the farm level, i.e. taking the dairy farm as unit of observation of the phenomena. The intra-farm variability in terms of animal traits has usually not taken into account, since inventories are generally performed by considering farm-average data. However, the effect of the inter-individual variability could be notable, also in aspects fundamental in terms of environmental footprint analysis such as enteric methane production (Garsworthy et al., 2012). Moreover, if the comparison of the environmental footprint of crossbred and purebred cows is under consideration, cows of different genetic groups should be kept in the same herds and managed and fed in the same way. Therefore, in such approach the unit of observation should be the individual cow, rather than, as usual, the farm. The use of the individual dairy cow as the reference unit of LCA modelling 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 a low number of cattle heads involved. Since crossbreeding programs, such as the above-mentioned ProCROSS, involve the animal scale rather than the farm one, the individual dairy cow would be the most adequate level to evaluate the potential effect on the environmental footprint of this crossbreeding scheme.

Therefore, the aim of this study was to compare the environmental footprint of crossbred cows from a 3-breed rotational scheme using VR, MO and HO bulls with that of HO purebred herdmates using an LCA-derived methodology, considering the individual dairy cow as the reference unit and the whole dairy cow lifespan as the reference period. Crossbred cows have been taken as a mixture of generations and sire breeds representing the 3-breed rotational system, whereas the comparison of the sire breeds within rossbreds was outside the scope of the study.

MATERIALS AND METHODS

Origin of the data

The data originated from the study of Piazza et al. (2022a), involving 791 dairy cows kept on two farms located in the Italian Po valley: for each cow, information about the genetic group, milk composition and curd yield (test-day milk samples), and body size measurements were available. In both farms, mating scheme was managed according to the following sequence: VR semen was used on HO purebred cows to produce the 1st generation (F1) of VR × HO crossbred cows, then MO semen was used on F1 cows to obtain the 2nd generation (F2) of MO × (VR × HO) crossbred cows, and HO semen was used on the F2 cows to produce the 3rd generation (F3) of HO × [MO × (VR × HO)] crossbred cows. The sequence then started again with VR and so on. However, in one herd a second crossbreeding program, in which VR and MO were reversed in the sequence (MO-VR- HO), was also used - i.e. MO semen was used on the HO cows to produce the F1 MO × HO, which were inseminated with VR semen to produce the F2 crossbred cows, which were inseminated with HO semen to produce the F3 crossbred cows, then the sequence was then repeated using MO sires to obtain the 4th generation (F4) crossbred cows, and so on.

The two farms were representative of the two most important dairy systems in Italy: one herd was located on the Lombardia plains (province of Brescia), in the protected designation of origin (**PDO**)

Grana Padano hard cheese production area; the other herd B was located in the Emilia-Romagna region (province of Modena), in the PDO Parmigiano Reggiano hard cheese production area. Within each herd, HO and CR were kept in free stalls with cubicles, reared and milked together, and were fed the same total mixed ration. The composition of the diets fed in the two farms are reported in Table 1.

Data editing and data set preparation

To compute the environmental footprint associated to the individual cows, the starting dataset was enriched with additional information as follows. Firstly, data about the major events occurred during the lifetime of each dairy cow (dates of birth, calving, dry-off and culling or death) were collected. Secondly, data about milk production during the whole career (yield of milk, fat and protein) were acquired from the official milk recording system.

Data derived from the lifetime events and milk production in career were merged with the initial dataset by using the dairy cow as the common variable. To obtain the final dataset we have retained all cows that respected the following conditions:

- culled or dead within the end of April 2021 (date of end 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;
- with information of their full career (production, milk nutrient contents, calving and dry dates);
- measured for heart girth, wither height, body length and BCS.

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

Lifetime and milk production. With regard to the lifetime events data, the heifer period (first calving date minus birth date), longevity (culling or death date minus birth date) and herd life (culling or death date minus first calving date) were calculated per each cow. Moreover, for each lactation, the lactating period was equal to the total days in milk (**DIM**), whereas the dry period was calculated as following calving date minus dry-off date. The herd life lactating period resulted as the sum of all the lactating periods (from the first to the last lactation), whereas the unproductive days as the sum of all the dry periods plus the heifer period.

With regard to milk production, the lifetime milk yield (**MYlife**) has been computed as the sum of the milk actually yielded by each cow during all the lactations performed. Milk fat and protein yielded per lactation have been calculated by multiplying the milk yield of each lactation by the average milk fat and protein content of each lactation. The lifetime milk fat and protein production have been computed by summing up the relative productions obtained in each lactation. The milk, fat and protein yielded per day of life have been computed as the amount yielded in career of each milk component divided by the lifetime duration. Moreover, the curd yield at test-day was obtained according to the 9-MilCA method proposed by Cipolat-Gotet et al. (2016) and fully described for this sample of cows by Saha et al. (2020).

Mean body weight and BCS estimation during life and heifer period. The procedure used to develop the equation used for predicting body weight (**BW**) from body measures has been described and discussed in the paper of Piazza et al. (2022a). Briefly, data on body measures – height at withers, body length and heart girth - and body weights were collected from a subsample of 227 cows, and then used together with parity and stage of lactation information in a multiple regression model in order to obtain an equation able to predict the test day body weight of all cows.

In this study, we have taken the dairy cow in her whole lifetime as reference unit for the environmental footprint assessment. However, several cow-related information concerned test-day data collected once per cow (predicted body weight, BCS, fresh curd yield), which - prior to further analysis - needed to be scaled in order to be referred to the dairy cow herd life.

For this purpose, the average BW during the whole cows' lifetime, from the first calving to the culling/death date (**BWCOW**) and during the heifer period (**BWHEI**, from birth to the first calving) were estimated. These weights were necessary in order to estimate dry-matter intake (**DMI**), as an individual direct measure of this trait was not available, and knowledge of average BW allows to compute the net energy requirements for maintenance (**NEm**) (NASEM, 2021). The estimation of

BW_{COW} was performed as follows. As the test-day BW was collected on dairy cows in 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 which included the fixed effect of the combined factor farm (2 classes) x genetic group (2 groups: CR or HO) x parity (3 classes: 1, 2 and >2 parity) x DIM (3 classes: 1 to 100, 101 to 200 and > 200 days 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 ≤100 DIM and for > 200 DIM classes were calculated as the ratio between the LSM of the two classes and the LSM of the reference class (Table 2). Then, the test-day BW actually measured on each cow was multiplied by the corresponding adjustment coefficient to obtain DIM-adjusted BW for all the cows of the study.

Thereafter, a similar procedure was used to project the DIM-adjusted BW to the different orders of parity spent by each cow during her whole life. Consequently, DIM-adjusted BW was analysed with a second GLM which included the fixed combined effect of farm (2 classes) x genetic group x parity class (3 classes), providing a coefficient of determination of 0.45. The projection coefficients, within each farm and genetic group, were calculated by dividing the LSM of DIM-adjusted BW of each class of parity by the LSM of DIM-adjusted BW of all the other parity classes, in order to obtain a set of projection coefficients useful to estimate for each cow an average weight adjusted for DIM for all the lactations performed by each cow (Table 3). So, if a cow had just 1 lactation in her career, her test-day BW was adjusted just for DIM class, if necessary (cows actually weighted outside the DIM interval 100 to 200). If a cow had 2 lactations and was actually weighted during her second lactation, the average weight of cows in the second lactation was her test-day BW adjusted to the 100-200 DIM class, and this weight was multiplied by the coefficient of projection from parity 2 to parity 1 to predict the average BW adjusted to the 100-200 DIM class in lactation 1, and so on. We assumed that cows reached their mature weight at 3rd parity, so if a cow had more than three lactations, the average weight for lactation >3 was considered equal to the average weight in lactation 3.

Last, 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 BCS during the whole cows' life (**BCS_{COW}**) 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 ($BCS_{100\text{-}200d}$) and of 0.34 for the model run for projecting $BCS_{100\text{-}200d}$ to the lactations actually performed by each cow.

The BW_{HEI} was computed for each cow as the difference between the average BW at first calving, estimated by adjusting the average 1st parity weight to the DIM class ≤100 (the DIM class closest to 1st calving), using the coefficients of adjustment reported in Table 3, and the BW at birth, computed according to NASEM (2021).

Estimation of fresh curd yield during life. The lifetime fresh curd yield (CY) was computed on the basis of MYlife and the adjusted CY. The latter was obtained from the test-day curd yield with a procedure similar to that adopted for the estimation of BW_{COW}, explained in the previous paragraph. Firstly, DIM at test-day were classified in 5 classes: <60 d, from 61 to 120 d, 121 to 180 d, 181 to 240 d, over 240 d. The LSM of CY were obtained from a GLM model testing the effect of the interaction between farm (2 classes) and test-day DIM classes. As previously described for BW, the adjustment coefficients were computed as the ratio between the LSM of CY for the nth class of DIM and the LSM of CY for the third class of DIM, taken as reference. The adjusted CY for each dairy cow was computed as the test-day CY multiplied by the correspondent adjustment coefficient. Finally, the lifetime cheese production was computed as MYlife multiplied by the adjusted CY.

Estimation of net energy requirements. The net energy (NE) requirements were estimated separately for the different lifetime periods of each cow, namely heifer, lactating and dry periods. The total daily NE requirement was computed as the sum of the NE requirements for growth and pregnancy - according to the equations reported by IPCC (2019) - for milk production - based on NASEM (2021) - and for maintenance, computed using two different approaches. The NEm was at first computed according to NASEM (2021) equation, using BW_{COW} for cow (lactation and dry periods) and BW_{HEI} for heifer, converted in metabolic weight (**MW**), as follows:

$$\mathbf{NEm\_}_{\mathbf{MW}} \text{ (MJ/d)} = 0.418 \times (BW_{COW/HEI})^{0.75} \text{ (NASEM, 2021)}$$

Secondly, as cow's average daily NEm is primarily due to the lean tissues (Agnew and Yan 2000; NASEM, 2021), the mean fat and body protein mass were computed according to equations proposed for dairy cows by Fox et al. (1999), cited by NRC (2016), and specifically modified to be referred to BW and BCS on a scale of 1 to 5, with the resulting equations:

$$\text{Body fat mass } (\mathbf{pBfat}, \text{kg}) = (0.06171 \times BCS - 0.0308706) \times BW_{COW};$$

$$\text{Body protein mass } (\mathbf{pBprt}, \text{kg}) = (-0.01287 \times BCS + 0.170174) \times BW_{COW};$$

Therefore, $NEm\__{MW}$ was computed just for the subset of HO cows, and then divided to the body protein mass of each cow for the subset of HO cows. The number obtained (0.631 MJ/kg of body protein) was used as coefficient and multiplied by the pBprt of each cow (HO and CR) to calculate the NEm based on protein mass (**$NEm\__{PM}$**).

The rationale and the entire procedure supporting the calculation of $NEm\__{PM}$ is fully described and reported by Piazza et al. (2022a).

Estimation of the carcass weight and carcass value of the individual dairy cows. The BW of the individual cow at culling was assumed to be equal to the estimated BW at the last lactation performed by each cow (i.e. estimated BW at first, second or third lactation for cows culled during the first, second or third lactation or later). The dressing percentage (DP) per genetic group was derived by a sub-sample of cull cows (n = 286, 114 HO and 172 CR) for which the actual carcass weight and carcass value were available (Piazza et al., 2022b). For this subsample, first individual DP was calculated by dividing CW by the estimated BW at culling, and then DP were averaged by genetic group (average DP resulted 0.419 and 0.454 for HO and CR, respectively). Average DP within each genetic group (HO and CR) was multiplied by estimated BW at culling to obtain an individual-estimated CW. As internal validation, the estimated CW of the cows of the sub-sample used for calibrating average DP has been compared with the actual CW measured on cull cows at slaughterhouse (Piazza et al., 2022b), and the difference between the estimated and calculated CW averaged 1.7 ± 58.9 kg. The carcass unit price per genetic group, derived by the study of Piazza et al. (2022b), 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 of cull cows of the study.

Life cycle assessment

Goal and scope definition. The computation of the environmental footprint 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 to a HO purebred mating scheme on the environmental footprint of the dairy cows during their entire lifetime. As mentioned above, the reference unit of the LCA model was the individual dairy cow, showed in Figure 1. Although dairy production is multifunctional, as culled cows and calves exceeding replacement needs are typically aimed to beef production, they can nonetheless be considered by-products of the dairy milk activity. For this reason, the whole impact of the dairy operations has been allocated to the milk, with no further allocation to the surplus animals' co-productions. Moreover, three different types of functional units (FU; i.e. the unit to which the impact was referred) were adopted. The first type dealt with a temporal point of view, related to the lifespan of cows, taking the lifetime and the day of life as FUs. The second one dealt with a productive point of view, and the yield of one kg of raw milk, of milk solids (fat plus protein) and of fresh cheese were taken as FUs. The third one was related to an economic point of view, and one euro of gross income and the impact per one euro of income over feed costs (IOFC) were taken as FUs.

With regards to the typologies of impacts, four impact categories were assessed: global warming (GWP, kg CO_2-eq), acidification (AP, g SO_2-eq) and eutrophication (EP, g PO_4-eq) potentials, and land occupation (LO, m^2/y). Moreover, the system boundaries were set to include the impact due to the dairy cow management throughout its lifetime (from birth to sale or death), the rearing of the replacement heifer, the handling of their manure, the production of the on- and off-farm feedstuffs consumed by the dairy cow and its replacement heifer, the production and use of energetic sources and bedding materials.

Life cycle inventory. The inventory for each individual dairy cow was constructed on the collection of two different types of variables, individual-based (if referred to the individual dairy cow) or farm-based (equal to all the dairy cows reared in the same farm) (Figure 1). The inventory computation with respect to the individual-based variables was based on the edited dataset of the individual dairy cows, whereas the farm-based inventory data were collected through a farm questionnaire and physical dairy farm inspection. The farm data (common to all cows, regardless to genetic group) dealt with information about the ingredient composition of the rations fed to the lactating and dry cows and to heifers (Table 1), the production of the on-farm feedstuffs and the amount of materials (energy sources and bedding materials) consumed by the farm (Table 4). Since both farms did not make significant changes in the farm structure and management in the last 10 years, farm-based data were assumed representative of the dairy cows' management with respect to their lifespan.

Daily feed intake of lactating cows, dry cows and heifers was computed at individual level as the ratio between the daily net energy (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 the single feedstuffs weighed by their relative inclusion in the ration. Chemical data of the single feedstuffs were derived from Sauvant et al. (2007) and INRA (2019), except for compound concentrates, for which commercial labels were used. Nitrogen input-output animal flow for each animal category was computed according to the procedure proposed by Ketelaars and Van der Meer (1999) (Table 5). Excretion was computed as intake minus retention. Intakes were computed as feed intake x crude protein (CP) dietary content/6.25, whereas retention was computed as the sum of the retentions for milk (protein content of milk / 6.38), growth and pregnancy (retention coefficients per livestock category were derived from Ketelaars and Van der Meer (1999).

The impact computation regarding the different impact categories was performed as follows.

Impacts related to the different time periods in which the lifespan of the dairy cow was divided - heifer period and cow period, comprising one or more lactations distributed into the lactating and the dry phase – were calculated separately. These different contributions were summed up to compute the impact of each dairy cow during her whole life. To account for the contribution of each cow to her future replacement, an extra quota of emission was computed by multiplying the amount of emission related to the production of heifer by a coefficient equal to 1.2 (one replacement heifer including a 20% of mortality or discard) divided by the number of lactations actually performed by each cow (to account for differences in longevity of cows, which clearly affect the burden of emissions due to replacement needs). All the equations were reported in detail in tables 5, 6 and 7. Methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) were the greenhouse gases included in the computation of GWP. Methane emission due to enteric fermentation was estimated by using the equation proposed by Ramin and Huhtanen (2013), whereas the CH_4 and N_2O emissions occurred during the management of the animal manure (housing plus storage) were based on the protocol published by the Intergovernmental Panel on Climate Change (IPCC, 2019), as well as the N_2O emission due to the fertilisers applied to the farm agricultural area destined to produce on-farm feedstuffs. Acidification potential included the emission of ammonia (NH_3) and nitrogen oxides, both related to the N volatilization phenomenon. The computation of the N volatilized during the manure management phase was based on European Environmental Agency protocol (EEA, 2019), whereas that occurred in the field on IPCC (2019). Volatilized N compounds, depositing on the soil, contribute also to EP, together with the P-related compounds. Equations and factors derived from Nemecek and Kägi (2007) were applied to calculate the P loss during the on-farm feedstuffs production, whereas IPCC (2019) were used for N loss through leaching estimation. The impacts related to the purchased input (off-farm feedstuffs, energetic and bedding resources, but also the external agricultural input used in the on-farm feedstuffs production, such as chemical fertilizers, pesticides, seeds) were computed on the basis of impact factor derived from Ecoinvent v3.6 (Werter et al., 2016) and Agrifootprint v5.0 (Blonk Consultants, 2020) databases implemented in Simapro v9.3 software (see Table 7).

Life cycle impact assessment. Each compound emitted, associated to the impact category to which it can contribute, was converted in the common measure of the impact categories through the application of characterization factors (e.g., conversion of CH_4 to CO_2-eq about GWP computation). Characterization factors for GWP were derived from Mhyre et al. (2013), whereas for AP, EP and LO from CML-IA (Oers, 2016). Last, according to the different functional units defined above, the following environmental footprint metrics were computed: i) related to the temporal dimension: each impact category per cow (whole lifetime) and per day of life (intensity of emission discounted by the longevity of animals); ii) related to the production dimension: each impact category per unit of raw milk, of fat plus protein yielded, and of fresh cheese yielded in the whole lifetime; iii) related to the profitability dimension: each impact category per unit of gross income and impact per unit of income over feed costs (as IOFC may assume positive or negative values, it is necessary to reverse the ratio for expressing the level of emission).

Statistical analysis

Traits related to the lifespan and career periods, body weight and body composition, milk and milk components yields and all environmental footprint metrics were analysed with a GLM model (version 9.4; SAS Institute Inc., Cary, USA) which included the fixed effects of the genetic group (2 groups, HO or CR), of farm (2 classes, herd A and B) and of the interaction farm x genetic group. Effects was declared significant at $P < 0.05$ and tendential for $P > 0.05$ but ≤ 0.10.

RESULTS

Herd life, milk production, and body-related traits

Raw means, standard deviation, and results of ANOVA are given in Table 8, 9 and 10 for herd life traits, milk production traits and BW-related traits respectively. On average, cows calved for the first time after almost 24 mo (coefficient of variation, CV: 9%) from birth, performed little more than 3 lactations, with a notable variation among cows (CV: 42%), had a herd life of 1126 d, of which almost 85% spent in lactation and 15% in dry period, with an overall lifespan close to 1840 days. Herd significantly affected all herd life traits, whereas genetic group significantly influenced the age at first calving, the number of lactation and the overall amount of days of life spent in milk, and tended to affect the whole lifespan of cows. Compared to HO (Figure 2), CR cows calved earlier (-2.2%), performed more lactations (+11.3%), and had a longer herd life (+8.3%), which determined a nominal longer lifespan as well (+3.9%).

Regarding the milk production (Table 9), the herd life yields averaged nearly 31,000 kg of raw milk, 2170 kg of fat plus protein and 5200 kg of fresh curd, with a notable variability ranging between 46 to 48%. On the other hand, per day of life, the same yields averaged 16.1 kg, 1.1 kg, and 2.7 kg, respectively for raw milk, fat plus protein and fresh curd, with a variability ranging from 24% to 27%. Genetic groups performances' were significantly different for fat plus protein yield in the whole life ($P<0.05$) and tended to be different also when fat plus protein yield was expressed per day of life ($P=0.07$). In particular, compared to HO (Figure 3), CR cows evidenced a milk fat plus protein nearly +8% greater when related to the whole lifetime (+174 kg) and nearly + 4% greater when related to the day of life; also the milk and fresh curd yields during herd life and per day of life were nominally greater on CR compared to HO.

The predicted average herd life BW and BCS of cows were nearly 684 kg and 3.33 respectively, and estimated mean body fat and protein masses were 120 and 87 kg respectively. The estimated mean daily feed intake during the heifer, lactating and dry periods averaged nearly 8, 23 and 11 kg respectively. Cows had a value of carcass of nearly 632 € with a CV of 13%, providing a gross income of more than 16 K€ per cow and an income over feed costs around to 9 K€/cow. The effect of the genetic group significantly affected most traits, with the exception of average BW, DMI during lactation and income metrics. In particular (Figure 4), crossbred cows had greater BCS (+10%), body fat and body energy content (+13.8 and (+8.7%, respectively), and a minor body protein content (-2.3%) than their HO herdmates. Crossbred cows (Figure 5) had lower DMI during heifer period (-2%), whereas variation in DMI during dry period depended on the way of computation of NEm. Moreover, crossbred cows reached a higher value of carcass (+21%) and tended to provide higher value for gross income and IOFC.

Environmental impact categories

The raw means, standard deviation and P-values of the fixed effects tested for the impact categories considered are reported in Tables 11, 12 and 13: for each impact category metric are provided two set of values, obtained considering the values of NEm computed using the MW or the pBprt.

On average, the emissions generated during lifetime of cows were close to 39 000 kg CO_2-eq, 780 kg SO_2-eq and 280 kg PO_4-eq, respectively for GWP, AP and EP, whereas the overall land occupation related to lifetime of cows averaged nearly 49500 m^2. Similarly, the average emissions per d of life resulted nearly 21 kg CO_2-eq (GWP), 420 g SO_2-eq (AP) and 150 g PO_4-eq (EP), and the amount of land required was close to 26.5 m^2. While the genetic group did not affect the impact values for lifetime even considering different NEm (on MW and on pBprt), the impact values per day of life was affected by different genetic groups when computed using NEm based on pBprt, in particular CR cows showed lower impact values (from -1.4% to -1.6%, $P<0.05$) compared to purebred HO.

Regarding the production-related FUs (raw milk, milk solids and fresh curd, table 12), mean impact values per 1 kg raw milk resulted nearly 1.4 kg CO_2-eq (GWP), 27 g SO_2-eq (AP), 10 g PO_4-eq (EP), and 1.7 m^2 (LO). When referred to 1 kg milk solids and 1 kg fresh curd, the mean impact values were 14.3 and 6.1 times greater than those expressed as raw milk respectively, with any difference between impact categories. For this reason, the CVs related to impact values per unit of milk solids and fresh curd were equal to that obtained for raw milk. Compared to HO (Figure 7), CR cows had nominal lower impact values per unit of raw milk and lower impact values for unit of milk solids, which were significantly lower when computed using NEm_{PM} (from -4.5% to -5.0%, $P<0.05$). Moreover, considering NEm_{PM}, a tendential decrease of the impact values for all the impact categories was observed for CR dairy cows with respect to HO ones also referring to the fresh crud product (from -3.3% to -4.4%, $P<0.10$).

Considering the impact metrics scaled on income traits (Table 13), statistical analysis did not evidence differences between genetic groups when emissions where computed using NEm based on MW, whereas differences reached the statistical significance when emissions where computed using NEm based on body protein mass. Indeed, CR cows evidenced a 3.5 to 4% lower impact per € of GI than purebred HO (Figure 7a) and provided a nearly 7% greater IOFC per unit of impact (Figure 7b).

DISCUSSION

Herd life, milk production and body related traits

The first part of this study regarded the preparation of the data useful for the computation of the environmental impact categories. This part analyzed a set of variables aimed at depicting the performances in the whole career of cows. In this study, compared to purebred HO cows, CR cows were found to have a longer productive career and lifespan. Crossbred cows are usually reported to have lower milk production but higher or comparable milk fat and protein contents compared to HO cows (Malchiodi et al., 2014; Shonka-Martin et al., 2019a; Saha et al., 2020). However, the longer herd life of CR in this study could have affected the lifetime milk production and the fat plus protein yields, since milk production of CR were greater in values and fat plus protein was significantly higher than HO herdmates. Also Heins et al. (2012) reported longer herd life for Scandinavian Red and MO × HO crossbreds compared with their purebred HO herdmates, and similar results were reported by Clasen et al. (2017) on Danish VR × HO crossbred cows in their whole career, from first calving to the fifth lactation, compared with their HO herdmates.

More days of herd life means more time to accumulate production and incomes from production. In this study CR cows tended to provide higher gross income and IOFC than HO cows. Shonka-Martin et al. (2019b) reported higher values of IOFC for CR cows derived from the same 3-breed rotation crossbreeding scheme, related to a lower DMI of CR compared to HO cows during the lactation period. In our study, IOFC was nominally greater for CR than HO cows, but the DMI of the lactating cows was comparable, although DMI for the heifer period was significantly lower. Moreover, the DMI for the dry period of CR cows resulted higher when referred to the MW, but lower if referred to the Bprt mass. In the study of Piazza et al. (2022a), different metrics have been compared to estimate production efficiency of cows, suggesting that referring production ability of cows to body composition and energy measures might be a more effective method to evaluate production efficiency. Shonka-Martin et al. (2019a) reported a similar suggestion in their study on 3-breed rotational CR cows in their first 150 d of lactation stating that CR cow had a greater feed efficiency than purebred HO cows due to the differences in the dry matter intakes and energy requirements of cows. As more energy is required to maintain body protein than fat mass (DiCostanzo et al., 1990), the different body composition of cows becomes relevant in the computation of the energy requirements, and also in the computation of the related variables, such as IOFC and the impact categories. The results of this study confirmed that CR cows had different body composition compared to HO cows, evidencing a higher BCS, greater Bfat and lower Bprt content but similar BW than purebred HO cows. These results are consistent with the results of previous studies on cows derived from the considered mating scheme (Hazel et al., 2017a; Shonka-Martin et al., 2019a; Hazel et al., 2020).

Crossbred cows had generally better body conditions compared to HO cows (Saha et al., 2018; Hazel et al., 2020), exhibiting a higher BCS, as confirmed also from this study. Hazel et al. (2021) compared the lifetime profit of purebred HO to 3-breed rotational crossbred cows, and they found that the revenue for the culled cows was greater (+16%) for the combined 3-breed crossbreds, in particular for MO sired crossbred cows. Moreover, Piazza et al. (2022) recently compared carcass attributes of 3-breed rotational crossbred cows to those of purebred HO cows, kept in the same farm, considering carcass weight, value and fleshiness and fatness scores. They found CR outperformed HO cows for all the traits considered, reaching a total carcass value of 100€ greater than purebred HO cows. In accordance with previous studies' results, compared to their HO herdmates, CR cows considered in this study reached a 21% greater cull cows value, which means about +115 € more revenue for the cow at the end of their career (Figure 5).

Environmental footprint

The main part of this study analysed the environmental footprint of two different genetic types (CR and HO) reared in the Italian intensive dairy system, adopting three different typologies of functional units (temporal, productive, economic) and two different methods to compute the dairy cows NE requirement for maintenance. The results obtained in this study, with respect to the environmental footprint, evidenced that CR dairy cows had the potentiality to reduce the environmental burden associated with the dairy milk production. Moreover, this potential reduction could be observable with respect to the different impact categories assessed (emission-related and resource-related). However, the choice of the FU and of the NEm computation methods could modify the degree of this mitigating potentiality.

Among the different FU adopted in this study, the results expressed per unit of milk solids demonstrated an interesting robustness to the NEm computation method chosen. In fact, CR dairy cows evidenced a lower impact per unit of milk solids with both NEm methods, although with a greater extent when NEm_{PM} was used. This result can be related to the combination of a greater milk fat plus protein production in herd life (denominator of the impact values) and similar herd life impact (numerator) associated with CR vs HO dairy cows. On the other hand, milk fat plus protein was the unique variable adopted as FU for which a significant difference between CR and HO dairy cows was observed, although CR dairy cows showed better absolute values with respect to all the other FUs (lifetime, raw milk, fresh curd, gross income and IOFC). In this sense, the reduction observed for CR dairy cows with respect to HO ones when adopting NEm_$_{PM}$ and referring the impact to life day and gross income was probably related to the modification in the lifetime impact values, that was lower when using NEm_$_{PM}$ than using NEm_$_{MW}$ (see Figure 6). The abovementioned decrease in DMI with respect to both the dairy cow periods (lactating and dry) observed for CR vs HO dairy cows was probably the main driver of the reduction in the lifetime impact values. About IOFC, the adoption of NEm_$_{PM}$ instead of NEm_$_{MW}$ altered not only the estimated feed intake, but also the related feed cost, increasing the IOFC of CR with respect to that computed for HO dairy cows, altering both the numerator and the denominator of the impact categories. For this reason, the method which NEm is computed with has to be considered a focus point in the dairy cows impact assessment.

The mitigating effect of increasing the output yield per animal has been well established in literature (Gerber et al., 2011; Lorenz et al., 2019). However, further increases in milk yields, starting from the high productive level already achieved (see Table 2), could be difficult to obtain and not always profitable (Moallem, 2016). Consequently, the longer herd life observed for CR compared to HO dairy cows gained interest. The potentiality of herd life duration to mitigate the environmental impact of milk production has already been explored (Grandl et al., 2019), although involving small dairy sample and only GWP category. The effect of a longer herd life could be observed at different scale. A first effect is related to the decrease in the unproductive part of dairy cow lifetime associated to greater herd life, i.e. days when animals consumed, generating impacts, but not produced. In these terms, the results were in accordance with the mitigating effect of diluting the maintenance animal requirements (Hristov et al., 2013) but this dilution was found with respect to diluting the animal requirements associated with unproductive life. The second one is related to the decreased need to rear replacement heifers, as fewer cows would be culled in a reference period of time.

The decrease in the replacement rate has been proven to have mitigating effects on the environmental footprint of dairy production, as young animals consumed feed resources, that must be produced, emitted enteric methane, and excreted nutrients (Hristov et al., 2013; Knapp et al., 2014). Enlarging the scale of analysis, a possible trade-off deals with the decrease in the meat provision from culled cows. Although not directly related to milk production, this trade-off has to be assessed as, in a steady-state level of the beef demand, this deficiency in beef output would be covered by an increase in the beef provision from suckler cow-calf systems, whose environmental footprint has been demonstrated to be greater than that associated with dairy-derived beef (De Vries et al., 2015). Thus, the mitigating effect of increasing the dairy cows' herd life could not be achieved (Vellinga and de Vries, 2018). However, this trade-off could be counterbalanced by three different factors. Firstly, CR culled cows' carcasses had a better conformation of HO ones (Piazza et al., 2022), improving the qualitative provision of beef co-products from dairy cows. Secondly, the use of beef semen to inseminate dairy cows can increase the beef output from dairy herds (Holden and Butler, 2018). Thirdly, the European consumers' attitude and willingness to pay for beef products have been shifting towards a supply from more sustainable production systems (Dudinskaya et al., 2021), so a reduction in the supply of dairy-beef would not automatically be covered by a production increase derived from beef systems associated with a greater environmental footprint. Furthermore, as an high culling rate could be considered an indicator of low animal welfare (Oltenacu and Broom, 2010), a decrease in culling rate could increase the social acceptance of dairy farming (Cardoso e al., 2016)

Individual dairy cow LCA approach

The application of the LCA methodology is usually performed to assess the environmental footprint of a product at the farm scale (McAuliffe et al., 2016; Baldini et al., 2017). In fact, the farm is the production unit in which the data needed for the inventory analysis is primarily collected and stored and the unit in which decisions aiming to mitigate the environmental footprint can be made and the LCA results can be used for communication to the market, consumers, and policymakers (e.g.

to produce an Environmental Product Declaration, EPD). However, the farm scale can have some limitations as well, such as the incapacity to consider the relationship between the farm and the local territory context, a limitation to which the territorial LCA aims to resolve (Loiseau et al., 2012), or the exclusion of the intra-farm variability due to the diversity of the animals reared.

The use of the individual animal scale for LCA analysis could be greatly data-demanding, as data has to be collected for each animal instead of collecting the average data at farm level. However, the few studies that have applied this scale to analyse the carbon footprint of dairy (Grandl et al., 2018; McAuliffe et al., 2018) highlighted that the individual animal scale could give deeper insights in the assessment of the 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 afforded to include in the environmental footprint assessment the potential differences between the CR and HO dairy cows in terms of the number of lactations, length of the herd life, amount of milk solids production, body composition, and economic value of the carcasses. On the other hand, data about farm organisation, manure management system and feedstuff production were obtainable only at farm scale, and must therefore be considered as management factors common to all cows in the herd. 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 the data availability. In this sense, farms implementation of precision livestock practices (Pahl et al., 2016) to monitor at individual level important variables such as the feed intake could enhance the power of the individual LCA models to capture the differences between animals and, at a higher level, genetic groups, and help to make decisions at farm level intended to decrease the environmental footprint associated to dairy production.

CONCLUSIONS

The results of this study showed that the crossbreeding program investigated affected positively several individual traits of cows, acting also on the environmental impacts of the cows. In general, compared to their purebred HO herdmates, 3-breed rotational crossbred cows had longer herd life (+4%), ended more lactations (+12%) and spent more day in milk (+8%). Moreover, they produced more fat plus protein during their herd life (+8%), had greater BCS (+10%) and reached a greater value of cull cows (+21%), achieving a greater lifetime gross income (+6%, namely +900 €/cow) with respect to the performances of their purebred HO herdmates. Regarding the environmental impacts, compared to HO cows, CR had comparable emissions per cow during their herd life, per day of life and per kg of milk produced, but lower emissions per kg of fat plus protein and per kg of fresh curd (-3.5 and -4%). In conclusion, this 3-breed rotational crossbreeding scheme can be regarded as an effective strategy to mitigate the emissions of GHGs and other pollutants of dairy cows reared in an intensive farming system.

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TABLES AND FIGURES

Table 1. Descriptive statistics of ingredients composition of the rations, and of their chemical composition, fed to each animal category (lactating cows, dry cows, heifers) in the two farms.

Variable	Farm A			Farm B		
	Lactating cow	Dry cow	Heifer	Lactating cow	Dry cow	Heifer
Ingredient composition, %						
Maize silage	20.6	11.6	3.2			
Sorghum silage	7.7		10.8			
Wheat silage	2.6		0.1			
Maize ears silage	19.3					
Grass silage	2.9	14.6	7.2			
Hay	0.3	7.7		10.4	48.1	19.5
Alfalfa hay	2.6		1.5	26.8	2.4	0.7
Wheat straw			4.3			
Maize flour	5.5		0.3	28.6	1.9	3.5
Barley grain				10.1	1.2	0.2
Sunflower meal			4.7			
Molasses	4.6		0.2			
Compound feed 1	24.4		1.3			
Compound feed 2	9.2		0.4			
Compound feed 3		3.1	0.7			
Compound feed 4				14.5	3.8	3.2
Compound feed 5				1.9	2.9	
Compound feed 6				1.2		
Compound feed 7				0.1	1.0	
Chemical composition[1]						
GE, MJ/ kg DM	18.16	16.65	17.4	18.15	17.57	18.04
DE, MJ/ kg DM	14.19	11.23	11.73	12.3	10.3	11.76
NE, MJ/ kg DM	7.23	5.46	5.88	6.8	5.43	5.81
CP, % DM	16.01	10.59	12.87	17.32	11.64	14.04
CF, % DM	12.36	27.47	27.87	15.49	28.95	24.57
EE, % DM	4.57	3.59	2.91	3.34	2.88	3.17
Ash, % DM	3.66	9.53	7.62	6.65	9.22	7.98
NDF, % DM	25.66	49.52	55.44	31.34	59.23	50.36
Starch, % DM	28.35	11.69	5.22	26.89	3.53	13

P, % DM	0.34	0.48	0.26	0.37	0.32	0.35
NSC, % DM	50.10	26.76	21.15	41.35	17.03	24.44
OMD, % DM	79.04	77.37	71.85	69.74	62.87	66.6

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

Table 2. Coefficients of adjustment of the test day body weight (BW) of the individual cows to obtain a BW adjusted at the 100-200 class of days in milk.

Farm × genetic group[1]	Parity	Class of days in milk 0-100	 100-200	 > 200
Farm A, CR				
	1	1.056	1.000	0.984
	2	1.036	1.000	0.966
	3+	1.037	1.000	0.982
Farm A, HO				
	1	1.048	1.000	0.958
	2	1.019	1.000	0.945
	3+	1.087	1.000	0.984
Farm B, CR				
	1	1.000	1.000	0.950
	2	1.032	1.000	0.988
	3+	1.032	1.000	1.000
Farm B, HO				
	1	1.000	1.000	0.971
	2	1.024	1.000	0.951
	3+	1.040	1.000	0.937

[1] Genetic group: CR= crossbreds, HO= Holstein.

Table 3. Coefficient of projection of the body weight (BW) of cows adjusted to the 100-200 class of day in milk, to obtain the projected BW at different parity orders.

Parity x±1 vs parity x	CR[1]	HO
Farm A		
From 1 to 2	1.150	1.092
From 1 to 3	1.150	1.197
From 2 to 1	0.869	0.915
From 3 to 1	0.870	0.835
From 2 to 3	1.000	1.096
From 3 to 2	1.000	0.912
Farm B		
From 1 to 2	1.139	1.065
From 1 to 3	1.245	1.155
From 2 to 1	0.878	0.939
From 3 to 1	0.803	0.866
From 2 to 3	1.094	1.084
From 3 to 2	0.914	0.922

[1] CR=crossbreds, HO=Holstein.

Table 4. Descriptive statistics of production inputs and yields for each feedstuff produced on-farms.

Crop	Product	Area	Fertilisation, mineral	Fertilisation, manure	Fertilisation, mineral	Fertilisation, manure	Pesticides	Yield
Unit		ha	kg N/ha	kg N/ha	kg P/ha	kg P/ha	kg/ha	q DM/ha
Farm A								
Maize	Maize silage	51	18	413	0	258	19	214
Maize	Maize ears silage	28	28	390	0	244	19	152
Grass	Grass silage	13	0	182	0	114	0	78
Grass	Hay	39	0	405	0	253	0	80
Wheat	Wheat silage	3.5	0	301	0	188	0	161
Sorghum	Sorghum silage	27	2	283	0	177	0	158
Alfalfa	Alfalfa hay	11	0	57	0	36	0	101
Farm B								
Barley	Barley grain	82	84	152	0	101	2	64
Grass	Hay	1	0	121	0	76	0	80
Alfalfa	Alfalfa hay	67	24	170	57	107	0	90

Table 5. Equations for the computation of nitrogen input-output flow for each animal category according to Ketelaars and Van der Meer (1999).

Variable	Unit	Acronym	Formula	Type
Dairy cow, herd life				
Nitrogen intake	kg	N_int_dairy	N_int_MC+N_int_DC	individual
Nitrogen retention, in milk	kg	N_ret_milk_dairy	prtlife/6.25;	individual
Nitrogen retention, in body weight increase	kg	N_ret_BW_dairy	(BW$_{\mathrm{COW}}$- BW$_{\mathrm{HEI}}$)*0.025;	individual
Nitrogen retention, during pregnancy	kg	N_ret_preg_dairy	(BW$_{\mathrm{BIRTH}}$*0.22/6.25)*(lact_n-1)	individual
Nitrogen retention	kg	N_ret_dairy	N_ret_milk_dairy + N_ret_BW$_{\mathrm{COW}}$ + N_ret_preg_dairy	individual
Nitrogen excretion	kg	N_excr_dairy	N_int_dairy-N_ret_dairy	individual
Heifer, from birth to the first calving				
Nitrogen retention, in milk	kg	N_ret_milk_HEI	0	individual
Nitrogen retention, in body weight increase	kg	N_ret_BW_HEI	(BW$_{\mathrm{HEI}}$-BW$_{\mathrm{BIRTH}}$)*0.027	individual
Nitrogen retention, during pregnancy	kg	N_ret_preg_HEI	(BW$_{\mathrm{BIRTH}}$*0.22/6.25)	individual
Nitrogen retention	kg	N_ret_HEI	N_ret_milk_HEI+N_ret_BW$_{\mathrm{HEI}}$+N_ret _preg_HEI	individual
Nitrogen excretion	kg	N_excr_HEI	N_int_HEI-N_ret_HEI	individual

Table 6. Equations for the computation of the emissions due to enteric fermentation and manure management production stage

Variable	Unit	Acronym	Formula	Tipo	Reference
Enteric fermentation					
CH$_4$	L/head/d	CH$_4$_ent_L	-64+26*DMI-0.61*(DMI-12.5)^2+0.25*OMD*10-66.4*EE/100*DMI-45 *(NSC/(NDF+NSC))	mixed	Ramin and Huhtanen, 2013
			DMI = dry matter intake, kg/head/day; OMD = organic matter digestibility of diet, %; EE = fat of diet, %; NSC = non fibre carbohydrate, %; NDF = neutral detergent fiber.		
	kg/head/d	CH$_4$_ent	0.716*CH$_4$_ent_L/1000	mixed	
Manure management					
CH$_4$	kg/head/d	CH$_4$_man	VS*(Bo(T)*0.67*∑ (MCF$_S$ /100) * MS$_S$	mixed	IPCC (2019)
			VS = (GEI*(1-DE/100)+(0.04 *GEI))*((1-ASH)/GE_DM)	mixed	Tier 1-2
			GEI: Gross Energy, MJ/day; DE: diet digestibility, %; ASH: ash content of manure; GE_DM: Gross Energy MJ/kg DM, Bo(T) = m^3 CH$_4$ / kg of VS excreted (0.24 cow, 0.18 heifer); MCF$_S$: methane conversion factor for manure management system S (Solid = 0.02, Slurry = 0.14); MS$_S$: fraction of livestock category handled using manure management S (50% solid; 50% slurry).		
N$_2$O direct	kg/head	N$_2$O_manu_dir	(N_excreted*0.005)*(44/28)	mixed	IPCC (2019) Tier 2
Nitrogen volatilisation		TAN_solid	(N_excr+N_bedding)*MS_solid*0.6		EEA, 2019
			N_bedding (kg) = straw (kg/head)*0.08/6.25		
		TAN_slurry	N_ excreted *MS_slurry*0.6		
		N_vol_solid_housing	TAN_solid*0.08		
		N_vol_slurry_housing	TAN_slurry*0.24		
		TAN_solid_storage	TAN_solid-N_vol_solid_housing		
		TAN_solid_storage	TAN_slurry-N_vol_slurry_housing		
		N_vol_solid_storage	TAN_solid_storage*0.32		

		N_vol_slurry_storage	TAN_slurry_storage*0.32		
N volatilised from manure management	kg/head	N_vol	N_vol_solid_housing+N_vol_solid_storage+N_vol_slurry_housing+N_vol_slurry_storage	mixed	
N_2O indirect	kg/head	N_2O_manu_indir	N_vol*0.01*(44/28)	mixed	IPCC (2019) Tier 2

Table 7. Equations for the computation of the emissions due to the on-farm crop production

Variable	Unit	acronym	Formula	Tipo	Reference
N_2O direct	kg	N_2O_dir_crop	(FSN + FON + FCR) * EF * 44 / 28 FSN = annual amount of synthetic fertiliser N applied to soils, kg N; FON = annual amount of animal manure, kg N; FCR = annual amount of N in crop residues, kg N; EF = 0.01 kg N-N_2O / kg N applied.	farm	IPCC, 2019
Nitrogen volatilisation		N_vol_crop	FSN × 0.11 + FON × 0.21	farm	IPCC, 2019
N_2O indirect, from N volatilised	kg	N_2O_indir_crop	N_vol_crop × 0.01 × 44 / 28	farm	IPCC, 2019
NO_3 leaching	kg	N_leach	(FSN + FON + FCR) × 0.24	farm	IPCC, 2019
N_2O indirect, form N leaching	kg	N_2O_indir_leach	N_leach * 0.011 * 44 / 28	farm	IPCC, 2019
P (kg) leaching	kg/ha		0.07	farm	Nemecek and Kagi, 2007
P (kg) run-off	kg/ha		0.175*[1+0.2/80*mineral P_2O_5 (kg/ha)+0.4/80*manure P_2O_5 (kg/ha)+0.7/80]	farm	Nemecek and Kagi, 2007

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Table 8. Raw means, standard deviation (SD) and results of ANOVA for herd life traits of 564 cows (279 purebred Holstein and 285 crossbred cows).

Variable	Means	SD	ANOVA P-values			RMSE[2]
			Herd	Genetic group[1]	Herd × Genetic group	
Age at first calving, d	715	64	<0.01	<0.01	>0.1	1.27
Number of lactations	3.15	1.33	<0.01	<0.01	>0.1	52
Lifespan, d	1841	478	<0.01	0.09	>0.1	388
Days in milk, d	955	394	<0.01	0.03	>0.1	93
Dry days, d	171	107	<0.01	>0.1	>0.1	113
Unproductive days, d	887	113	0.01	>0.1	>0.1	472

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

Table 9. Raw means, standard deviation (SD) and results of ANOVA for lifetime production of milk, fat plus protein and fresh curd yield of 564 cows (279 purebred Holstein and 285 crossbred cows).

Variable	Means	SD	ANOVA P-values			RMSE[2]
			Herd	Genetic group[1]	Herd × Genetic group	
Lifetime production, kg/cow:						
Milk yield	31140	14484	>0.1	>0.1	>0.1	14459
Fat plus protein yield	2168	996	>0.1	0.05	>0.1	993
Fresh curd yield	5185	2494	>0.1	>0.1	>0.1	2495
Production per day of life, kg/d:						
Milk yield	16.13	3.96	>0.1	>0.1	>0.1	3.96
Fat plus protein yield	1.12	0.27	>0.1	0.07	>0.1	0.27
Fresh curd yield	2.69	0.73	0.04	>0.1	>0.1	0.73

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

Table 10. Raw means, standard deviation (SD) and results of ANOVA for the average lifetime predicted body weight (pBW), body condition score (BCS), predicted body fat (pBfat) and protein content (pBprt), predicted total body energy content (pBEn), estimated dry matter intake (DMI) during heifer, lactation and dry periods and income over feed cost computed using net maintenance energy requirement (NEm) based on metabolic weight ($IOFC_{MW}$) or on body protein mass ($IOFC_{PM}$), and cull cows' value and gross income.

Variable	Mean	SD	ANOVA P values			RMSE[2]
			Herd	Genetic group[1]	Herd × Genetic group	
Body attributes:						
pBW, kg	684	60.1	<0.01	>0.1	0.04	58.9
BCS	3.33	0.37	<0.01	<0.01	<0.01	0.31
pBfat, kg	120	23.4	<0.01	<0.01	<0.01	21.0
pBprt, kg	87	6.6	>0.1	<0.01	>0.1	6.5
pBEn, MJ/kg	6667	990	<0.01	<0.01	<0.01	908
DMI computed on NEm based on MW:						
DMI heifer, kg/d	8.00	0.59	<0.01	<0.01	<0.01	0.55
DMI lactation, kg/d	22.89	1.92	>0.1	>0.1	>0.1	1.93
DMI dry, kg/d	10.88	0.77	<0.01	<0.01	>0.1	0.75
DMI computed on NEm based on Bprt:						
DMI lactation, kg/d	22.78	2.02	>0.1	>0.1	>0.1	2.02
DMI dry, kg/d	10.76	0.84	<0.01	<0.01	>0.1	0.83
Economic traits, €/cow:						
Cull cows value	632	84.6	0.01	<0.01	>0.1	59.1
Gross income	16357	8155	<0.01	>0.1	>0.1	7340
$IOFC_{MW}$	8993	5994	<0.01	>0.1	>0.1	5171
$IOFC_{PM}$	9011	5973	<0.01	>0.1	>0.1	5154

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

Table 11. Raw means, standard deviation (SD) and results of ANOVA for the impact categories associated with lifetime and per day of life computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt).

Variable	Unit	Mean	SD	ANOVA P-value			RMSE[2]
				Herd	Genetic group[1]	Herd × Genetic group	
NEm based on MW							
Lifespan impact categories[3]:							
GWP	kg CO_2-eq	39026	10911	<0.01	>0.1	>0.1	10707
AP	kg SO_2-eq	781	245	<0.01	>0.1	>0.1	233
EP	kg PO_4-eq	281	107	<0.01	>0.1	>0.1	86
LO	m^2	49644	17740	<0.01	>0.1	>0.1	15390
Impact categories per day of life:							
GWP	kg CO_2-eq/d	21.2	1.4	<0.01	0.10	0.01	1.4
AP	g SO_2-eq/d	420	40	<0.01	>0.1	<0.01	31
EP	g PO_4-eq/d	150	31	<0.01	>0.1	<0.01	11
LO	m^2/d	26.6	4.3	<0.01	>0.1	0.01	2.2
NEm based on Bprt							
Lifespan impact categories:							
GWP	kg CO_2-eq	38957	10971	<0.01	>0.1	>0.1	10763
AP	kg SO_2-eq	779	247	<0.01	>0.1	>0.1	235
EP	kg PO_4-eq	280	108	<0.01	>0.1	>0.1	87
LO	m^2	49542	17874	<0.01	>0.1	>0.1	15502
Impact categories per day of life:							
GWP	kg CO_2-eq/d	21.1	1.5	<0.01	0.01	0.02	1.41
AP	g SO_2-eq/d	419	42	<0.01	0.02	0.01	32
EP	g PO_4-eq/d	150	32	<0.01	0.05	<0.01	12
LO	m^2/d	26.5	4.4	<0.01	0.07	0.01	2.3

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

[3] GWP: global warming potential, AP: acidification potential, EP: eutrophication potential, LO: land occupation

Table 12. Raw means, standard deviation (SD) and results of ANOVA for the impact categories associated with the yield of raw milk, fat plus protein and fresh curd computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt).

Variable	Unit	Mean	SD	ANOVA P-value			RMSE[2]
				Herd	Genetic group[1]	Herd × Genetic group	
NEm based on MW							
Raw milk[3]							
GWP	kg CO_2-eq/kg	1.40	0.39	>0.1	>0.1	>0.1	0.39
AP	g SO_2-eq/g	27.6	6.9	<0.01	>0.1	>0.1	6.8
EP	g PO_4-eq/g	9.80	2.93	<0.01	>0.1	>0.1	2.27
LO	m^2/kg	1.74	0.46	<0.01	>0.1	>0.1	0.77
Fat plus protein							
GWP	kg CO_2-eq/kg	20.0	5.4	>0.1	0.06	>0.1	5.4
AP	g SO_2-eq/g	394	95	<0.01	0.09	>0.1	93
EP	g PO_4-eq/g	140	41	<0.01	0.07	>0.1	31
LO	m^2/kg	24.8	6.3	<0.01	0.05	>0.1	5.5
Fresh curd							
GWP	kg CO_2-eq/kg	8.48	2.54	0.04	>0.1	>0.1	2.54
AP	g SO_2-eq/g	167	47	<0.01	>0.1	>0.1	45.3
EP	g PO_4-eq/g	60.0	20.1	<0.01	>0.1	>0.1	15.7
LO	m^2/kg	10.6	3.2	<0.01	>0.1	>0.1	2.74
NEm based on Bprt							
Raw milk							
GWP	kg CO_2-eq/kg	1.39	0.39	>0.1	>0.1	>0.1	0.39
AP	g SO_2-eq/g	27.5	6.8	<0.01	>0.1	>0.1	6.7
EP	g PO_4-eq/g	9.78	2.91	<0.01	>0.1	>0.1	2.24
LO	m^2/kg	1.73	0.46	<0.01	>0.1	>0.1	0.40
Fat plus protein							
GWP	kg CO_2-eq/kg	19.9	5.4	>0.1	0.03	>0.1	5.4
AP	g SO_2-eq/g	392	94	<0.01	0.02	>0.1	92
EP	g PO_4-eq/g	140	40	<0.01	0.02	>0.1	30
LO	m^2/kg	24.7	6.3	<0.01	0.02	>0.1	5.4
Fresh curd							
GWP	kg CO_2-eq/kg	8.46	2.53	>0.1	0.08	>0.1	2.52
AP	g SO_2-eq/g	167	47	<0.01	0.06	>0.1	45
EP	g PO_4-eq/g	59.6	20.0	<0.01	0.08	>0.1	15.6
LO	m^2/kg	10.5	3.2	<0.01	0.07	>0.1	2.7

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

[3] GWP: global warming potential, AP: acidification potential, EP: eutrophication potential, LO: land occupation

Table 13. Raw means, standard deviation (SD) and results of ANOVA for the impact categories associated with gross income and income over feed cost computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt).

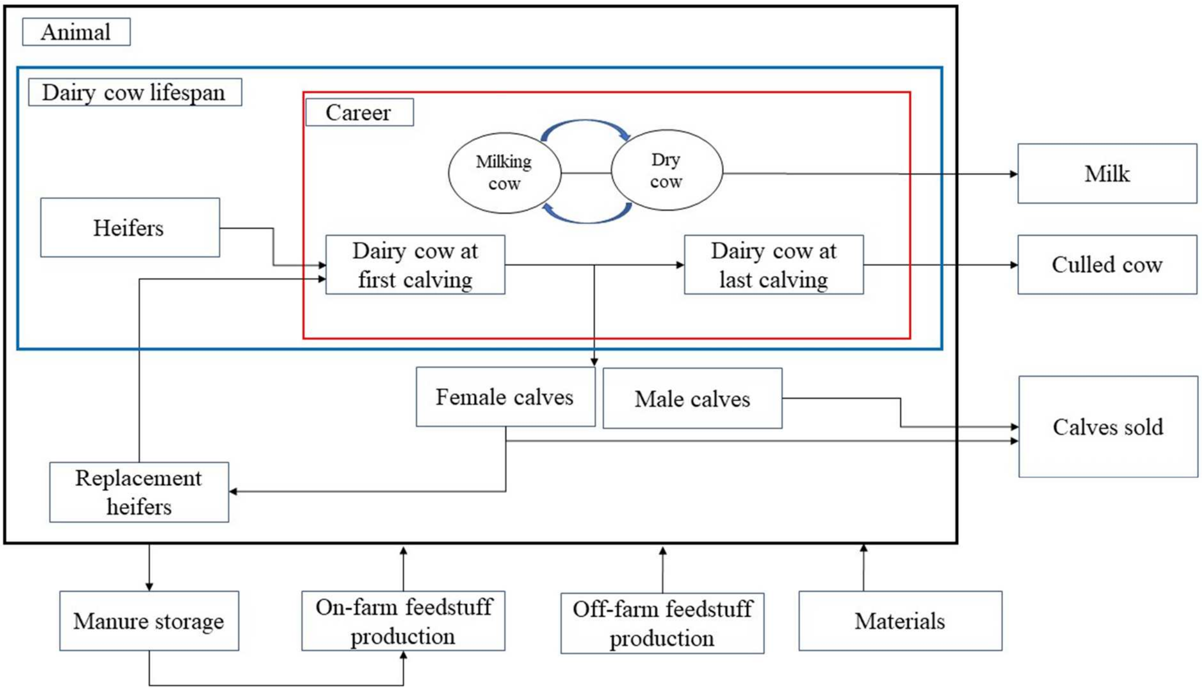
Variable	Unit	Mean	SD	ANOVA P-value			RMSE[2]
				Herd	Genetic group[1]	Herd × Genetic group	
NEm based on MW							
Gross income[3]							
GWP	kg CO_2-eq/€	2.74	0.93	<0.01	>0.1	>0.1	0.75
AP	g SO_2-eq/€	53.6	15	<0.01	>0.1	>0.1	12.6
EP	g PO_4-eq/€	18.5	3.9	>0.1	>0.1	>0.1	3.9
LO	m^2/€	3.32	0.76	<0.01	>0.1	>0.1	0.73
Income over feed cost							
GWP	€/kg CO_2-eq	0.21	0.10	<0.01	>0.1	>0.1	0.08
AP	€/g SO_2-eq	10.5	4.6	<0.01	>0.1	>0.1	3.6
EP	€/g PO_4-eq	28.8	11.1	<0.01	>0.1	>0.1	10.1
LO	€/m^2	0.16	0.06	<0.01	>0.1	>0.1	0.06
NEm based on Bprt							
Gross income							
GWP	kg CO_2-eq/€	2.74	0.93	<0.01	0.07	>0.1	0.74
AP	g SO_2-eq/€	53.4	14.8	<0.01	0.04	>0.1	12.4
EP	g PO_4-eq/€	18.5	3.8	>0.1	0.05	>0.1	3.8
LO	m^2/€	3.31	0.75	<0.01	0.05	>0.1	0.72
Income over feed cost							
GWP	€/kg CO_2-eq	0.21	0.10	<0.01	0.05	>0.1	0.07
AP	€/g SO_2-eq	10.5	4.6	<0.01	0.04	>0.1	3.6
EP	€/g PO_4-eq	29.0	11.0	<0.01	0.04	>0.1	10.1
LO	€/m^2	0.16	0.06	<0.01	0.03	>0.1	0.06

[1] Holstein or crossbred cows.

[2] RMSE: root mean square error.

[3] GWP: global warming potential, AP: acidification potential, EP: eutrophication potential, LO: land occupation

Figure 1. System boundaries for the computation of the environmental footprint of crossbred and Holstein Friesian individual dairy cows along their lifespan (N=564).



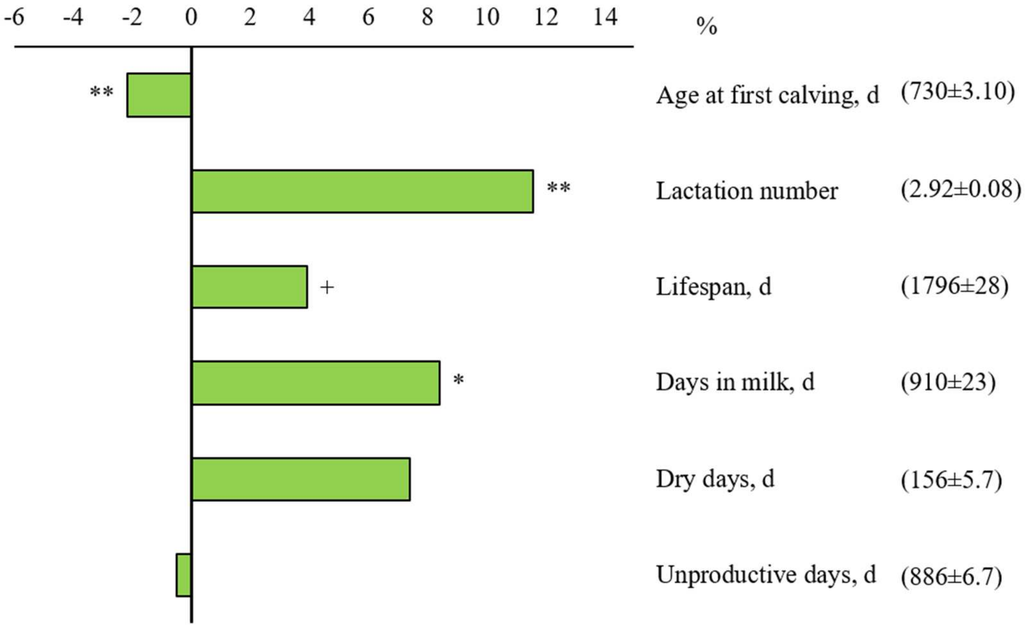
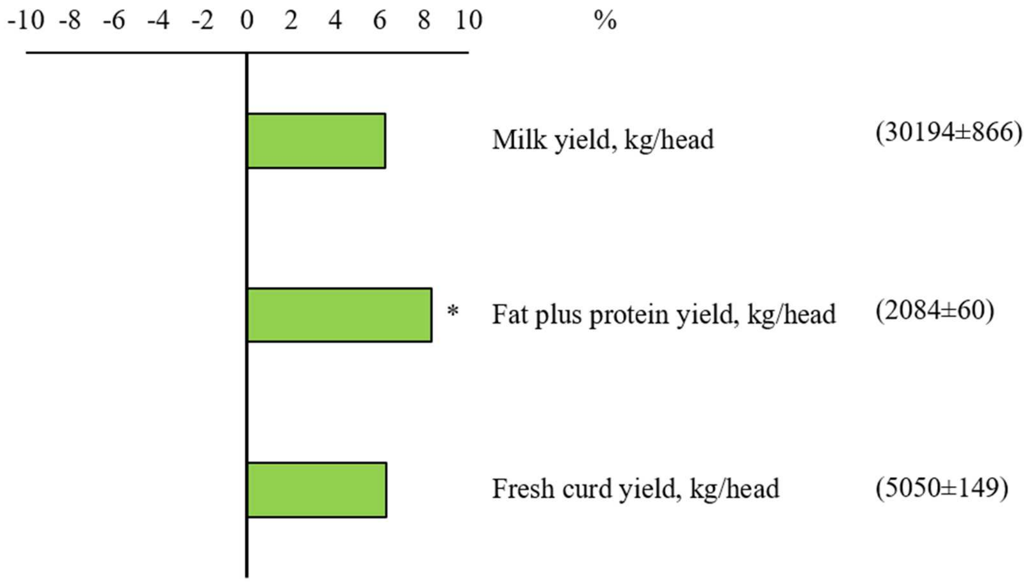


Figure 2. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for herd life traits (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** P<0.01, * P<0.05, + P<0.1).

a) Herd life



b) Day of life

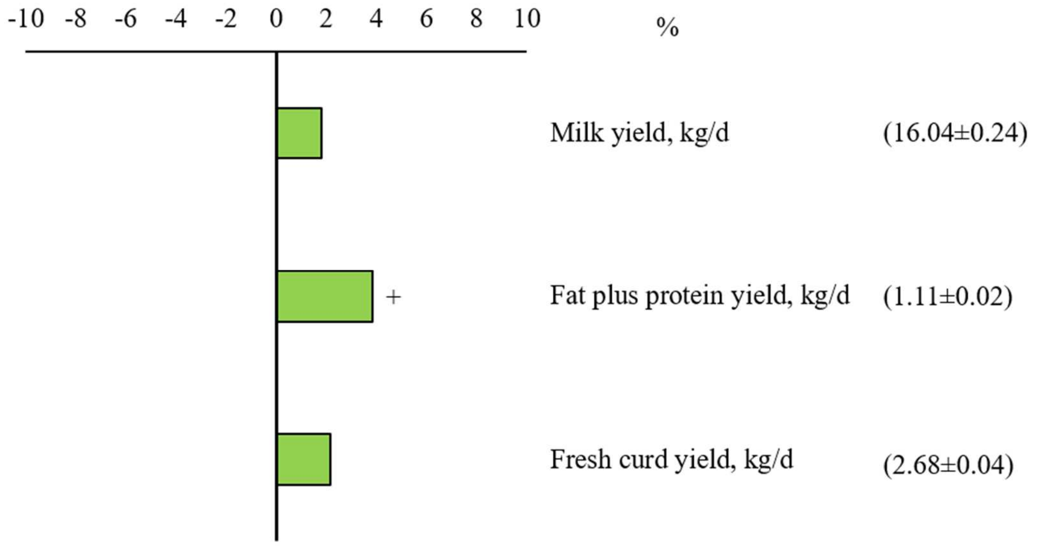


Figure 3. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for yield of raw milk, fat plus protein and fresh curd in herd life (a) and per day of life (b) (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** P<0.01, * P<0.05, + P<0.1).

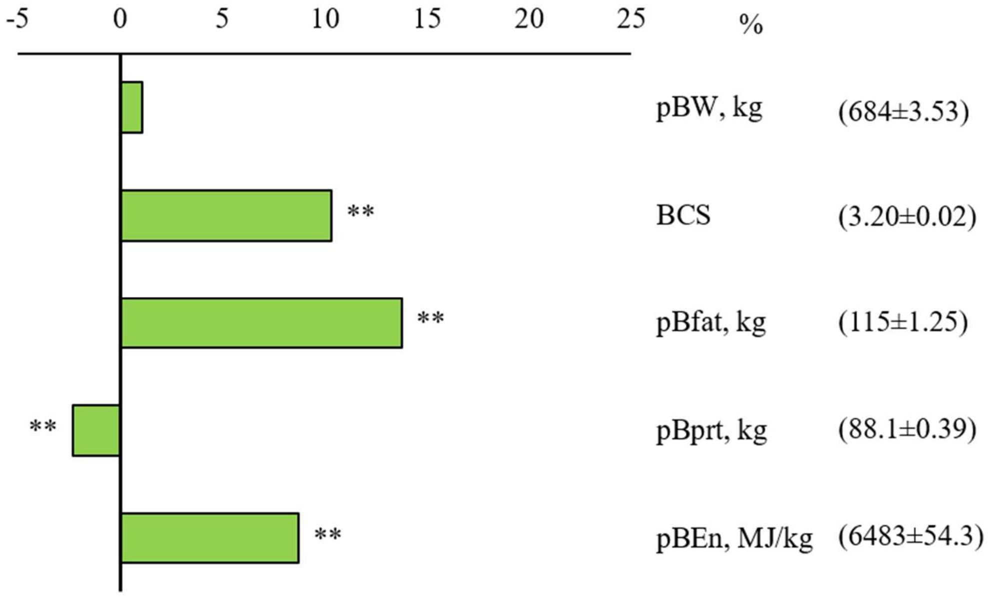


Figure 4. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for the average predicted body weight (pBW), body condition score (BCS), body fat (pBfat) and protein content (pBprt), and total body energy content (pBEn) (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** P<0.01, * P<0.05, + P<0.1).

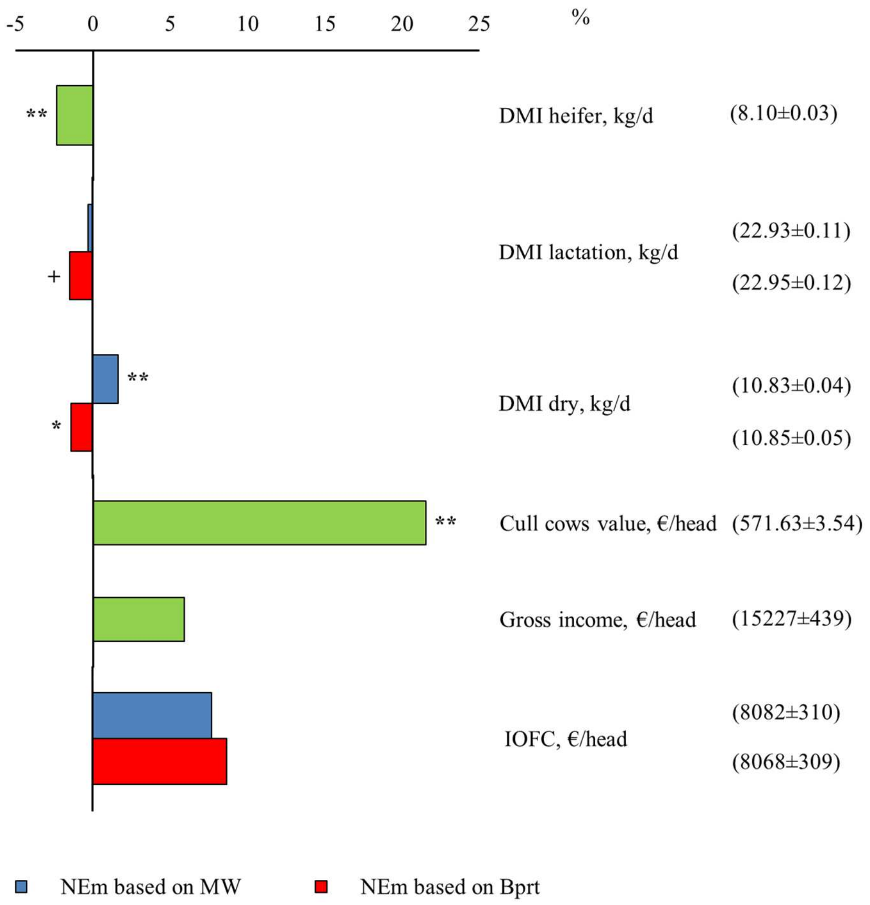
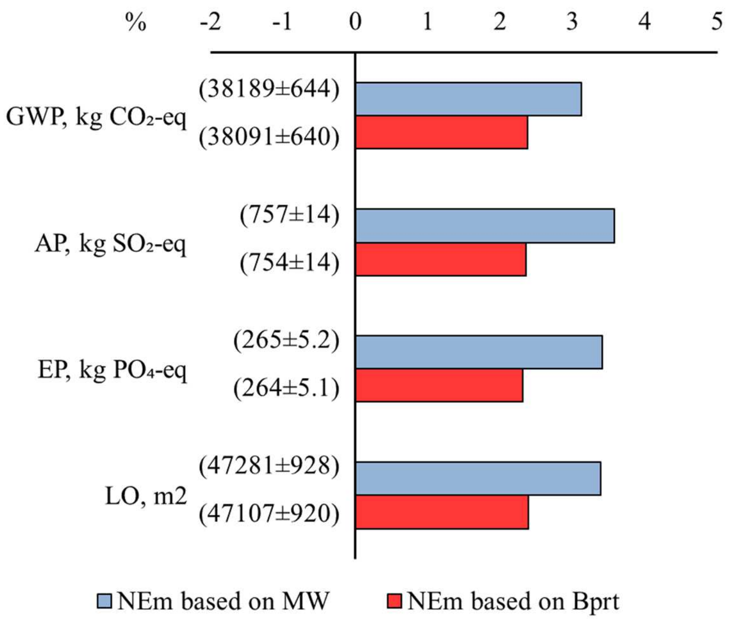


Figure 5. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for the estimated dry matter intake (DMI) during heifer, lactation and dry periods and income over feed cost (IOFC) computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt), and cull cows' value and gross income (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** P<0.01, * P<0.05, + P<0.1).

a) Impact for herd life



b) Impact per day of life

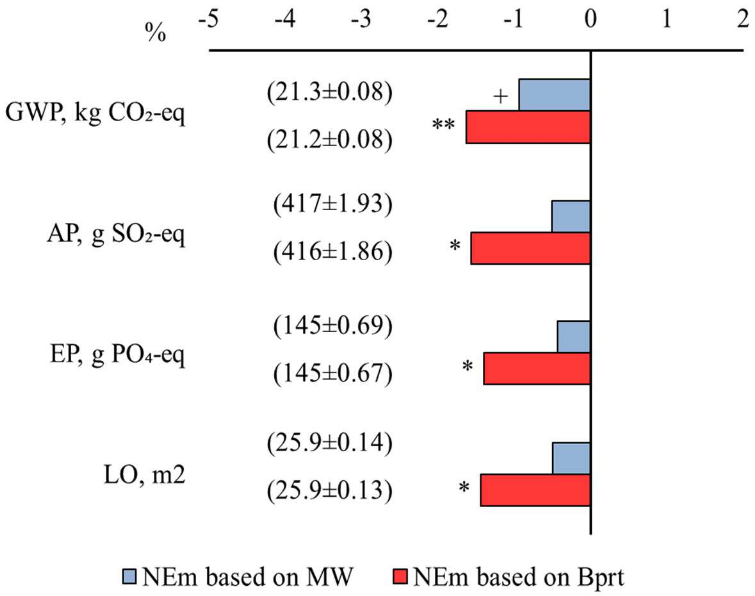
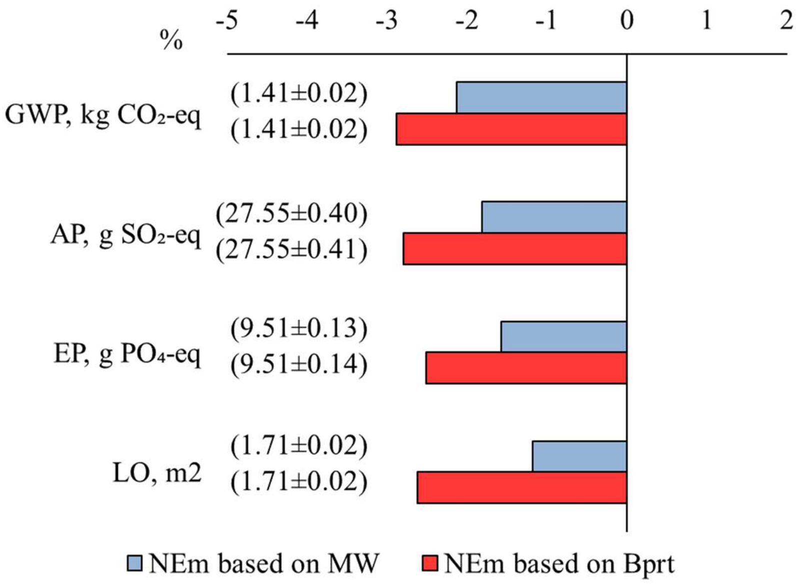
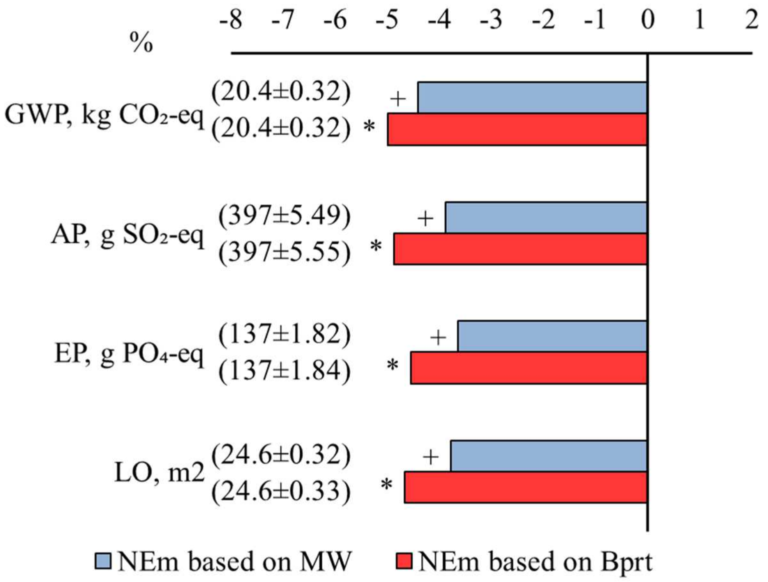


Figure 6. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for the impact categories (global warming, GWP; acidification potential, AP; eutrophication potential, EP; and land occupation, LO), associated with herd life (a) and day of life (b) computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt) (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** $P<0.01$, * $P<0.05$, + $P<0.1$).

a) Impact per kg of milk



b) Impact per kg of fat plus protein



c) Impact per kg of fresh curd

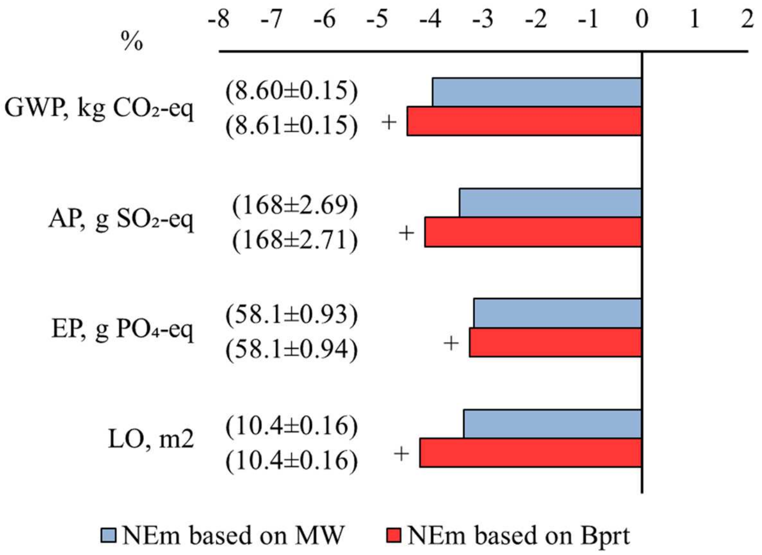
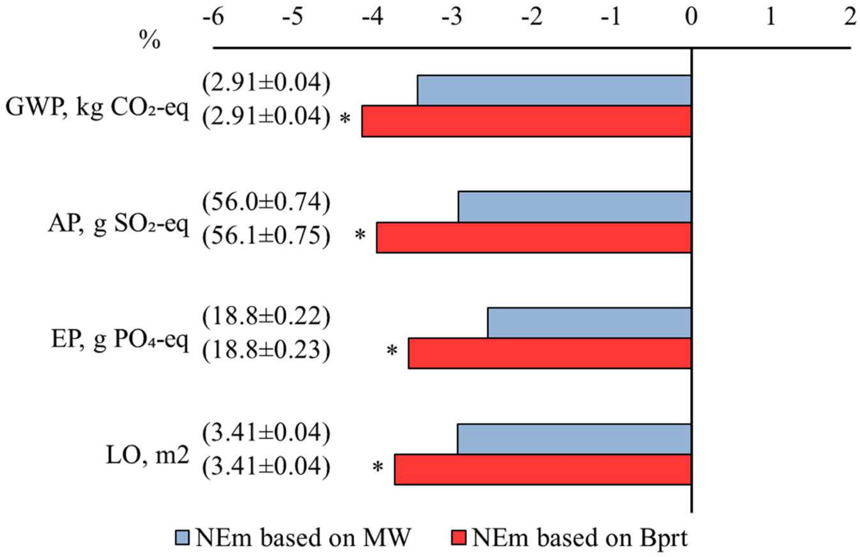


Figure 7. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for the impact categories (global warming, GWP; acidification potential, AP; eutrophication potential, EP; and land occupation, LO) associated with the yield of raw milk (a), fat plus protein (b) and fresh curd (c) computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt) (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** $P<0.01$, * $P<0.05$, + $P<0.1$).

a) Impact per € of gross income



b) IOFC per unit of impact

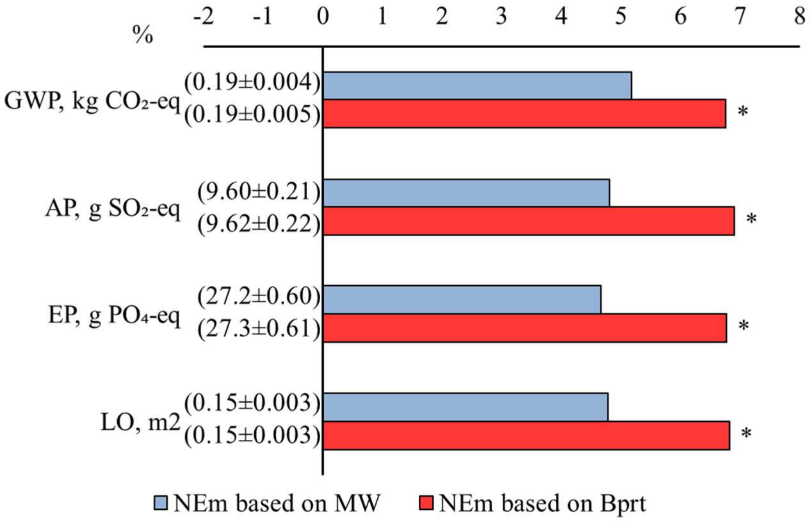


Figure 8. Percentage deviation of least square means of crossbred cows with respect to least square means of Holstein cows for the impact categories (global warming, GWP; acidification potential, AP; eutrophication potential, EP; and land occupation, LO) associated with gross income (a) and income over feed cost (IOFC) (b) computed using net maintenance energy requirement (NEm) based on metabolic weight (MW) or on body protein mass (Bprt) (between brackets LS means and SE of Holstein cows). Asterisks refer to significant differences between crossbred cows and purebred HO cows (** $P<0.01$, * $P<0.05$, + $P<0.1$).

GENERAL CONCLUSIONS

The results obtained from this set of contributions partially confirmed results from previous studies, on the other hand highlighted the potential of the use of the considered 3-breed rotational crossbreeding system. Each chapter of this dissertation showed different aspects of this crossbreeding program effects on dairy cows and farming management of two herds of Northern Italy, moving from the most specialized trait of milk to the whole career performances of crossbreds compared to HO cows.

Within the ProCROSS crossbreeding system, different generations of CR cows were found to produce lower volumes of milk and higher protein content than HO ones, confirming results from previous studies. However, CR showed to have similar mineral profiles of milk compared to HO cows, but a great variation in the profiles among different combinations of VR, MO and HO breeds, particularly when comparing VR- with MO-sired cows. The mineral composition of milk affects the nutritional and technological properties of milk, which are important features involved in the cheese-making procedures. Moreover, crossbred cows were also found to have better carcass attributes with respect to HO cows, in particular they were heavier, with better conformation and condition scores, fetched higher prices and had a higher total value (+ €109/cow). Thanks to the better body condition, CR had also a lower risk to face urgent unscheduled culling with respect to their HO herdmates, which can cause a drastic reduction of the salvage value of cull cows. Furthermore, compared to HO cows, CR had a similar body weight, but greater BCS, and lower estimated body protein mass suggesting that crossbred cows could also have lower energy requirements for maintenance. Indeed, scaling milk production on estimates of energy requirements for maintenance taking into account differences in body composition revealed to be a more accurate measure to compare production performances of cows derived from this crossbreeding scheme and purebred HO cows.

In addition, CR outperformed purebred HO cows in terms of herd life traits, such as age at first calving, lifespan and days in lactation, with a comparable production of milk in life but greater production of fat plus protein of milk, confirming the results found in previous studies. Exploring cows' performances in terms of environmental impact, CR emissions were higher for herd life compared to HO cows, due to the longer lifespan and productive life, but comparable per kg of milk produced in life and lower per kg of fat plus protein produced in life and per day of life. Moreover, CR cows environmental impact was minor per € of gross income, and the income over feed costs was higher per unit of emission with respect to purebred HO herdmates.

The results of this thesis suggested that ProCROSS crossbreeding program is able to affect positively milk composition and technological properties, economic incomes derived from culling and production efficiency metrics of cows. In addition, it has the potential to be considered as an effective strategy to mitigate the emissions of GHGs and other pollutants of dairy cows and to improve the sustainability of the intensive dairy farming systems.

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