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CICLO XXVI

FERTILITY, MILK PRODUCTION AND TECHNOLOGICAL PROPERTIES OF MILK OF 2 AND 3-WAY CROSSBRED COWS COMPARED WITH PURE HOLSTEIN

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ABTRACT

Crossbreeding programs in dairy cattle currently stand as a viable alternative in the dairy industry, because they may alleviate problems related to elevated homozygosis, which has occurred as a result of intensive selection programs using pure breeds.

The data utilized of the set of analysis presented hereby, was collected from four commercial dairy farms located in northern Italy between 2007 and 2013. All the farms had a common management system and were using the same 3-way rotational crossbreeding program, including Holstein(HO), Montbeliarde(MO), Swedish Red (SR). The farmers were using also 2-way rotational crossbreeding program, including Holstein(HO) and Brown Swiss (BS).

The most common reason for the interest in the use of cross breeding programs by dairy producers is the need to improve cow fertility, health and survival, traits that have progressively deteriorated during the last few decades. A common index used for the evaluation of fertility is the interval between calving and conception, more commonly known as days open. The reproductive performance of crossbred cows has been evaluated by many authors. However, many of these studies reported a value of days open truncated at 250 d. This approach could reduce fertility differences between breed groups. Therefore, the availability of time related variables, such as days open, are influenced by culling time of the cow and by the time of data collection. Data from those cows for which the insemination event, or the conception, have not occurred by the time of culling or data recording, are missed, which leads to some bias on fertility parameters. The objective of the second chapter was to investigate the effect of crossbreeding on fertility traits, studying reproduction time interval traits through survival analysis and success traits and inseminations to conception through logistic regression of heifers and primiparous cows. Results from both cows and heifers suggested that crossbreeding can improve fertility of purebred dairy herds. Crossbred animals showed early insemination after calving and also became pregnant before purebred Holstein cows. Crossbred cows showed a higher probability to conceive at the first insemination, and required less inseminations to achieve conception. The favorable effect in heifers was less pronounced than in primiparous cows.

Differences in milk yield between pure HO cows and crossbreds has been well characterized, however the information available on milk lactation curves of crossbred cows, such as peak yield, time at peak, and persistency, is less abundant. The objective of chapter 3

was to evaluate milk curve parameter of primiparous HO and crossbreds cows, using Wilmink function (1987) and modified Wilmink function , in order to include the effect of pregnancy. Milk lactation curves parameters showed different values between pure HO and crossbred cows. Holsteins showed a higher level of production than all crossbred cows. On the contrary, the increase in milk yield towards the peak was not different between pure HO and crossbreeds. Some crossbreeds showed a difference in the persistency of the lactation The effect of pregnancy on milk production was lower for MO × HO and MO ×(SR × HO) crossbreds cows than for pure Holstein, but the gestation started to affect milk production earlier for these two crossbreds. The use of pregnancy on the model showed a general increase of the accuracy of milk production prediction after the milk peak, relative to the classic Wilmink model.

The effect of pregnancy is also reported to had an effect as well on milk yield, as on milk component. Furthermore, as reported before, crossbreds cows showed higher reproductive ability then pure HO, and became pregnant before, so that the effect of gestation on the production start earlier in the lactation than for pure Holsteins. The aim of chapter 4 was to compare milk production and milk components of pure HO and crossbred cows, accounting for the effect of pregnancy stage in the model. Pure HO cows showed higher milk yield than all the crossbreds cows. The superiority of HO for yield traits was found also for protein and fat yield, which were higher for HO cows than all crossbreeds, except for BS × HO crossbreds that showed not differences in these traits, when compared with pure HO. However, this inferiority in production was accompanied by a higher milk quality for crossbreds cows. All crossbreds showed higher protein content than pure HO cows and SR × HO crossbred s cows showed also higher fat content than pure HO. Lower values of SCS were reported for SR × HO and BS × HO crossbreds cows, compared with pure HO. Whether the benefit from increased fat and protein content in milk can offset a reduced yield of milk and milk components will depend on specific pay systems.

The marketing of cheese has grown in recent years, and cheese plays an important role in the economics of dairy production. So that is important to consider the quality of milk not only in terms of protein and fat content, but also to consider the aptitude of milk to be transformed into cheese. The objective of the last chapter was to evaluate the effect of crossbreeding on milk quality traits, traditional milk coagulation properties and curd firmness model obtained from individual milk samples. Relative to pure HO, the crossbreds used in our study showed more desirable MCP, showed a faster coagulation time and higher curd firmness values. Furthermore, differences between crossbreds for these parameters were also found. Our results suggest that the use of crossbred animals can impact milk yield, milk composition and its MCP. Depending on the intended use of milk, different types of sires can be chosen to direct the efforts of farm crossbreeding programs.

RIASSUNTO

L'uso di un piano di incrocio fra vacche da latte risulta essere una valida alternative per gli allevamenti, in quanto può aiutare ad alleviare problemi dovuti all'elevata omozigosi, causata da una spinta selezione nelle razze in purezza.

I dati utilizzati per questa tesi sono stati raccolti in quattro diversi allevamenti in nord Italia, e riguardano animali presenti in azienda tra 2007 e il 2013. Gli allevamenti avevano in comune lo stesso tipo di programma di gestione aziendale e utilizzavano lo stesso programma di incrosio a rotazione a tre vie, con razze Holstein (HO), Swedish Red (SR) e Montbeliarde.(MO) Inoltre, negli allevamenti erano anche presenti incroci ottenuti con l'uso di razza Bruna Alpina.(BS).

La ragione più comuni per la quale gli allevatori si interessano a piani di incrocio fra razze da latte, è la necessità di migliorare la fertilità, la salute e la sopravvivenza degli animali in allevamento. Un comune indice usato per valutare la fertilità è l'intervallo parto-concepimento, comunemente chiamato anche days open. Le performance riproduttive degli incroci sono state valutate da diversi autori. Molti di questi studi utilizzano però un analisi che stabilisce un limite massimo di 250 giorni di days open, e questo tipo di approccio può ridurre le differenze fra gli incroci e gli animali puri. Inoltre, la disponibilità di variabili temporali, come i days open, sono influenzate dal momento in cui l'animale viene eliminato e dal momento in cui avviene la raccolta dati. Tutti quegli animali per i quali non c'è stata un 'inseminazione, o un parto, al momento dell'eliminazione dell'animale, o della raccolta dati, non vengono considerati nell'analisi, il che può portare qualche bais nella valutazione dei parametri legati alla fertilità. L'obiettivo del secondo capitolo era quello di valutare l'effetto del crossbreeding sui caratteri legati alla fertilità di manze e vacche primipare, analizzando i caratteri di tipo temporale tramite analisi di sopravvivenza, e caratteri di tipo successo/non successo e numero di inseminazioni, tramite analisi di regressione logistica. .I risultati, sia per le manze che per le primipare, mostrano che gli incroci possono aumentare la fertilità a livello aziendale. Gli animali meticci hanno infatti mostrato migliori risultati sia per l'intercallo parto-prima inseminazione, che per l'intercallo parto-concepimento, e a questi animali è stata inoltre attribuita una più alta probabilità di concepire alla prima inseminazione e un numero inferiore di inseminazioni per concepimento. L'effetto favorevole degli incroci è risultato più pronunciato nelle primipare, che nelle manze.

La differenza in termini di produzione di latte fra HO ed incroci è stata ben caratterizzata, ma le informazione sul tipo di curve di lattazione degli incroci, come il picco di lattazione, il momento in qui questo avviene e la persistenza, sono meno numerose. L'obiettivo del capitolo 3 è stato quello di valutare i parametri relativi alle curve di lattazione di vacche primipare HO e dei relativi incroci, utilizzando la classica funzione descritta da Wilmink (1987) ed una funzione modificata, nella quale è stato considerato anche l'effetto della gravidanza. Il livello di produzione di latte è risultato essere più alto per le HO, rispetto a tutti gli incroci, mentre il parametro che descrive l'incremento verso il picco, non ha evidenziato particolati differenze fra incroci e vacche pure. Per la persistenza della lattazione sono state trovate alcune differenze fra gli animali puri e gli incroci. Inoltre, l'effetto della gravidanza sulla produzione di latte è risultata essere inferiore per gli incroci MO × HO e MO ×(SR × HO) rispetto agli animali puri, ma l'effetto significativo della gestazione sulla produzione per questi due incroci iniziava prima che per le HO. Il modello che tiene conto della gravidanza ha mostrato una miglior capacità predittiva per il latte dopo il picco, rispetto alla classica funzione di Wilmink.

Oltre ad avere un effetto sui parametri relativi alla curva di lattazione, la gravidanza influisce anche sulla produzione di latte e dei suoi componenti, sia in termini quantitativi che percentuali. Inoltre, come riportato in precedenza, gli incroci hanno dimostrato migliori performance riproduttive, rimanendo gravide prima rispetto alle vacche HO. L'effetto della gestazione sulla produzione inizia quindi in una fase della lattazione precedente per gli incroci. L'obbiettivo del capitolo 4 era quello di comparare vacche HO con relativi incroci per la produzione e la composizione del latte, considerando nel modello l'effetto della gravidanza. Le HO hanno mostrato una produzione di latte più alta rispetto a tutti gli incroci ed anche la quantità di grasso e proteina prodotti è risultata maggiore per gli animali puri, ad eccezione dell'incrocio BS × HO , che non ha mostrato differenze significative in termini di produzione grasso e proteina rispetto alle HO in purezza. Se le HO sono risultate superiori in termini quantitativi, gli incroci si sono dimostrati migliori per i parametri qualitativi. La percentuale di proteina era più alto per tutti gli incroci rispetto agli animali in purezza,. e gli incroci SR × HO hanno mostrato anche un maggior contenuto di grasso nel latte. Inoltre, sia gli incroci SR × HO , che quelli BS × HO, avevano un più basso valore di SCS, rispetto alle HO. Gli incroci hanno quindi mostrato una produzione inferiore rispetto agli animali puri, ma posso incrementare la qualità del latte, soprattutto in termini di proteina percentuale, il che, in alcuni sistemi di pagamento del latte, può avere anche un riscontro economico.

Il mercato legato alla produzione di formaggio è stato caratterizzato da un incremento negli ultimi anni, ed il mercato del formaggio riveste un ruolo economico importante nel mercato del latte. Parlando di qualità del latte non è quindi importante parlare solo di contenuto in grasso e proteina, ma anche dell'attitudine casearia del latte. L'obiettivo del capitolo 5 era quello di valutare l'effetto del crossbreeding sulla qualità del lattee sulle proprietà coagulative del latte, utilizzando dati ottenuti da analisi su latte individuale I risultati

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Chapter 1

General Introduction

INTRODUCTION

During the last half of century the 21th century, the dairy industry worldwide has undergone dramatic transformations. The adoption of new technologies for faming of dairy animals, as well as changes in consumer preferences have shaped the modern dairy industry. The most notorious changes are perhaps those associated with milk production, which are a result of long-term processes such as adoption of technological innovations, changes in the production system, and specialization (USDA, 2002).

By the year 2000, milk production in the United States alone had increased by 45% relative to 1975 (USDA, 2002). Importantly, this increase has been achieved with a lower number of cows (9 *vs.* 11 million; Bauman *et al.* 2006), which reflects the remarkable increments in individual production efficiency. A better understanding of lactation biology, dairy cow physiology and nutrition practices have been responsible for much of this gains in productivity. However, genetic programs have undoubtedly played a major role in these changes, as they have allowed increased individual milk production of cows, resulting in elevated farm productivity. Intensive genetic selection programs have been responsible for the improvement in the milk production potential of dairy cows, and have resulted in a steady increased milk production per cow. One unwarranted consequence of higher milk yield per cow, however, has been the deterioration of reproductive efficiency, as it is argued that aggressive genetic selection for increased milk production is antagonistically genetically correlated with health and fertility (Veerkamp *et al.* 2001).

On these lines, it may seem paradoxical that the effort to increase the efficiency of milk production can simultaneously reduce the efficiency of reproduction. A recent review by Berry (2008) addressed these problems and further proposes that the successful implementation of new genomic tools available to improve productivity and profitability of dairies can only be properly exploited if these are accompanied by correct breeding programs that do not focus on the intensive selection of single traits. As discussed by Mc Parland *et al.* (2007), aggressive selection on a narrow breeding goal typically reduces population genetic diversity, leading to increased inbreeding that negatively impacts animal health, fertility and survival. From this, it appears that improvements in overall efficiency of cows to meet current and future population demands, can only come from more sound strategies that consider cow's overall performance, without deterioration of fertility or health.

Selection programs and inbreeding

Selection in the context of anthropic genetics refers to the systematic and non-random selection of individuals for reproduction, which carry gene (s) of interest, with the purpose of altering the gene frequencies within a population. The selection of the individuals which are to reproduce will depend on the objectives that are being pursued (i.e. the phenotype benchmark), such as the overall improvement of traits related to better health, production or reproduction, etc, and will ideally result in the permanent modification of these traits in the targeted population. Selection objectives must be clearly defined, and can be established by considering some basic factors, which usually include animal species, animal breed, and economic context (*i.e.* the phenotype the market values best), to give direction to the breeding program by establishing the improvement goals.

In its most simple definition, inbreeding is the mating of related individuals within a given population. This is opposition to outcrossing, in which the individuals are only distantly related, at best. In terms of population genetics, inbreeding is a reproductive system in which the individuals chosen for mating express a trait at levels which are superior to the average of the population for a given trait. These "top" individuals will thus pass on their genes and increase the frequency of the alleles associated with their phenotype in the target population, and as a result, the genetic differences within the population will be reduced (higher inbreeding value than the progenitor population). The end result is an increased degree of relation between the individuals of the targeted population, and an overall reduction of heterosis. Inbreeding increases the probability that the two copies of a given gene will be identical (homozygous) in the individuals of a population.

Inbreeding programs have been extensively used to drive genetic improvement in dairy cattle during the past decades. This has led to rapid genetic progress, evidenced by substantial increases in the yields of milk and milk components (Shook, 2006). Genetic progress has been achieved through the use of highly selected bulls as sires in artificial insemination (AI) schemes, and has also resulted in the accumulation of inbreeding, as expressed by increased inbreeding indexes during that period of time (Wiegel, 2001). Although inbreeding has had positive effects on milk production traits, as breeding indexes increased, simultaneous negative effects have been reported in other traits, which is often refered to as inbreeding depression. Inbreeding has been reported to decrease cow survival (Bonczek and Young, 1980), single lactation production (Hermas et al., 1987; Short et al., 1992), fertility (Hodges et al., 1979; Hudson et al., 1984), and to increase somatic cell count (Miglior et al., 1992). Inbreeding has thus become an important consideration among the large population of Holsteins in the US (Smith et al., 1998) and the implementation of alternative to overcome the issues has become highly relevant.

Crossbreeding and heterosis

In opposition to inbreeding, crossbreeding is a type of mating system that uses individuals not closely related to each other, that is, whose relation is lower than that of the average pair of individuals in a given population. This often translates to the mating of different breeds or genetic lines of individuals. The main goal behind crossbreeding is to achieve increased heterosis, which then results in the expression of hybrid vigor (i.e. phenotypic superiority) of the crossbred individual, relative to its parents. Falconer and Mackay (1996) defined heterosis or hybrid vigor as the difference between the hybrid and the mean of the two parents (which belong to different breedlines).

When considered in the context of elevated inbreeding and homozygosis, crossbreeding can provide its benefits beyond the mere (although very significant) effects of hybrid vigor. By mating distantly related inbred lines, crossbreeding is simultaneously restoring some of the lost heterozygosis to the genetic pool of the population. This idea was clearly summarized by Dickerson (1973), who suggested that the positive effects of crossbreeding are also due to the recovery from accumulated effects of reduced heterozygosity or random drift in gene frequency due to limited size of the population (i.e. from inbreeding depression) in the parental stock.

At the light of these potential benefits, the idea of using crossbreeding programs has become increasingly attractive for the dairy industry over the last few decades. However, the idea of crossbreeding in dairy cattle is not a new one, as it has been part of the dairy landscape for almost a century. Wentworth (1927) highlighted its numerous benefits and emphasized its potential as a tool to improve farm income; all of this even before any effects of inbreeding accumulation could have been observed.

The implementation of a crossbreeding program in a dairy herd starts with the choice of sires of one or more breeds, to mate with the existing female population. The type of crossbreeding program (2- or 3- breeds) that is chosen will determine the types of breeds needed and will also determine the degree at which the hybrid vigor can be conserved in the subsequent generations. When designing a crossbreeding program, one of the key goals is to maintain the hybrid vigor, which is responsible for the superior phenotype, to its possible maximum. It is known that the maximal expression of hybrid vigor is obtained in the hybrid (F1) from two pure parental breedlines, and that this vigor value is decreased in the subsequent generations (Hansen, 2008). It has been shown that in practice, the use of a three-way crossbreeding program, in which breeds are utilized alternately, is the best possible scheme to maximize the expression of hybrid vigor in a population over time (Hansen, 2008).

Finally, when such a program is being planned, some standard considerations in the crossing breed should be taken into account, including genetic progress for yield traits, yield levels in comparison with those of HO cows –which has become the reference breed in dairy cows-, demonstrated heterosis when crossed with Holstein (HO), and superiority in maternal performance (McAllister, 2002).

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Chapter 2

Analysis of reproduction time intervals, fertility success and number of inseminations of purebred Holsteins and of two- and three-breed crossbred heifers and cows obtained using Swedish Red, Montbeliarde and Brown Swiss sires

ABSTRACT

The objective of this study was to compare fertility traits of heifers and primiparous cows of Swedish Red × Holstein (SR × HO, n = 643, and 581, respectively), Montbéliarde × Holstein (MO × HO, n = 241 and 114, respectively), Brown Swiss × Holstein (BS × HO, n = 58 and 50, respectively) and MO × (SR × HO) (n = 241 and 139, respectively) crossbred cows with purebred Holstein (HO) heifers and cows (n = 2,549 and 2,553, respectively for heifers and cows). Animals were born between 2007 and 2011 and belonged to 4 herds located in northern Italy. Heifers were compared for age at first service (AFS), age at first conception (AFC), interval between first service and conception (IFC), non-return rate at 56 days after first service (NR56), conception rate at first service (CR), and number of inseminations to conception (INS). The same traits were evaluated for primiparous cows, with the exception of AFS and AFC, and days at first service (DFS) and days open (DO) were evaluated instead. The AFS, AFC, IFC, DFS and DO traits, being continuous variables, were analyzed under a proportional hazards cox model, that properly accounts for censoring among cows that were culled or failed to conceive. The NR56, CR, and INS were analyzed as binary traits, using the logistic regression. For heifers, crossbreeds SR × HO had a higher risk of having the first service and conceiving earlier than purebred HO, with an hazard ratio (HR) equal to 1.31 for AFS and 1.34 for AFC, respectively. Similarly, MO × (SR × HO) crossbreeds were different from pure HO heifers in this regard (HR = 1.18 and 1.24, respectively). For the primiparous cows, all crossbreeds showed significant differences for DFS, DO and IFC relative to purebred HO, except for the crossbreed between BS × HO. In fact, MO × HO, SR × HO and MO × (SR × HO) crossbreed cows showed and increased chance of having fewer DFS (HR = 1.40, 1.30 and 1.27, respectively), fewer DO (HR = 1.59, 1.43 and 1.58, respectively), and fewer IFC (HR = 1.52, 1.26 and 1.39, respectively) than pure HO cows. All crossbred genotypes, including BS × HO cows, showed a higher probability for increased NR56, CR, and for having a smaller INS than pure HO cows. Results indicate a higher reproductive potential for the crossbreed cows compared with purebred Holsteins.

INTRODUCTION

Reproductive efficiency in dairy cows is decreasing worldwide (Lucy, 2001). Norman et al. (2007) reported unsatisfactory reproductive performance as the primary reason for culling cows in the first three lactations. To overcome this problem, many of the leading dairy countries have incorporated fertility traits into national genetic evaluation systems

(VanRaden, 2004; Miglior et al., 2005). Many studies reported a negative correlation between milk production and fertility traits (Pryce et al., 2002, 2004; Tiezzi et al., 2011), indicating that selecting animals for milk production during the past decades could have negatively affected fertility, especially through a worsening of cows' body condition (Tiezzi et al., 2013). However, other factors are associated with reduced fertility over time, included increasing herd size, greater use of confinement housing, labor shortages, higher inbreeding coefficient, and global warming (Lucy, 2001). Crossbreeding programs in dairy cattle stand as a viable alternative in the dairy industry, because they may alleviate problems related to elevated homozygosis, which occur as a result of intensive selection of pure breeds. Weigel and Barlass (2003) performed a comprehensive analysis to report the experiences of US dairy producers that were using crossbreeding programs. Most of respondents cited the need to improve cow fertility, health and survival, as the reason for their interest in crossbreeding. Hains et al. (2012) recently reported the results of a five-lactation study, on production and fertility traits of pure Holstein (HO), Montbeliarde (MO) × HO, Scandinavian Red (SCR) × HO, and Normande (NO) × HO, while few information is available on three-way crossbred cows. Crossbred cows showed shorter DO, when compared with purebred HO. In addition, groups differed in their change in DO across lactation number. The NO × HO crossbreds had a rather constant DO across lactations, MO × HO crossbreds tended to have fewer DO as lactation number increased, and SCR × HO crossbreds tended to have an increase in DO as lactation progressed. However, as in other studies with crossbred cows, DO data was truncated at 250 d (Heins et al., 2006; Blöttner et al., 2011; Bjelland et al., 2011), an approach used by the Animal Improvement Programs Laboratory of the USDA for routine genetic evaluations in the United States (VanRaden et al., 2004). The use of this procedure could reduce fertility differences between breed groups (Heins et al., 2006). Another fertility measure commonly used to evaluate reproduction performance are the number of the days between the calving and first service (DFS), and the interval between the first service and conception (IFC). Some authors reported a difference on DFS between pure HO and crossbred cows, with fewer DFS for crossbreds (Heins et al., 2006; Walsh et al., 2008; Blöttner et al., 2011). However, DFS do not correspond exactly to the ability of the cow to resume cyclic activity after calving, but is a combination between the interval between calving and first heat and the voluntary waiting period, that is the moment in which cows are intentionally not inseminated. The voluntary waiting period can be altered by management decisions related to cow partum health, but are also influenced by the season, the parity and the milk yield during early lactation (DeJarnette et al., 2007). The availability of those time related variables are influenced by culling time of the cow and by the time of data collection. Data from those cows for which the insemination event, or the conception, have not occurred by the time of culling or data recording, are missed (censored records), which leads to some bias on fertility parameters. Several methods have been proposed to deal with the analysis of censored data. Linear Gaussian models for handling right-censored data were developed by Korsgaard et al. (1998) and Sorensen et al. (1998) using the technique of data augmentation under a Bayesian framework (Tanner and Wong, 1987). This approach provides all posterior conditional distributions in a standard form after updating censoring records with values sampled from a left-truncated density and has been successfully applied to the analysis of fertility traits in dairy cattle (Chang et al., 2006; González-Recio et al., 2006; Hou et al., 2009). Variation of non-Gaussian censored variables can be investigated using survival analysis techniques (Cox, 1972; Prentice and Gloeckler, 1978; Ducrocq et al., 1988) because of their ability to properly account for censored and uncensored records and to make use of all available information (Vargas et al., 1998; Allore et al., 2001; Schneider et al., 2005).

The reproductive ability of cows can also be expressed in success traits, such as number of inseminations to conception (INS), conception at first service (CR), and non-return to first service (NRx), which is determined by whether another service follows within a pre-determined number of days or not, usually 56 or 90 days. These type of traits are also computable for heifers, thus allowing a comparison between cows and heifers and also the estimates of these traits could be biased by culling or incomplete data.

The objective of the present study was to investigate the effect of crossbreeding on fertility traits, using data from two- and three-way crossbreds heifers and cows, obtained from Swedish Red (SR), Monbeliarde (MO) and Brown Swiss (BS) sires and HO dams studying reproduction time interval traits through survival analysis and success traits and inseminations to conception through logistic regression.

MATERIALS AND METHODS

Data Collection

Fertility measurements both from heifers and primiparous cows, were retrieved from the management software Afifarm, version 3.06 (S.A.E. Afikim, Israel). All animals were born in four herds located in northern Italy between 2007 and 2011. All the farms were using the same 3-way rotational crossbreeding program, including Holstein (HO), Swedish Red

(SR) and Montbeliarde (MO) to obtain two breed SR × HO and three breed MO × (SR × HO) crossbred cows. The farmers were using also MO × HO and Brown Swiss (BS) × HO crossbreeds, which were included in the analysis. A total of 3,483 purebred HO heifers were compared with 634 SR × HO, 241 MO × (SR × HO), 126 MO × HO, and 58 BS × HO crossbred heifers and a total of 2,549 purebred Holstein cows (HO) were compared with 581 SR × HO, 139 MO × (SR × HO), 114 MO × HO, and 50 BS × HO crossbred cows.

Editing Procedure and Definition of Traits

Fertility traits for heifers were age at first service (AFS), age at first conception (AFC), interval between first service and conception (IFC), conception rate at first service (CR), non-return rate at 56 days after first service (NR56) and number of inseminations to conception (INS). For primiparous cows, the same traits were evaluated, except for AFS and AFC, and days at first service (DFS) after first calving and days open (DO) were evaluated instead. The CR and NR56 were coded as discontinuous variables (0-1), where 1 was assigned when pregnancy at first service was confirmed for CR and when a second insemination within 56 d from the first service did not occur, for NR56. Pregnancy was confirmed with subsequent calving or, when not available, with a pregnancy diagnosis by the veterinarian. If the first insemination was not available, or the days between the first insemination and data collection were less than 56, the insemination event was considered as a missing record. If data corresponding to the subsequent calving were available, the pregnancy was required to be between 30 d to the average of pregnancy days, calculated for each breed group. If the pregnancy time was out these limits, data was considered to be missing. Inseminations occurring within 6 d from each other were considered as a single service and only the information from the last breeding was included. Inseminations to conception (INS) was considered as an ordinal categorical variable with 5 classes. The last class was an open class from 5 or more inseminations. Cows without a diagnosis of pregnancy or a subsequent calving after the last service were penalized by adding a penalty service, as previously described by Hou et al. (2009).

All time variables were analyzed with a survival analysis, which allowed us to use censored records. Heifers' AFC and AFS were required to be less than 650 and 750 d, respectively, and IFC was restricted to be between 0 and 356 d, both for heifers and cows. Cows' DFS was required to be between 0 and 250 d and DO between 20 d and 365 d. Data below the lower limit were excluded from the analysis. Data above the upper limit were replaced with the value of the upper limit and considered as censored records. Cows that were in the herds but had not been inseminated by the time of the data collection were considered as censored for AFS if more than 20 d had passed between calving and data collection and first service was replaced as the last day available; otherwise, records were deleted. Heifers and cows not present in the herd at the moment of sampling were considered as a censored record if culling time occurred after an age of 450 d for heifers and after 65d after calving for primiparous cows (about the average for AFS and DFS respectively), and days at first service was replaced with the culling date. Heifers and cows culled before those time limits were not considered in the analysis. For animals without pregnancy test, or with an abortion following the pregnancy diagnosis, data were considered to be censored, and DO, AFC and IFC were calculated from the data of the last insemination event available. For those cows that didn't had the first insemination, IFC was considered as missing value and not used in the analysis.

Statistical Analysis

The fertility traits AFS, AFC, DFS and DO intervals were analyzed using a survival analysis methodology. Preliminarily, the survivor function for the general population was estimated by the Kaplan-Meier method (Kaplan and Meier, 1958). The linear regression of $\ln\{-\ln[S(t)]\}$ on $\ln(t)$, where $S(t)$ is the Kaplan-Meier estimated survivor function, was considered to check the suitability of the assumption of a Weibull baseline hazard (Ducrocq et al., 1988). Because the relation between $\ln\{-\ln[C(t)]\}$ and ln(t) was not linear, the assumption of a Weibull distribution function for the baseline was rejected and the model used for modeling the fertility traits was a proportional hazard model (Cox, 1972). The PHREG procedure of SAS (SAS Institute, 2011) was used, considering the fixed effects of herd, breed group and season-year of birth or season of calves, respectively for heifers and for cows. The IFC was analyzed with the same model but considering the fixed effect of season-year of first insemination. The CR, NR56 and INS traits were analyzed with a logistic analysis using the LOGISTIC procedure of SAS and considering the fixed effects of herd, breed group and season-year of first calf. For INS, probabilities modeled are cumulated over the lower ordered values.

RESULTS AND DISCUSSION

Descriptive Statistics of reproduction time interval traits, and of fertility success traits

Descriptive statistics for reproductive traits related to time are reported in Table 1, and those describing success traits are shown in Table 2. On average, uncensored heifers received the first insemination at the age of about 14 mo. and became pregnant at about 15 mo., with an IFC of 27 d. Censored records had higher values and greater variation than uncensored records. and represented less than 1% of the total records for AFS. A higher proportion of censored records was found for AFC and IFC (6.96% and 6.14%, respectively).

Table 1. Descriptive statistics of age at first service (AFS), age at conception (AC), interval between first service – conception (IFC), days at first service (DFS), and days open (DO) for heifers and primiparous cows.

			Uncensored records		Censored records	
	N	Censored	Mean	SD	Mean	SD
Heifers						
AFS, d	4543	0.99%	443.18	35.82	587.82	64.37
AC, d	4543	6.96%	470.28	56.67	516.08	88.55
IFC, d	4498	6.14%	27.18	44.99	56.69	73.84
Primiparous cows						
DFS, d	3433	6.41%	63.71	19.27	84.02	72.65
DO, d	3433	21.64%	104.08	61.12	130.48	95.71
IFC, d	3213	21.58%	41.58	57.49	76.71	87.38

In a previous study, Vargas et al. (1998) reported 3% censored records for AFS, using data from heifers that were at least 390 d old. The censored data represented a greater proportion of all animals in the case of primiparous cows (6.41%, 21.64% and 21.58% for DFS, DO and IFC, respectively), in relation with the higher culling rate of cows than of heifers, and reflecting also the greater bias that could affect fertility estimates if these data are not taken into account in the analysis. The higher proportion of censored information in data from cows suggests that the adoption of survival analysis is perhaps more useful to study reproductive performance in cows than in heifers. The mean of DFS was 64 d for uncensored records and 84 d for censored records. Conception occurred on average 104 d after calving for uncensored records and 130 d for censored records, with an IFC of 41 and 77 d, respectively. Similar to heifers, variation in primiparous cows was higher for censored than for uncensored records.

Moving to success traits (Table 2), on average, heifers needed 1.8 inseminations to conception and CR and NR56 showed similar values (55% and 58%, respectively). In the case of cows, INS was on average 2.4 and the CR and NR56 averaged 38% and 41%. Because a large proportion of the data corresponded to purebred HO animals, the herd average was likely influenced mainly by the HO performance. Reproductive performance values from the farms used in the current study seem rather high when compared with other reports from literature. Normande et al. (2009) reported average values of 85 d for DFS, 142 d for DO, 2.5 INS and 34% CR for first-lactation US HO cows in 2006.

Table 2. Mean ad number of observation (N) for conception rate a first service (CR), non return at 56d (NR56) and number of insemination to conception (INS) for heifers and primiparous cows.

	Heifers		Primiparous cows	
	N	Mean	N	Mean
CR	4498	0.55	3043	0.38
NR56	4498	0.58	3211	0.41
INS	4498	1.80	3213	2.42

Kaplan-Maier survival curves for AFS and AFC (Figure 1a and 1b) were different among breed groups. At 15 months of age, more than 80% of the BS × HO and SR × HO crossbreds, 70% of the MO × (SR × HO) crossbred, 64% of the MO × HO crossbreds and only 54% of the pure HO heifers received the first insemination. However, at the same age, less than 40% of the pure HO heifers were pregnant, versus more than 60% of BS × HO and SR × HO crossbreds heifers, and around 50% of MO × HO and MO × (SR × HO). About 50% of the HO heifers and 60% of the SR × HO crossbred heifers were pregnant after the first service (Figure 1c). The other crosses showed intermediate values. Differences among breed groups were not very evident (Figure 2a) in terms of DFS, probably because of a common voluntary waiting period. The proportion of pregnant females at first insemination was lower in primiparous cows than in heifers (Figure 2c). In fact, only 33% of the purebred HO cows and around 50% of the crossbred cows were pregnant with the first service.

Considering Kaplan-Maier survival curves for DO (Figure 2b), at 100 d postpartum, the proportion of pregnant cows was 49% for pure HO, around 65% for BS × HO and SR × HO crossbred cows, and around 75% for MO × HO and MO × (SR × HO) crossbred cows.

Figure1. Kaplan-Meier survival curves for age at first service (AFS), age at first conception (AFC) and interval between first insemination and conception (IFC) for pure Holstein cows (HO), Brown Swiss x HO crossbreds (BS x HO), Montbeliarde x HO (MO x HO), Swedish Red x HO (SR x HO) and MO x (SR x HO) crossbreds heifers

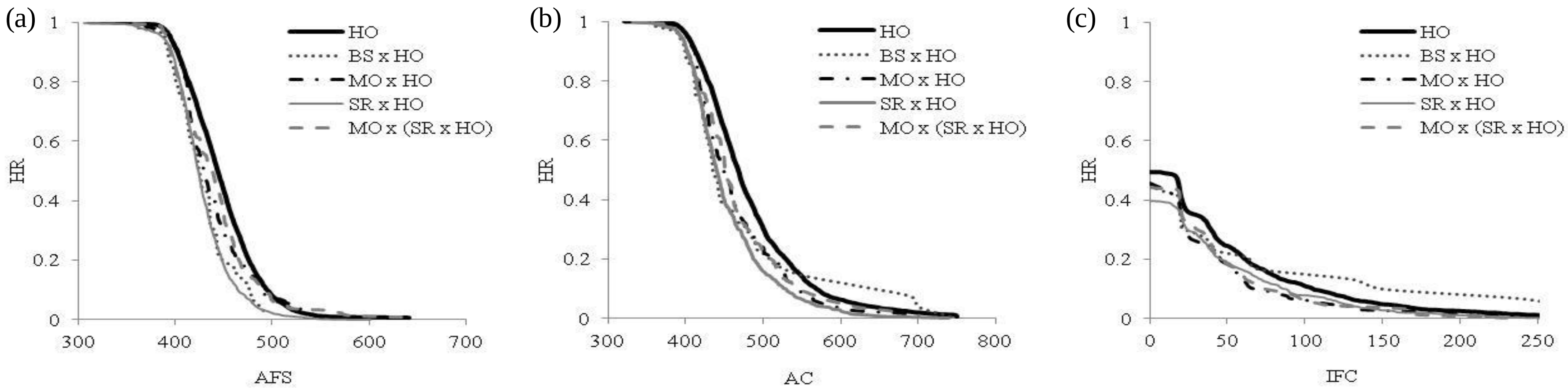
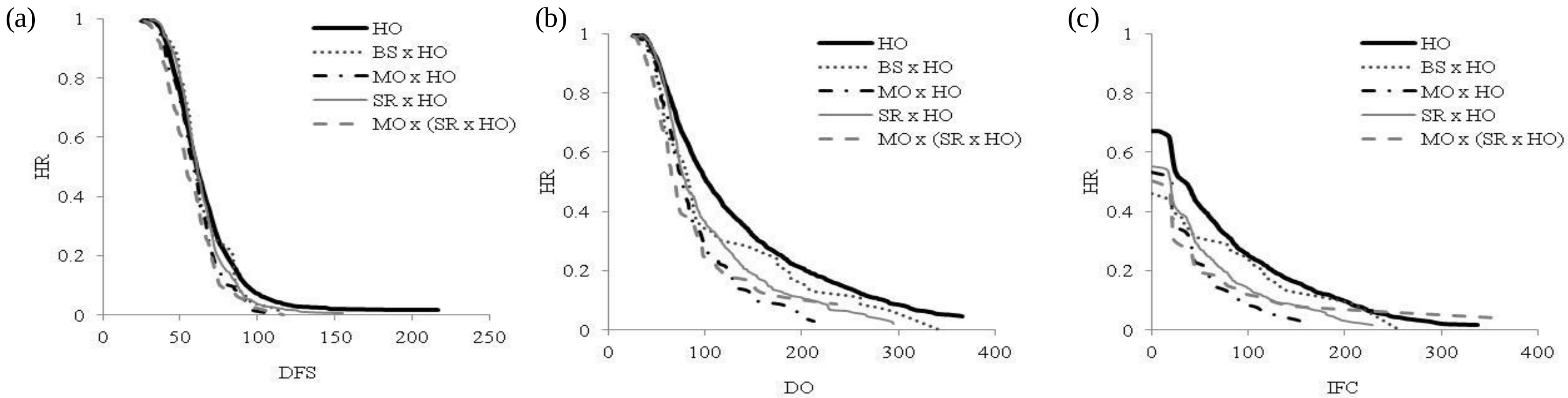


Figure 2. Kaplan-Meier survival curves for days at first service (DFS), days open (DO) and interval between first insemination and conception (IFC) for pure Holstein cows (HO), Brown Swiss x HO crossbreds (BS x HO), Montbeliarde x HO (MO x HO), Swedish Red x HO (SR x HO) and MO x (SR x HO) crossbreds cows.



Heins et al. (2008), found a positive effect of crossbreeding but a lower proportion of pregnant cows before 100 d postpartum for both purebred HO and crossbred JE × HO cows (31 and 41%, respectively). In another study, Olson et al. (2011) reported frequency of pregnant cows similar to those of the present study but at 150 days after calving instead of 100 days for first lactation of purebred HO and crossbreds JE × HO cows (56 and 66 % , respectively).

Effect of breed combination on fertility traits

Differences over reproduction time interval traits comparing crossbreds and purebred HO were greater in primiparous cows than in heifers (Table 3). However, some differences were found also among heifers. The SR × HO and MO × (SR × HO) crossbred heifers showed a higher risk to be inseminated (HR = 1.31 and HR = 1.18, respectively) and to become pregnant (HR = 1.34 and HR = 1.24, respectively) with time than pure HO heifers ($P < 0.05$). However, HR of IFC was closer to 1.00 for all crossbred combinations and significantly different only for SR × HO crossbred heifers ($P < 0.05$), also because of the greater number of observations. With the exception of BS × HO, the crossbred heifers tended to have a higher risk to become pregnant since first insemination than purebred HO. In agreement with these results, all crossbred heifers showed numerically (Table 4) a higher risk of non-return within 56 d, and of become pregnant after first insemination and to have less inseminations to conception than purebred HO. However, only SR × HO crossbred heifers were significantly different from purebreds with 46% higher probability to have NR56, 54% higher probability to have CR, and 50% higher probability to have less INS than purebred HO heifers.

Crossbreed primiparous cows were significantly different compared with purebred HO ($P < 0.05$) for all considered traits (Tables 3 and 4), except for BS × HO crossbreds, which showed no significant differences for DFS, DO and IFC, when compared with HO cows (Table 3). Also Blöttern et al. (2011) showed no difference for DFS and DO, when comparing pure HO cows with BS × HO primiparous cows. However, in the same study, the authors showed a difference in DFS between those two breed group in their second and third lactation, in favor of crossbreds cows. In another study, considering records of 5 lactation, Dechow et al. (2007) imputed 21 d more of DO to pure HO compared with BS × HO crossbreds. However, in the same study no differences for age at first calving were reported. Those results suggest that probably the reproductive potential of this crossbreds may increase over parity. However, in our study, BS × HO showed higher probability for NR56, CR, and

INS than purebred HO with odds ratio (OD = 2.17, 2.29 and 1.80; respectively) very close to those found for other crossbred combinations (Table 4).

Table 3. Estimated Hazard Ratio (HR) and 95% confidence interval (95%CI) of breed group effects for reproduction time interval traits of heifers (age at first service - AFS, age at first conception - AFC, and interval between first service and conception – IFC) and of primiparous cows (days to first service - DFS, days to conception - DO, and IFSC)

	Heifers		Primiparous cows	
Trait [1]	HR[2]	95%CI	HR[2]	95%CI
AFS/DFS[2]				
HO	1		1	
BS x HO	0.90	0.69 – 1.17	1.17	0.88- 1.56
MO x HO	0.93	0.78 – 1.12	1.40	1.15 - 1.69
SR x HO	1.31	1.19 – 1.43	1.30	1.15 - 1.43
MO x (SR x HO)	1.18	1.03 – 1.35	1.27	1.05 - 1.53
AC/DO[3]				
HO	1		1	
BS x HO	0.90	0.68 – 1.20	1.23	0.91 - 1.64
MO x HO	1.11	0.92 -1.33	1.59	1.30 - 1.94
SR x HO	1.34	1.22 – 1.46	1.43	1.29 - 1.58
MO x (SR x HO)	1.24	1.07 – 1.42	1.58	1.28 - 1.94
IFC				
HO	1		1	
BS x HO	0.91	0.68-1.20	1.09	0.8 1 -1.47
MO x HO	1.12	0.93-1.26	1.52	1.24 - 1.86
SR x HO	1.12	1.02-1.35	1.26	1.14 - 1.39
MO x (SR x HO)	1.10	0.96 -1.26	1.39	1.13 - 1.72

[1]HO: Holstein; BS: Brown Swiss; SR: Swedish Red; MO: Montbèliarde
[2]HR>1 (HR<1) means a higher (lower) risk of being inseminated/become pregnant with time than Holsteins
[3] AFS refers to heifers; DFS refers to primiparous cows.
[4] AC refers to heifers; DO refers to primiparous cows.

This is in contrast to Blöttern et al. (2011), who reported no significance difference for number of inseminations in the first three calvings for these crossbred, compared with pure HO. The MO x HO, SR × HO and MO × (SR × HO) crossbred cows showed higher risk to be inseminated early after calving (HR = 1.40, 1.30 and 1.27, respectively), and higher risk to had conception after calving (HR = 1.59, 1.43 and 1.58, respectively) than purebred HO cows (Table 3).

Table 4. Estimated Odds Ratio (OR) and 95% confidence interval (95%CI) of breed group effects for success traits (non returned after 56 d from first service - NR56, conception rate at first service - CR), and for number of inseminations to conception (INS)

	Heifers		Primiparous cows	
Trait [1]	OR [2]	95%CI	OR [2]	95%CI
NR56				
HO	1		1	
BS x HO	1.18	0.69 - 2.03	2.29	1.27 - 4.13
MO x HO	1.16	0.80 - 1.69	1.74	1.17 - 2.58
SR x HO	1.46	1.21 - 1.76	1.37	1.12 - 1.66
MO x (SR x HO)	1.04	0.79 - 1.38	1.99	1.34 - 2.95
CR				
HO	1		1	
BS x HO	1.29	0.75- 2.20	2.17	1.21 - 3.90
MO x HO	1.11	0.77 -1.60	1.76	1.18 - 2.62
SR x HO	1.54	1.28 - 1.86	1.44	1.18 - 1.76
MO x (SR x HO)	1.22	0.92 - 1.60	2.26	1.52 - 3.36
INS				
HO	1		1	
BS x HO	1.29	0.77 - 2.16	1.80	1.06 - 3.04
MO x HO	1.16	0.82 - 1.64	1.85	1.30 - 2.65
SR x HO	1.50	1.26 - 1.80	1.59	1.33- 1.89
MO x (SR x HO)	1.22	0.94 - 1.60	2.02	1.42 - 2.85

[1]HO: Holstein; BS: Brown Swiss; SR: Swedish Red; MO: Montbèliarde

[2]OR>1 (OR<1) means a higher (lower) probability of become pregnant or to have less (more) inseminations than Holsteins

Contrary to our findings in heifers, IFC was also different for all those crossbreds compared with purebred HO cows ($P < 0.05$). The MO × HO crossbred cows had 52% higher risk to conceive since the first service than purebred HO and SR × HO, and MO × (SR × HO) crossbreed cows had 26% and 39% more probability to occur in a pregnancy since the first insemination. Also, Heins et al. (2006) reported lower DFS and DO for NO × HO and MO × HO primiparous cows than pure HO cows, but no differences were reported for DFS between SCR × HO crossbreds cows and pure HO cows. Walsh et al. (2008) reported a higher DFS for HO cows then MO × HO cows (73 and 68 d, respectively), but no differences for the same traits between NO × HO and HO were found. In the same study, no differences were reported neither for DO nor INS between HO and crossbreds cows. However, a 13 week breeding season was imposed, so the maximum value of DO was limited, which could decrease potential differences between breed groups. For example, in a study using JE × HO crossbreds, Heins et al. (2008) found no differences when comparing those crossbreds with HO for the proportion of cows pregnant within 120 d of lactation, but a difference was reported when comparing HO with JE × HO crossbred cows pregnant within 150 and 180 d of lactation, reporting values of 59% and 61% for HO cows, and 75% and 77% for JE × HO cows. In the same study, fewer DO for JE × HO crossbred than pure HO cows (-23 d) were reported, however, conception rate at 6 insemination was not different between those two breed groups.

In accordance with our observations for time related reproductive traits, MO × HO, SR × HO and MO × (SR × HO) crossbred primiparous cows showed a higher probability for NR56 (OD = 1.74, 1.37 and 1.99, respectively) and CR (OD = 1.76, 1.44 and 2.26, respectively) than purebred HO cows. Heins et al. (2006) showed a higher CR for MO × HO and NO × HO compared with pure HO cows and a tendency of SR × HO cows to have a higher CR than pure HO cows. Contrary to those result, Walsh et al. (2008) reported no differences between purebred HO cows and MO × HO and NO × HO crossbred cows, for CR and also for INS, which, on the contrary, showed favorable values for crossbred cows in our study. The MO × HO, SR × HO and MO × (SR × HO) had a higher probability to have lower INS than purebred HO (OD = 1.30, 1.33 and 1.42).

Finally, although significant, differences in reproductive performance between HO and the crossbreeds were smaller in heifers than in primiparous cows (Tables 3 and Table 4). Other studies using pure breeds reported moderate genetic relationships between heifers and lactating cows, suggesting that fertility is indeed a different trait when evaluated on heifers and lactating cows, and that gene expression in heifers might be different from that of lactating cows (Tiezzi et al., 2012). If the reproductive performance of heifers is not affected by milk production, the fertility of lactating cows reflects the better ability of the cow to conceive when milk yield hinders reproductive physiology. Several studies reported a higher milk yield, combined with decreased reproductive performance for purebred HO, when compared with crossbred cows (Dechow et al. 2007; Heins et al., 2008; Heins et al., 2012). This suggests a disadvantage of purebred HO, which may be due partially to the effect of increased nutrient demands to support greater milk yield during early lactation. However, Walsh et al. (2008) reported no differences between NO × HO and MO × HO crossbred cows, and purebred HO cows for milk yield, but favorable values of DFS for crossbred cows. This suggests a difference in reproductive physiology between pure HO and crossbreds, and a better ability of these cows to respond to the high metabolic demands of milk production.

CONCLUSIONS

Our results indicate that, taking also into account the censored records (culled animals, incomplete data, etc.), crossbred cows had a greater reproductive potential when compared with purebred Holstein. Crossbred animals showed an early insemination after calving and also became pregnant before purebred Holstein cows. Crossbred cows showed a higher probability to conceive at the first insemination, and required less inseminations to achieve conception. Also for heifers the crossbreds tended to be numerically superior to purebreds, but this superiority was significant only for Swedish Red x Holstein crossbred heifers. This favorable effect in heifers was less pronounced than in primiparous cows, suggesting that the positive effect of crossbreeding on fertility is fully shown in response of cows to the demands of high milk production. Results from both cows and heifers in the present study suggest that crossbreeding can improve fertility of purebred dairy herds.

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Chapter 3

Effect of crossbreeding on days open and milk lactation curves parameters

ABSTRACT

Pure Holstein cows (HO, n = 1,632) were compared with Brown Swiss (BS) × HO (n = 48) crossbreds cows, Montbeliarde (MO) × HO (n = 62) crossbreds cows, Swedish Red (SR) × HO (n = 376) crossbreds cows, and MO × (SR × HO) (n = 66) for days open and milk curve parameters. 755,720 test day records for milk were analyzed using Wilmink function (WIL) and modified Wilmink function (PREG), in order to include the effect of pregnancy. Crossbreds cows showed fewer DO than pure HO, except for the BS × HO crossbred cows, for which no differences were found. Milk lactation curves parameters showed different values between crossbred cows and pure HO cows. Crossbred cows showed higher level of production (parameter **a**) than all crossbred cows. SR × HO crossbred cows showed a faster decrease rate of milk production after the peak than pure HO cows, and faster increase towards the lactation peak (parameter **b**), than pure HO, when estimated by the PREG model. MO × (SR × HO) crossbreds cows showed a more persistent lactation than pure HO, and BS × HO tended to have a higher persistency than pure HO cows. The effect of pregnancy on milk production was lower for MO × SR and MO ×(SR × HO) crossbreds cows than for pure HO, however, the effect of gestation on milk production started earlier for these two crossbreds. The use of pregnancy in the model increased the accuracy of milk production prediction after the milk peak, relative to the classic WIL model.

INTRODUCTION

The difference in milk yield between pure Holstein (HO) cows and crossbreds has been well characterized. Some studies imputed lower milk production on crossbreds cows when compared with pure HO (Heins et al., 2008, 2011; Heins and Hansen 2012) and other authors did not find differences between crossbreds and HO cows for milk yield (Walsh et al, 2008; Blöttner et al. 2011b), which may due to also to the use of different types of crossbreeds in these studies. However, the information available on milk lactations curves of crossbred cows is less abundant. Heins et al (2006) provided a preliminary assessment of the lactation curves of crossbred cows, and plotted the milk curves from pure HO and Normande × HO, Montbeliarde × HO, and Scandinavian Red × HO crossbred cows. These plots suggested that breed groups were similar for persistency of production throughout lactation. However, statistical analysis to assess the differences was not provided. In a study evaluating models for fitting curves of pure line and crossbreds cows, Batra (1986) suggested there was little evidence of nonadditive genetic variation associated with the coefficients of lactation curves, and the heterosis effects were reported to be not significant for most of the parameters.

Different models have been proposed to describe milk lactation curves. This models differed in the number of parameters and also in the degree of their relationships with the main features of a typical lactation pattern, such as peak yield, time at peak, and persistency. Three of the classic models developed specifically for lactation curves, and that e×plain these parameters, are Wood (1967), Willmink (1987), in with three parameters are associate with this pattern, and the logarithm-based model developed by Ali and Schaeffer(1987), that describe the milk curve with 5 different parameters. These mathematical functions could also be used to describe individual milk curves and to evaluate the effects of environmental factors, by comparing curve parameter values (Tekerli et al. 2000, Strucken et al., 2011).

The effects of pregnancy on milk yield have been reported in literature (Olori et al., 1997; Roche, 2003), and they become important in the last part of the gestation. This effect is due to a combination of the higher energy and nutrient requirements for the fetus (Bell et al., 1995), as well as to hormonal changes during this period. A way to incorporate the effect of pregnancy in the evaluation of milk yield, is the use of days open (DO). The effect of DO does not only affect milk yield, but DO also have a significant effect on the coefficients of the parameters used to describe the lactation curve (Batra, 1986; Sanders,1928). The persistency of the lactation curve is lower for cows with lower DO then cows with a longer DO. (Bhimanova et al. 2009; Brotherstone et al. 2004). However, the use of DO in the model can cause some bias due to the fact that cows with higher yield during early lactation have longer DO, so that using DO is influenced by this correlation (Lee et al., 1997). Bohmanova et al. (2009) reported that the use of pregnancy stage seems to better account for the effect of gestation than DO, and different model choices intended to account for the effect of gestation on milk production, have also an effect on ranking of both 305-d milk yield EBV and persistency EBV.

Considering these effect of pregnancy on milk yield and on the shape of the milk curve, it is advisable to account for pregnancy in the predictive models, in order to make a better comparison between crossbred cows and pure HO. Crossbreds cows showed fewer DO when compared to pure HO (Dechow et al., 2007; Heins et al., 2011), therefore the effect of pregnancy for crossbreds cows appears earlier in the lactation, compared with pure HO cows.

The aim of this study was to compare milk lactation curves of pure HO and crossbreds cows, using a mathematical model to describe the milk curves and considering the effect of pregnancy.

MATERIAL AND METHODS

Data collection

Test-day information for milk yield was collected every day directly in the farm by the management software Afifarm, version 3.06 (S.A.E. Afikim, Israel). Milk yield records correspond to data collected from first lactation cows, from 2008 to 2013, in four different herds located in northern Italy.

Only cows with at least 150 days in milk were considered and every cow required to have at least one record in the first 30 d of lactation. Herd-test-days for which less than 10 records were available, were excluded from the analysis, and DIM was required to be between 5 and 340 d. Only cows with fertility records available, such as insemination and calving dates, were included in the analysis. If data corresponding to the subsequent calving was available, the pregnancy was required to be between 30 d to the average of pregnancy days, calculated for each breed group. If the pregnancy time was out these limits, the cows were considered to be missing because the stage of pregnancy was not reliable. Gestation length was required to not exceeded 240 d, and beyond this limit, records were not considered in the analysis to avoid overlapping with the dry period, which should start right after this point. Milk data corresponding to periods of disease or any confirmed heath problem (mastitis, digestion problems, locomotion problems, etc) after veterinary checks, was excluded from the analysis, in order to avoid confounding effects of pregnancy and health disorders on milk production. Following data editing, 755,720 test day for milk from 1,632 pure Holstein (HO) cows, 48 Brown Swiss (BS) × HO crossbreds cows, 62 Montbeliarde (MO) × HO crossbreds cows, 376 Swedish Red (SR) × HO crossbreds cows, and 66 MO × (SR × HO) were analyzed.

Statistical analysis for DO

Cows were considered pregnant if a subsequent calving, or a pregnancy diagnosis by the veterinarian, were available, and the DO were calculated as the interval between calving and the last insemination. A fixed value of 250 was used if DO were higher than 250 d. In the same way, cows not pregnant by 250 DIM were assigned this 250 d value, as used by the Animal Improvement Programs Laboratory of USDA for routine genetic evaluations for cow fertility in the United States (VanRaden et al., 2004). 89 cows that were not pregnant and that were not at least 250 DIM by the time of data collection, were not considered for the analysis of DO. DO were analyzed using the GLM procedure of SAS (SAS Institute, 2011). The model for the statistical analysis included the effect of herd, breed combination and year-season of calving.

Milk lactation curve analysis

The differences in milk curve between crossbreeds and pure Holstein were analyzed using a random regression model based on the Wilmink (1987) function, with a NLMIXED procedure of SAS. The mathematic function proposed by Wilmink (1987) is described as follows:

$$Y_{DIM} = a + b \times \exp^{-k \times DIM} + c \times DIM \quad [WIL],$$

where parameter **a** is associated with the level of production, parameter **b** with the production increase towards the peak, parameter **c** with the production decrease after the peak, and **k** is a constant factor associated with the moment of the peak yield. **k** was fixed at 0.05 for all breed groups, as the original value proposed by Wilmink. Each parameter of the Wilmink function was estimated for each breed group and contrasts were computed for parameter differences between HO and crossbreds cows.

In order to estimate the effect of pregnancy, a modified Wilmink function was used, which can be described as follows:

$$Y_{DIM} = a + b \times \exp^{-k \times DIM} + c \times DIM + d \times PREG \quad [PREG],$$

where **d** represents the effect of pregnancy on production. PREG was estimated as DIM - (DO – *sp*), where *sp* is the starting point for the effect of pregnancy on milk production, and DO is days open. The *sp* value of the pregnancy effect, as well as the parameter **d**, were estimated for each breed, and HO parameters were compared with those of crossbreds.

RESULTS AND DISCUSSION

Effect of breed composition on DO

All the effects included in the model significantly affected DO (data not shown). Crossbred cows showed fewer DO when compared with pure HO, except for the BS × HO crossbred cows, for which no differences were found. In agreement with these results, Blöttner et al. (2001a) reported no differences for DO between BS × HO crossbred cows and pure HO cows. However, Dechow et al. (2007) reported 17 fewer days open (DO) for BS ×

HO crossbreds, when compared with pure HO, and Brandt et al. (1974) reported 34 fewer DO for BS × HO crossbreds, than for pure HO cows. The first generation crossbreds cows MO × HO and SR × HO reached the conception 25 d and 19 d before pure HO cows ($P <$ 0.001). Second generation of crossbreds showed similar results to those of the first generation, with -23 d of DO ($P < 0.01$) than pure HO cows. Heins et al. (2012) reported that first lactation Normande × HO, MO × HO and Scandinavian Red × HO crossbred cows had -20 d, -26 d and –12 d of DO, relative to pure HO cows, respectively.

Table1. Number of observation, lsquares means and standard errors of means (SE) for days open (DO) for pure Holstein *vs*. Crossbred cows.

Breed Group[1]	N	DO, d	SE	Contrast[2]
Pure HO	1,929	117	1.5	-
BS × HO	48	111	9.2	ns
MO × HO	102	92	6.3	***
SR × HO	532	98	2.9	***
MO × (SR × HO)	83	94	7.1	**

[1]HO-Holstein; BS- Brown Swiss; MO-Montbèliarde; SR-Swedish Red;
[2]Reference group pure HO cows - [3]**$P < 0.01$; ***$P < 0.001$;

Effect of breed composition and pregnancy on milk curve parameters

The level of production (parameter **a**) was higher in pure HO cows than in crossbred cows ($P < 0.001$). The highest differences between HO and crossbreds were found for BS × HO crossbred cows, followed by MO × (SR × HO), MO × HO, and SR × HO crossbreds cows. Contrary to our results, Blöttner et al (2011b), reported not difference in milk production between HO and BS × HO crossbreds cows. However, our results are agreement with other studies, that showed a higher milk yield for HO cows then crossbred cows, using MO, Scandinavian Red and Normande sire (Heins and Hansen, 2012). Changes on parameter **a** estimates with the PREG function were small among all the different breed groups.

The increase toward peak production (parameter **b**), was not different between pure HO and crossbreds cows, except for SR × HO crossbreds cows, which showed a faster increase towards the peak, but only in the model in which the effect of pregnancy was considered.
The persistency (parameter **c**) was lower in the crossbred SR × HO than in pure HO cows, and higher for SR × (MO × HO) cows than pure HO cows. These results do not agree with what

Table 2. Estimate values and standard errors (SE) of 3-parameter estimated using classic Wilmink function for pure Holstein cows *vs*crossbreds.

	Pure HO		BS × HO		MO × HO		SR × HO		MO × (SR × HO)	
Traits[1]	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
a	36.66	0.32	27.90***	1.18	33.36***	0.80	35.48**	0.39	30.73***	0.90
b	-18.66	0.23	-17.89	1.45	-18.35	1.00	-18.27	0.43	-20.39	1.09
c	-0.05	0.001	-0.047†	0.004	-0.047	0.003	-0.051**	0.001	-0.039**	0.003

HO-Holstein; BS- Brown Swiss; MO-Montbèliarde; SR-Swedish Red;
† < 0.10 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Estimate values and standard errors (SE) of 4-parameter and of the starting point (*sp*) for the effect of pregnancy on milk production, estimated using modified Wilmink function for pure Holstein cows *versus* crossbreds.

	Pure HO		BS × HO		MO × HO		SR × HO		MO × (SR × HO)	
Traits	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
a	36.33	0.30	28.17***	1.10	33.08***	0.77	34.37***	0.38	30.95***	0.85
b	-16.71	0.22	-15.09	1.42	-15.17	0.97	-15.76*	0.42	-16.90	1.07
c	-0.040	0.001	-0.033†	0.004	-0.038	0.003	-0.043*	0.001	-0.028***	0.003
d	-0.083	0.001	-0.087†	0.002	-0.062***	0.002	-0.082	0.001	-0.073***	0.002
spt	160	0.24	147***	1.22	148***	1.13	161	0.41	146***	1.11

HO-Holstein; BS- Brown Swiss; MO-Montbèliarde; SR-Swedish Red;
† < 0.10 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

was suggested by the study of Heins et al (2006). The authors provided a preliminary assessment of the lactation curves of pure HO and Normande × HO, MO × HO, and Scandinavian Red × HO crossbred cows, and suggested that the breed groups were similar for persistency throughout lactation. However, because statistical differences were not provided, a real comparison is not possible.

The variation of the persistency (parameter **c**) estimated with PREG model and with the WIL model was different between breeds (Figure 1). The parameter **c** estimated with PREG model was 30% higher than the one estimated by WIL model for BS × HO and MO × (SR × HO), and + 15% higher for pure HO and SR × HO crossbred cows. The MO × HO crossbreed was intermediate, with a change on the parameter of 20%.

The average of residual of the PREG model was smaller across lactation compared with the classic WIL function (Figure 1.) The residuals for the predicted milk after 50 DIM estimated with the PREG function were lower than those obtained with the classic WIL function (Figure 2), which tends to overestimate milk yield in mid lactation and to underestimate it in late lactation. This is probably due to the ability of the PREG model to account for the effect of pregnancy on milk yield after peak lactation. According to Haile- Mariam et al. (2003), when the effect of pregnancy is not included in the model, milk yield is often overestimated at the beginning and underestimated at the end of lactation.

The estimated start point of the effect of pregnancy was similar to that reported in other studies. Coulun et al. (1995), reported a noticeable effect of pregnancy around the 20th week of gestation and imputed an important effect of the pregnancy (>1 kg/d) after the 25th of gestation. Similar to our results for pure HO cows, Strandberg and Lundberg. (1991) estimated that pregnancy started to affect milk yield around 160 d after conception by -0.1 kd/d Finally, the estimated effect of pregnancy (parameter **d**), was slower in SR × HO and SR × (MO × HO) than in pure HO cows ($P < 0.001$) and tended to be higher for BS × HO crossbred compared with pure HO cows ($P < 0.10$). MO × HO and MO × (SR × HO) showed an earlier start of the pregnancy effect than pure HO cows (-12 d and 14d, respectively; $P < 0.001$), so it is possible that the lower **d** value is due also to a bigger spread of days of gestation. However, the effect of pregnancy started early also for BS × HO crossbred cows (-13 d, P < 0.001) than HO cows, but the estimated effect of pregnancy (parameter **d**) tended to be higher for BS × HO crossbred cows (P < 0.10), compared with pure HO cows.

Figure 1. Estimated milk curve of pure Holstein (HO), Brown Swiss × HO crossbreds cows (BS × HO), Swedish Red × HO crossbreds cows (SR × HO), Montbeliarde × HO crossbred cows (MO× HO) and MO × (SR × HO) cows, using classic Wilmink(1987) function [- - -] and a modified willmink function that considered the effect of pregnancy [——].

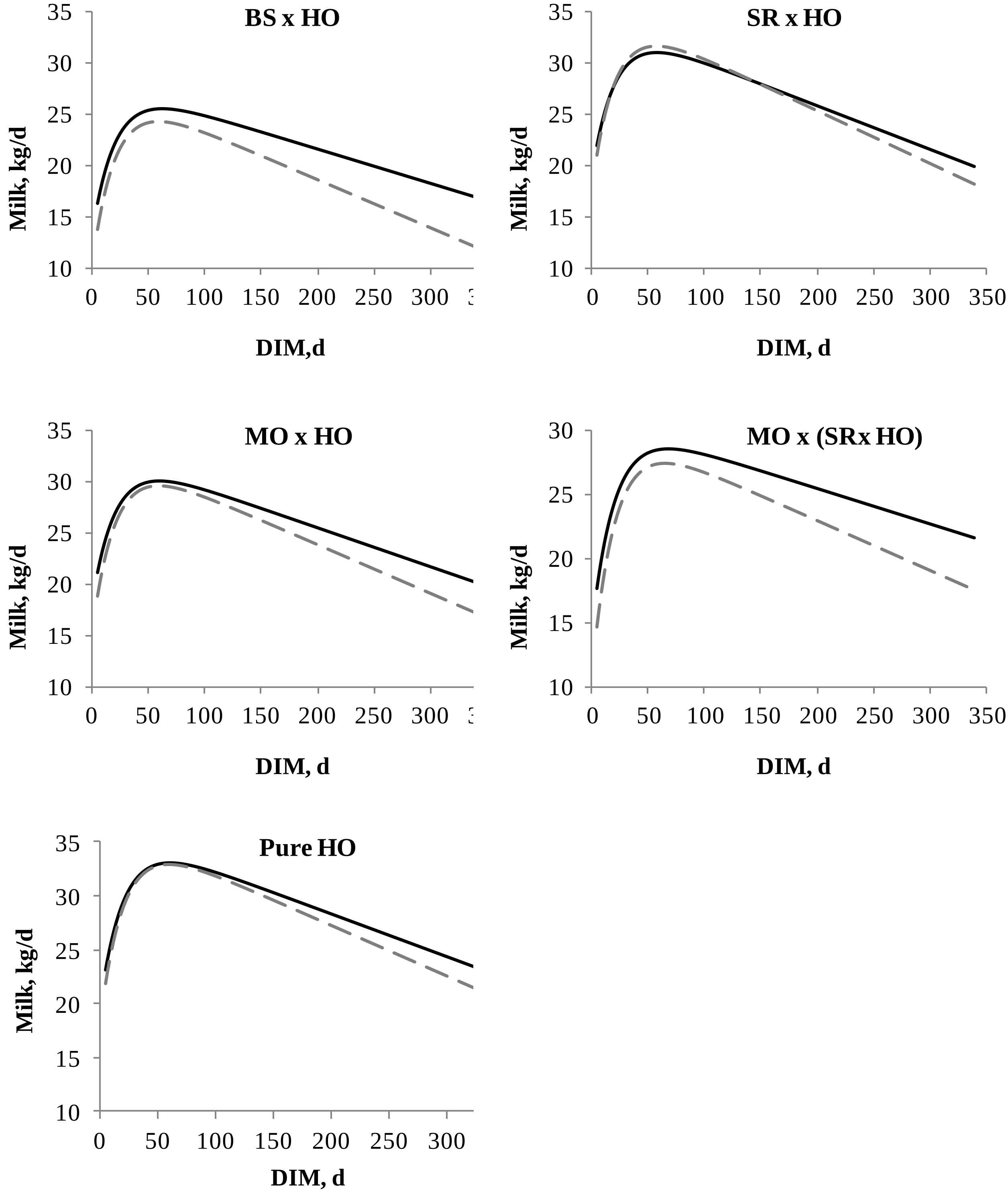
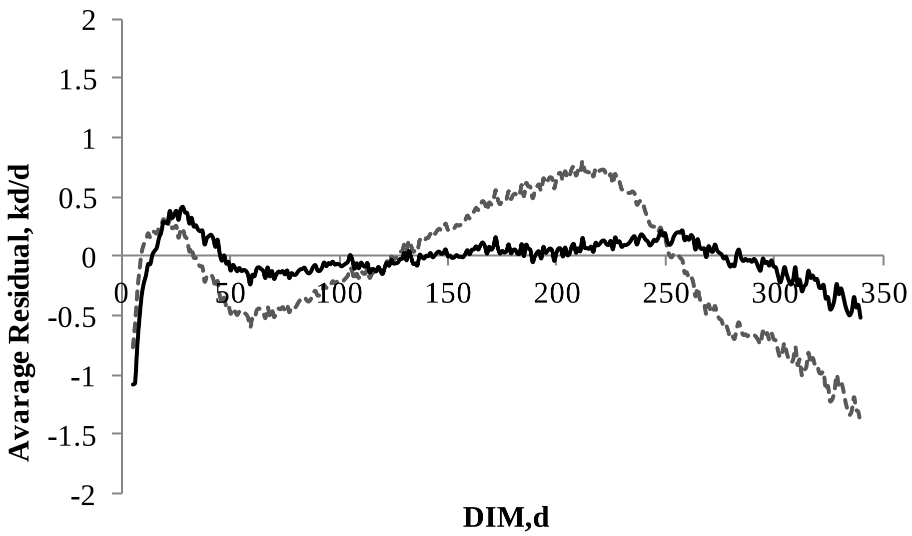


Figure2. Distribution of average residual (kg/d) for DIM 5 to 340 for Wilmink function [- - -], and for modified function [——].



This difference could be maybe explained from the likely differences on calf weight at birth, as more energy required for bigger calves. McCanadlish reported a correlation between mature weight of the dam and birth weight of the calf, that was around 6-8% of the dam weight. Blöttner et al (2011a) reported higher weight for BS × HO first lactation cows, compared with pure HO cows (621 kg *vs* 594 kg, respectively). However, in the same study, no differences were reported in calf weight at birth of HO-sired calves from BS × HO Holstein crossbred dams and pure HO calves.

CONCLUSIONS

Crossbred cows showed fewer DO when compared with pure HO, which suggests that for a better comparison of milk curves, the effect of pregnancy should be taken into account in the predictive model. Results show that crossbred cows had different milk curves that pure HO cows. Overall, crossbreds showed a lower milk production level (parameter a) than pure HO cows, but no differences were found for the increase toward the peak (parameter b), with the exception of SR × HO crossbreds cows, which showed a faster increase than pure HO, but only when estimated by the PREG model. MO × (SR × HO) crossbreds cows showed a more persistent lactation than pure HO, and opposite SR × HO crossbred cows showed a faster decrease rate of milk production after the peak than pure HO cows. The effect of pregnancy on milk production was lower for MO × SR and MO ×(SR × HO) crossbreds cows than for pure HO, but the gestation start to affect milk production earlier in these two crossbreds. The use of pregnancy on the model increased the accuracy of milk production prediction after the milk peak, relative to the classic WIL model.

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Chapter 4

Milk yield, protein and fat content of crossbred dairy cows obtained from Brown Swiss, Swedish Red and Montbéliarde sires compared to Holstein Friesian cows.

ABSTRACT

Pure Holstein (HO; n = 1,632), first generation crosses between Brown Swiss and HO (BS × HO; n = 48), Montbeliarde and HO (MO × HO; n = 62), Swedish Red and HO (SR × HO; n = 376), and first generation crosses between MO and SR × HO (MO × (SR × HO); n = 66) were compared for milk, fat and protein yield, fat and protein percentage, and somatic cell score (SCS). A total of 755,720 test-day information records for milk yield and 16,570 for fat and protein content and SCS were taken from first lactation cows milked in four herds in northern Italy. Production traits and SCS were analyzed accounting for pregnancy stage in the model. Stage of pregnancy previously detected and a start point for the effect on production was previously estimated for each trait and was fixed to be 70d for fat and protein content and 150d of gestation for milk, fat, protein yield, and SCS. Pure HO showed higher level of milk production than all the crossbreds cows, but a lower protein content. Fat content was higher for SR × HO crossbreds cows than pure HO. Pure HO showed higher protein and fat yield then all the other animals, except for BS × HO crossbreds that showed not differences in these traits, when compared with pure HO. Finally, SCS was lower for BS × HO and SR × HO crossbreds cows, than pure HO cows.

INTRODUCTION

During the last few years, production and milk quality of crossbred cows has been extensively characterized (Heins et al., 2006; Prendiville et al., 2010, Dechow et al., 2007), with contrasting results across crossbreed types. Walsh et al. (2008) reported no differences in milk production parameters between pure Holstein (HO) and Montbeliarde (MO) × HO and Normande (NO) × HO) cows during the whole lactation, except for fat yield, which was lower for MO × HO crossbreds than pure HO cows. In a subsequent study, Heins and Hansen (2012) utilized the same crosses, as well as Scandinavian Red (SCR) × HO crossbreds cows, and reported favorable results for milk, fat and protein yield for pure HO across 5 lactations. Heins et al. (2011) reported higher milk and protein yield for HO than for Jersey (JE) × HO cows during the first three lactations, and a difference in fat yield in the third lactation, in favor of pure HO cows, thus confirming the results reported in a previous study for first lactation cows (Heins et al., 2008). Comparing pure HO with Brown Swiss (BS) × HO crossbreeds cows, Blöttner et al. (2011b) reported no significant differences for milk, protein and fat yields over the first three lactations.

Even if the use of crossbred cows in dairy herds could negatively impact milk production, US dairy producers are currently more interested in the potential positive effects of crossbreeding on cow fertility, health and survival (Weigel and Barlass, 2003). Dechow et al. (2007) reported 17 fewer days open (DO) for BS × HO crossbreds, when compared with pure HO. This results confirmed previous studies (Brandt et al. 1974) reported BS × HO crossbreds had 34 fewer DO than pure HO. Heins et al (2008) reported a tendency of JE × HO crossbreds to have fewer mean DO, achieving conception 23 d before pure HO cows. In the same study, the proportion of cows pregnant at 150 and 180 d postpartum was significantly higher for JE × HO crossbreds than pure HO. At 150 d postpartum, 75% of JE × HO were pregnant compared with only 59% of pure HO, and at 180 d postpartum, 77% of JE × HO were pregnant compared with 61% of pure HO. In a more recent study, Olson et al. (2011) confirmed a tendency of these crossbreds to have favorable fertility than pure HO cows, even if the results were not statistically significant. Heins et al. (2012) reported that NO × HO, MO × HO and SCR × HO crossbreds had 12 – 26 fewer days open across 5 lactations, than pure HO cows.

The differences in DO between crossbreds and pure HO cows, and the significant effect of DO in current lactation that has been documented to decrease milk yield (Lee et al., 1997; Makusaan McDaniel, 1996), suggests that the appropriate adjustment for breed group differences in days open might bring the production of the crossbred groups closer to the production of pure HO cows (Heins et al. 2006). However, Lee et al. (1997) suggest that the use of days open alone is probably not an accurate means of assessing the effect of pregnancy, because cows with higher yield during early lactation have longer DO, so that using DO accounts for both effect of pregnancy and of higher production of these cows. Because of this, standardization of 305-d yield for current DO should be based on methods that consider and remove the effect of early yield to prevent for this environmental effect. The effect of pregnancy on production was found to be not linear during gestation, being close to zero until the in the late period of the gestation, around day 190 of pregnancy (NRC, 2001). Bell et al (1995) estimated that the net energy requirements for conceptus growth during in mature HO increased from 567 kcal/d to 821 kcal/d between 190 d and 270 d gestation. In the same study the rate of change in protein requirements corresponds to the rates of accretion from 62 to 117 g/d. However, other authors reported noticeable divergence between pregnant and not pregnant cows around 100 days of gestation (Olori et al., 1997; Roche, 2003), and also the effect of gestation started in a different moment for fat and protein content, increasing from 77 and 133 d of gestation, respectively (Roche, 2003).

The objective of this study was to compare pure Holstein with 2 and 3–way program crossbred cows for milk production traits and somatic cell count, investigating the effect of pregnancy.

MATERIAL AND METHODS

Data collection

Test-day information for milk yield, and for fat and protein concentration and SCC were recorded on first parity cows from 2008 to 2013 in four herds in northern Italy. Milk yield records were collected every day directly from the farm, and milk composition parameters were obtained from samples collected during monthly test-day milk recording by the breeders association. All traits were collected from the management software Afifarm, version 3.06 (S.A.E. Afikim, Israel).

Milk data from sick cows (mastitis, digestion problems, lameness, etc) was deleted from the analysis. Fat content was required to be between 0.5 and 7 % (as is basis) and protein content was required to not exceed 6 %. Only cows with fertility records available were included in the analysis. Cows were considered pregnant if a subsequent calving, or a pregnancy diagnosis by the veterinarian were available, and the stage of pregnancy was calculated as days from the last insemination. If data corresponding to the subsequent calving was available, the pregnancy was required to be between 30 d to the average of pregnancy days, calculated for each breed group. If the pregnancy time was out these limits, the cows were considered to be missing because the stage of pregnancy was not reliable. Days of pregnancy were required to not exceed 240 d of gestation, and records beyond this limit were not considered in the analysis. Only cows with at least 150 d in milk were considered and only days in milk (DIM) between 5 and 340 d were used. Each herd-test-day was required to have at least 10 records. Following the editing process, 755,720 test day records for milk, 16,570 test day records for milk quality and SCS from pure 1,632 Holstein (HO) cows, 48 Brown Swiss (BS) × HO crossbreds cows, 62 Montbeliarde (MO) × HO crossbreds cows, 376 Swedish Red (SR) × HO crossbreds cows, and 66 MO × (SR × HO), were analyzed. Fat and protein yields were calculated by multiplying milk yield and the relative percentages of fat and protein. To normalize the distribution of the data, somatic cell count was log-transformed to somatic cell score (SCS) according to the formula by Ali and Shook (1980): $SCS = \log_2(SCC/100{,}000) + 3$.

Statistical Analysis

Milk production traits and SCS were analyzed using the MIXED procedure of SAS (SAS Institute, 2011) with the following linear model:

$$y_{ijk} = \mu + HTDi + DIM + DIMb + Breed_j + PREG + Cow_k(Breed_j) + e_{ijk},$$

where y_{ijk} is the dependent variable; μ is the overall mean; HTDi is the fixed effect of the ith herd-test-day (i = 1 to 4443); DIM is the days milk used as covariate; DIMa is the EXP($^{-0.05*DIM}$) use as covariate; $Breed_l$ is the fixed effect of the *l*th breed combination (*l* = 1 to 7); PREG I the effect of pregnancy used as covariate; $Cow_k(Genotype_l)$ is the random effect of the kth cow nested within the lth genotype (m = 1 to 2782) N ~ (0, σ2cow(breed)); e_{ijk} is the random residual N ~ (0, σ2e). Significance of genotype effect was tested on the cow within breed group variance. Contrasts between least squares means of traits for HO versus BS × HO, MO × HO, SR × HO, and MO × (SR × HO) were estimated, and a 5% level was referred to for testing if means were significantly different.

The effect of pregnancy (PREG) was assumed to be zero until the day of pregnancy in which this condition was estimated to change and to have effect on the evaluated traits. This starting point was estimated for each variable with a non linear procedure and was estimated to be at 150 d of pregnancy for milk, fat and protein yield, and for SCS, and at 70 d of pregnancy for fat and protein content. The function used to estimate the pregnancy effect was a modified Wilmink (1987) function, described as follows:

$$Y_{DIM} = a + b \times \exp^{-k \times DIM} + c \times DIM + d \times PREG$$

where parameter **a** associated with the level of production, parameter **b** with the production increase toward peak, parameter **c** with production decreasing after the lactation peak. Parameter **k** is a constant factor assumed to be -0.05, and it is associated with the moment of the peak yield. Parameter **d** represents the effect of pregnancy on production. PREG was estimated as DIM - (DO - knot), where knot is the starting point of pregnancy, estimated for each variable, and DO is days open.

RESULTS AND DISCUSSION

Descriptive statistics of studied traits are summarized in Table 1. On average, cows produced 29.09 kg/d of milk per day with 3.69 % of fat and 3.50 % of protein. Mean values of fat and protein yield were 1.10 kg/d and 1.04 kg/d, respectively, and average SCS was 2.71.

Table 1. Descriptive statistics of milk yield, protein and fat content, protein and fat yield, and Somatic Cell Score (SCS).

Trait[2]	n	Mean	SD	P1	P99
Milk yield, kg/d	736,453	29.09	6.91	11.60	43.82
Fat, %	16,098	3.69	0.80	1.91	5.85
Protein,%	16,120	3.50	0.33	2.76	4.42
Fat, kg/d	16,098	1.10	0.33	0.43	2.13
Protein, kg/d	16,120	1.04	0.21	0.44	1.48
SCS	16,120	2.71	1.61	-0.18	7.48

[1]P1 = first percentile; P99 = 99th percentile

The sources of variation included in the statistical model are shown in Table 2. All the effects in the model affected all the studied traits ($P < 0.001$). Stage of lactation was a major source of variation for all studied traits. Pregnancy affected also all studied traits and was a higher source of variation for milk and protein yield. Breed group and HTD were less important than DIM and pregnancy stage, but nevertheless affected all the studied traits.

In agreement with other studies (Heins et al., 2006, 2012), pure HO cows had higher milk yield than BS × HO crossbreds (-1.51 kg/d; $P < 0.05$), MO × HO (-1.80 kg/d; $P < 0.001$), SR × HO crossbred cows (-1.82 kg/d; $P < 0.001$). Even after adjusting for the effect of pregnancy, pure HO cows showed a higher milk production than all crossbreed cows. However, these results may be due also to a differential effect of pregnancy across breeds. In a previous study, Malchiodi et al. (unpublished data), found a lower effect of pregnancy on milk production for MO × HO and MO × (SR × HO) crossbred cows, when compared with pure HO. BS × HO crossbreds, MO × HO crossbreds, and SR × HO crossbreds showed higher protein content (-0.11, -0.08, and -0.15 %, respectively; $P < 0.001$), when compared to pure HO. Heins and Hansen (2012) reported similar results, with higher means for protein content in NO × HO, MO × HO, and SCR × HO crossbreds, compared with pure HO cows (+0.17% , +0.09% and +0.11%, respectively). In another study, Brandt et al. (1973) reported similar result for BS × HO crossbred cows, which had higher protein contents, compared with pure HO (+0.12%). However, in that study, a higher fat content for BS × HO was also reported (+0.4%). This is in contrast with our results, in which fat content was different only when comparing pure HO cows with SR × HO crossbred cows (+0.18 kg/d; $P < 0.001$).

Table 2. Results from ANOVA (*F*-value and significance) for milk yield, protein and fat content, protein and fat yield, and Somatic Cell Score (SCS).

Trait	HTD		BREED		DIM1		DIM		PREG	
	F value	*P* Value	*F* Value	*P* Value	*F* value	*P* Value	*F* value	*P* Value	*F* value	*P* Value
Milk, kg/d	17.94	***	19.62	***	202,807	***	14,966.80	***	3,442.60	***
Fat,%	26.34	***	10.96	***	789.62	***	354.47	***	82.17	***
Protein, %	47.41	***	49.5	***	1447.39	***	6581.63	***	165.71	***
Fat, kg/d	29.26	***	8.48	***	172.12	***	722.54	***	106.13	***
Protein, kg/d	17.94	***	5.25	***	2,059.11	***	678.09	***	1,127.98	***
SCS	5.07	***	6.56	***	149.5	***	290.8	***	8.57	**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Number of observations, last squares means and standard errors of means (SE) for milk yield, milk quality traits and SCS for pure Holstein versus Crossbred cows

Traits	Pure HO (n = 1632)		BS × HO (n = 48)		MO × HO (n = 62)		SR × HO (n = 376)		MO × (SR × HO) (n = 66)	
	Mean	SE	Mean difference[1]	SE	Mean difference[1]	SE	Mean difference[1]	SE	Mean difference[1]	SE
Milk, kg/d	29.82	0.11	-1.51*	0.70	-1.80***	0.48	-1.82***	0.23	-1.90***	0.54
Fat,%	3.66	0.01	0.05	0.07	0.05	0.06	0.18***	0.03	- 0.03	0.06
Protein, %	3.47	0.01	0.11***	0.03	0.08**	0.02	0.15***	0.01	0.09***	0.02
Fat, kg/d	1.10	0.01	-0.04	0.03	-0.06**	0.02	-0.03**	0.01	-0.10***	0.02
Protein, kg/d	1.05	0.01	-0.02	0.02	-0.05**	0.02	-0.03***	0.01	-0.04*	0.02
SCS	2.83	0.03	-0.42*	0.17	0.03	0.15	-0.32***	0.07	0.08	0.15

[1]Last square means of crossbred cows are expressed as the difference from pure Holsteins;
HO-Holstein; BS- Brown Swiss; MO-Montbèliarde; SR-Swedish Red;
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Our results are in agreement, however, with those of Walsh et al. (2008), who reported no differences between HO and MO × HO crossbreds cows for fat content. However, in the same study, no differences were reported between HO cows and MO × HO crossbreds for protein content and protein yield, in contrast to our results, in which protein yield was higher for HO cows than for MO × HO (+0.05 kg/d; $P < 0.01$), and SR × HO (+0.03 kg/d; $P < 0.001$). In accordance with our results, Heins et al. (2006), reported differences for protein yield between HO and MO × HO , SCR × HO and NO × HO crossbred cows (+12, +8, and +28 kg/cow, respectively), for the 305-d actual production during first lactation. In the same study, HO cows showed higher fat yield then MO × HO and NO × HO crossbred cows (+12 and +27 kg/cows, respectively). In our study, fat yield was higher for pure HO than MO × HO and SR × HO crossbreds (+0.06 kg/d and +0.03 kg/d, respectively; $P < 0.01$). In accordance with these results, Walsh et al. (2008) reported higher complete lactation fat yield for pure HO cows than MO × HO crossbreds (+ 0.07 kg/cow). In accordance with the results reported by Blöttner et al. (2011), no differences were found between pure HO cows and BS × HO crossbred cows, both for fat and protein yield. However, in the same study Blöttner et al. (2011) reported no difference between HO and BS × HO crossbreds cows for milk yield. Similarly, Swalve et al. (2008) reported no difference in milk yield for BS ×HO cows and HO cows in first and second lactations, but, contrary to our results they also indicated higher fat and protein yield for BS × HO cows in first lactation, when compared with pure HO.

BS × HO and SR × HO crossbreds cows showed lower values of SCS (-0.42 and -0.32, respectively; $P < 0.05$ and $P < 0.001$), when compared with pure HO. This results disagree with other studies that reported no significantly differences for SCS, when compared pure HO cows with BS × HO crossbreds (Dechow et al. 2007;. Blöttner et al. 2011b). Similar to our results, Walsh et al. (2007) reported no differences between HO and MO × HO for SCS. In another study, Heins et al. (2012) reported significant differences between pure HO cows and MO × HO crossbreds and SR × HO crossbred cows (+0.28 and +0.20, respectively) during the first lactation, but no differences were reported between pure HO cows and NO × HO crossbreds. In the same study, this result in favor of crossbreds cows were confirmed also across 5 lactations.

In contrast to the numerous reports on one-way crossed found in literature, information regarding 2-way (or more) is very limited. MO × (SR × HO) crossbreds showed lower milk, fat and protein yield (-1.9, -0.04, and -0.10 kg/d, respectively; $P < 0.05$), than pure HO cows. Heins (2007), reported results from a study involving HO, MO, SCR and NO breeds in a 2-breed and 3-breed crossbreding program. However, because of a difference in the time of calving, a comparison between second-generation first-lactation cows with contemporary pure HO cows was not possible, and thus, only a comparison between 2-breed and 3-breed crossbreds was reported. The production of 2-breed and 3-breed crossbreds was very similar, and not differences were reported for milk, fat and protein yield. This suggested that the reduced HO in 3-breed crossbreds (25%HO) did not lowered the production capability. Hazel et al. (2013) reported results from MO x HO and MO x (JE x HO) crossbreds cows. MO x HO crossbred cow tended to have lower 305-d milk volume then pure HO, and MO ×(JE x HO) crossbreds had lower milk yield, compared with pure Holsteins. No differences between pure HO and these two crossbreds were reported for fat and protein yield, and for SCS. In our study, second generation MO × (SR × HO) crossbreds showed not differences in milk yield when compared with the first generation SR × HO crossbreds (data not shown). However, protein and fat content, as well as fat yield, were higher for SR × HO crossbreds than for MO × (SR × HO) crossbreds. Finally, SCS was higher in the second generation crossbred cows than in the first generation of crossbred, suggesting more data has to be collected to better characterize the effects of using 3-way crossbreeding programs.

CONCLUSIONS

Our results show that pregnancy affects all milk production traits. Even when considering effect of pregnancy, HO cows showed higher milk production compared with all other crossbreds, and were also superior for protein and fat yields. One exception was the BS × HO crossbred cows, for which protein and fat production was similar with pure HO cows. However, crossbreds cows showed higher contents of milk protein, and, particularly, SR × HO cows showed higher fat content, compared with pure HO.

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Chapter 5

Milk quality, coagulation properties and curd firmness modeling of purebred Holsteins and first/second-generation crossbred cows from Swedish Red, Montbeliarde and Brown Swiss bulls

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ABSTRACT

The objective of the present study was to investigate how the crossbreeding of Holstein (H) cows with bulls from Nordic and Alpine European breeds affect milk quality traits, traditional milk coagulation properties (MCPs) and curd firmness model obtained from individual milk samples. A total of 506 individual milk samples were collected from evening milkings (2-3 sampling days per herd) at three commercial farms located in the province of Modena, Northern Italy, between February and March of 2013. Over the past decade, the three farms have followed crossbreeding programs in parts of their herds, while the remainder of the animals consisted of purebred H. The basic scheme was a three-breed rotation based on the use of Swedish Red (S) semen on H cows (S × H), the use of Montbeliarde (M) semen on first-cross cows [M × (S × H)], and the use of H semen in the third cross. In all herds, a smaller proportion of purebred H were mated to M and Brown Swiss (B) bulls, and these first crosses were mated to S and M bulls, respectively. Milk samples were analyzed for milk composition and MCPs, and parameters for curd firmness were modeled. Our results revealed that different sire breeds were characterized by specific technological aptitudes, but that these were not strictly related to other milk quality traits. Furthermore, the favorable characteristics (in terms of the quality and technological properties of milk) could be maintained in the third generation of three-way crosses without negative effects on milk yield, even though the H heritage had been reduced from 50% to 25%. Our finding, therefore, suggest that different types of sires can be chosen (depending on the intended use of the milk) to assure the optimization of farm crossbreeding programs. However, further studies are needed to better understand the genetic bases of the technological properties of milk from different breeds, as well as the possible maternal or dominance effects of different breed combinations.

INTRODUCTION

Crossbreeding programs in dairy cattle are a viable strategy in the milk industry, as they may alleviate the fertility and longevity problems that can occur as a result of selection programs in dairy breeds (Heins and Hansen, 2012). However, questions have been raised regarding the need for information on optimum breed utilization (McAllister, 2002).Weigel and Barlass (2003) performed a comprehensive analysis in US dairy producers that were using crossbreeding programs. Most of respondents cited the need to improve cow fertility, health and survival as the reasons for their interest in crossbreeding. Many also emphasized the need to improve the milk fat and protein contents.

The milk production and milk quality of crossbred cows has been extensively characterized (Heins et al., 2006; Prendiville et al., 2010, Dechow et al., 2007). When compared with pure Holstein (H) cows, crossbred cows are generally characterized for producing less quantity of milk, fat and protein (kg), with higher concentrations of milk fat and protein (%). However, the extent of these differences may vary by the crossbreed type. Heins et al. (2006) evaluated the milk yield and milk quality of Scandinavian Red × H, Montbeliarde × H, and Normande × H crossbreds compared with purebred H. The authors reported that the milk, fat and protein yields were higher for purebred H versus the crossbreds, except for the case of Scandinavian Red × H, in which no significant difference was observed in the fat and protein + fat yields compared with pure H cows. In another study, Blöttner et al. (2011b) reported no difference between Brown Swiss × H crossbreeds cows and purebred H cows with respect to the protein and fat yields over the first three lactations. The effect of crossbreeding on dairy cows have also been analyzed across different environmental conditions (Penasa et al., 2010; Kargo et al., 2012; Vance et al., 2012).

The pricing systems for milk and milk products, which may differ substantially, determine the relative values given to milk volume and milk composition. For example, when calculating cheese merit economic values ($), milks high in protein (%) receive a higher price while those with a high fluid volume receive a penalty. In addition to specific milk pricing, farm profitability can also be impacted by the use of crossbreeding. VanRaden and Sanders (2003) estimated that the expected profitability of crossbred cows was higher in terms of both Net Merit$ and Cheese Merit$, when Brown Swiss × H and Jersey × H crossbreds were compared with purebred H. Along these lines, Heins et al. (2012) found that lifetime production of Montbeliarde × H and Scandinavian Red × H was higher than that of pure H (by 50% and 44%, respectively) and that the profit per cow-day was also superior for these crossbreds (by 5.3% and 3.6%, respectively).

As reported by the International Dairy Federation (2012), the marketing of cheese has grown in recent years, and cheese plays an important role in the economics of dairy production. Some technological properties of milk are particularly important for producing traditional cheeses according to the Protected Designation of Origin (PDO) designated by the E.U. The strict definitions of the processing techniques and conditions that may be used to produce PDO cheese do not allow the use of modern technology to overcome inadequacies in the characteristics of the utilized milk; thus, top-quality milk must be guaranteed (Martin et al., 2003; Saccà et al., 2003; Summer et al., 2003; Malacarne et al., 2006). The technological properties of individual milk samples have traditionally been analyzed through the assessment of milk coagulation properties (MCPs; Annibaldi et al., 1977; McMahon and Brown, 1984) and, recently, through model cheese production (Bittante et al., 2013a; Cipolat-Gotet et al., 2013; Ferragina et al., 2013). The most commonly analyzed MCPs are coagulation time and curd firmness, which are obtained during the 30-min period from the addition of the enzyme (rennet) to milk. These parameters are not available for milk samples that do not coagulate by the time the analysis is finalized, making MCPs less useful when the incidence of non-coagulating (NC) milk is significant. This often happens with milk from Holstein cows and some North European breeds (Ikonen et al., 1999), which are often used in crossbreeding schemes. The presence of NC milk is not only a problem in the dairy industry; it is also an issue for researchers, because it can cause biased estimations of phenotypic or genetic parameters if proper statistical models are not used (Ikonen et al., 1999; Cecchinato and Carnier, 2011; Cecchinato, 2013). Recent studies (Cipolat-Gotet et al., 2012; Cecchinato et al., 2013) in which the lactodynamographic test was prolonged to 90 min after rennet addition demonstrated that all samples coagulated, and the so called "NC" samples should more properly be defined as very late coagulating samples. To overcome these limitations, it was recently suggested that researchers should prolong the lactodynamographic test and model all of the point observations collected during the analysis for each individual sample (Bittante et al., 2012).

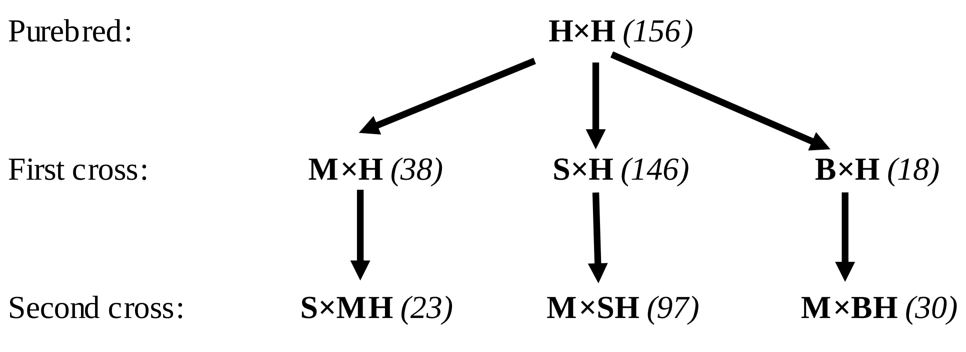
Milk coagulation properties are influenced by various environmental and genetic factors. Among the genetic factors, the MCPs of ruminants are most strongly influenced by the animal species, with the animal breed as the second most influential factor. Some breeds of central southern Europe, especially the Alpine region, generally show better MCPs than those originating in northern Europe (Bittante et al., 2012). However, while the effects of various breeds on MCPs have been well characterized, the effects of crossbreeding have not yet been properly addressed. In a feeding experiment, Kreuzer et al. (1996) compared Jersey × H crossbred cows with the corresponding pure parental breeds, and found that the MCPs of crossbreds were more similar to those of Jersey milk compared to H milk. Tyrisevä et al. (2004) reported on the MCPs of Finnish Ayrshire × H crossbreds cows. However, the studied crossbred cows all came from a Finnish Ayrshire and H dam or sire and an unknown second parent, limiting the authors' ability to make inferences with respect to specific crossbreeds. Therefore, the aim of this study was to investigate the effect of crossbreeding H cows with bulls from Nordic and Alpine European breeds on milk quality traits, traditional MCPs, and curd firmness parameters modeled from individual milk samples.

MATERIALS AND METHODS

Milk Sampling

Individual milk samples (n = 506) were collected from the evening milkings of three commercial farms located in the province of Modena, Northern Italy, between February and March of 2013 (2-3 sampling days per herd). All herds were managed according to the rules for producing the PDO cheese, Parmigiano-Reggiano, and thus had similar management conditions: silage, pasture and fresh herbage were not allowed, and the rations (fed as a total mixed diet) were based on dry roughage, concentrates and added water. In total, 506 individual milk samples were collected. Over the past decade, the three farms have followed programs in which parts of their herds were crossbred, while the remainder consisted of purebred H. The basic scheme was a three-breed rotation based on the use of Swedish Red (S) semen on H cows (S × H), the use of Montbeliarde (M) semen on first-cross cows [M × (S × H)], and the use of H semen in the third-generation crosses.

Figure 1. Crossbreeding scheme and, within brackets, number of cows sampled for milk quality, coagulation traits and individual curd firmness modeling analyses (H: Holstein; M: Montbeliarde; S: Swedish Red; B: Brown Swiss).



In all herds, a smaller number of purebred H cows were mated to M and Brown Swiss (B) bulls, and these first crosses were mated to S and M bulls, respectively (Figure 1). After collection, milk samples (without preservative) were refrigerated (4°C) and taken to the Cheese-Making Laboratory of the DAFNAE (Department of Agronomy, Food, Natural

Resources, Animals and the Environment) at the University of Padova (Italy) for analysis. All samples were processed within 24 hours from the collection.

Analysis of Milk Quality Traits

Individual milk subsamples were analyzed for fat, protein, casein and lactose contents using a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark). Somatic cell count was obtained from a Fossomatic FC counter (Foss Electric A/S, Hillerød, Denmark) and log-transformed to somatic cell score (SCS) according to the formula proposed by Ali and Shook (1980): SCS = 3 + $\log_2$(SCC/100,000). Milk pH was recorded using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain).

Analysis of Milk Coagulation Properties

Milk coagulation properties (MCPs) were obtained using two mechanical lactodynamographs (Formagraph; Foss Electric A/S, Hillerød, Denmark). Two sub-samples (10 mL) of every milk sample were heated to 35°C and mixed with 200 µL of a 1.2% (wt/v) rennet solution (Hansen Standard 215, 215 IMCU/ml; with 80±5% chymosin and 20±5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted in distilled water (to yield 0.051 IMCU × mL^{-1}). The milk sub-samples were then fitted into racks with 10 cuvettes each. The observation period lasted 60 min, starting right after rennet addition. The instrument recorded the width (in mm) of the oscillatory graph every 15 sec during testing and directly provided the traditional MCP traits: rennet coagulation time (RCT, min), defined as the time from enzyme addition to gelation of the milk; curd-firming time (k_{20}, min), defined as the time from gelation to the time at which the width of the graph attained 20 mm; and the width of the graph at 30 min (a_{30}, mm), 45 min (a_{45}, mm) and 60 min (a_{60}, mm) from rennet addition, which is a measure of the extent of curd firmness.

Modeling the Curd Firmness of Individual Milk Samples

Curd firmness (CF) was measured every 15 sec for 60 min, for a total of 240 recorded CF values per sub-sample. The equations proposed by Bittante (2013b) for modeling of CF were used to analyze the data. The first equation represents a basic three-parameter asymptotic model used to describe a curd-firmness track obtained after 30 minutes of analysis, and was previously explained in detail by Bittante et al. (2011). The second equation is an extended four-parameter model intended to describe a 90-minute track. We tested both models because our analysis was performed over 60 min. The performance of the models was evaluated based on convergence and the standard errors estimated for the individual equation parameters. The three-parameter model was chosen, as it provided higher convergence values than the four-parameter model. The three-parameter model is given as follows:

$$CF_t = CF_P \times (1 - e^{-k_{CF} \times (t - RCTeq)})$$

where CF_t is the curd firmness at time t (mm), CF_P is the asymptotical potential maximum value of curd firmness (mm), k_{CF} is the curd-firming instant rate constant (%×min^{-1}), and RCT_{eq} is the rennet coagulation time (min). This model uses all available information to estimate the three parameters, so that these are not single-point measurements as those achievable with traditional MCPs. The CF_t observations available for each sub-sample were fitted with curvilinear regressions using the non-linear procedure (PROC NLIN) of SAS (SAS Institute Inc., Cary, NC). The parameters of each individual equation were estimated by employing the Marquardt iterative method (350 iterations and a 10^{-5} level of convergence). In some cases (often late coagulating sub-samples) the data failed to converge (7 of 1,012 sub-samples).

Statistical Analysis

Seventeen sub-samples failed to coagulate by the end of the analysis, and were thus considered to be missing for all parameters. Similarly, a_{30} and a_{45} were considered to be missing if a sub-sample did not coagulate after 30 or 45 min, respectively, from the start of the analysis. Sub-samples that needed more than 50 min to achieve coagulation were considered non-coagulating samples (16 on 1012 sub-samples). Sub-samples that failed to converge were considered to be missing for all modeled parameters. Sub-samples that met the convergence criterion but did not coagulate within 50 minutes (10 of 1012 sub-samples) were treated as non-converging and were excluded from further analysis, because late coagulation caused problems with the estimations of CF_p and k_{CF}. The traditional MCP data and curd-firmness modeling parameters were analyzed using the MIXED procedure of SAS (SAS Institute, 2008) with the following linear model:

$$y_{ijklmno} = \mu + HTD_i + DIM_j + Parity_k + Breed_l + Cow_m + Pendulum_n + e_{ijklmno},$$

where y_{ijklm} is the dependent variable; μ is the overall mean; HTD_i is the fixed effect of the *i*th herd-test-day (*i* = 1 to 7); DIM_j is the fixed effect of the *j*th class of days in milk (*j* = 1 to 5;

class 1: < 60d, class2: 60-120, class3: 121-180 class4: 181- 240 class5: > 240); $Parity_k$ is the fixed effect of the kth parity (k = 1 to 3 and more); $Breed_l$ is the fixed effect of the lth breed combination (l = 1 to 7); Cow_m is the random effect of the mth cow (n = 1 to 506); $Pendulum_n$ is the fixed effect of the nth pendulum (m = 1 to 20); e_{ijklmo} is the random residual. Cow and residuals were assumed to be independently and normally distributed with a mean of zero and variance σ^2_{cow} and σ^2_erespectively and REML was used as the method of estimation of variance components.

Analysis of variance was performed on milk yield, milk-quality traits, SCS and pH, using the GLM procedure of SAS (SAS Institute, 2008). The linear model included the fixed effects of HTD, DIM (5 classes of 60 days), parity and breed combination.

Orthogonal contrasts were estimated between least squares means of traits for the effects of DIM: a) linear component; and b) quadratic component. Orthogonal contrasts were estimated between least squares means of traits for the effects of parity: a) primiparous vs. secundiparous and pluriparous cows; and b) secundiparous vs. pluriparous cows. Finally, orthogonal contrasts were estimated between least squares means of traits for the effects of breed combination: a) the effect of crossbreeding (H vs. all crossbred cows); b) the effect of generation (first-cross vs. second-cross cows); c) the effect of a Nordic sire in the first cross (S × H vs. M × H + B × H); d) the effect of Alpine sire in the first cross (M × H vs. B × H); e) the effect of Nordic sire in the second cross (S × MH vs. M × SH + M × BH); and f) the effect of maternal grand-sire in second-cross cows with Alpine sire (M × SH vs. M × BH).

RESULTS

Descriptive Statistics and Sources of Variation for Milk Traits

Descriptive statistics of the studied milk-quality traits are summarized in Table 1. On average, cows produced 31.8 kg/d of milk containing 4.09% fat, 3.71% protein and 2.71% casein. The mean values of SCS and lactose were 2.56 and 5.00%, respectively. The observed coefficients of variation were high for SCS (72%), intermediate for milk yield (31%) and fat content (21%), and low (less than 10%) for all of the other quality traits. Regarding samples, 1.7% failed to coagulate within 60 min from rennet addition, and 12.1% failed to coagulate within 30 min of analysis (late coagulating milk samples). On average (including samples that coagulated after 30 min), coagulation occurred at 20.86 min from rennet addition, with a k_{20} of 4.87 min, and a_{30} and a_{45} were 34.44 and 44.60 mm, respectively (Table 1).

Table 1. Descriptive statistics of milk yield, quality, coagulation traits and parameters of curd firmness modeling[1]

Trait[2]	n	Mean	SD	P1	P99
Milk yield, kg/d	506	31.82	9.95	11.78	55.20
Milk quality traits					
Fat,%	504	4.09	0.86	2.09	6.36
Protein, %	506	3.76	0.34	2.88	4.56
Casein, %	506	2.94	0.27	2.21	3.57
Lactose, %	504	5.00	0.27	4.16	5.46
SCS, units	506	2.56	1.84	-0.47	7.43
pH	506	6.47	0.08	6.29	6.68
Milk coagulation traits[2]					
RCT, min	979	20.86[4]	6.95	10.45	45.2
k_{20}, min	959	4.87	2.38	2.00	13.0
a_{30}, mm	900	34.44	14.9	1.12	61.3
a_{45}, mm	977	44.6	12.5	2.68	64.7
Curd firmness model[3]					
RCT_{eq}, min	977	21.4	6.62	11.52	43.54
CF_P, mm	977	50.4	8.09	26.94	67.56
k_{CF}, % × min^{-1}	977	12.6	5.65	2.81	34.09

[1]P1 = first percentile; P99 = 99th percentile.
[2]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition; a_{45} = curd firmness 45 min after rennet addition.
[3]CF_P = asymptotical potential value of curd-firmness; k_{CF} = curd-firming instant constant rate; RCT_{eq} = rennet coagulation time estimated using the equation.
[4]The late-coagulating samples (RCT > 30 min) are 12% of all samples and are included in the average.

Coefficient of variation for MCP ranged from 28% (for a_{45}) to 49% (for k_{20}). With respect to the parameters obtained from the individual equations describing CF (Table 1), CF_P averaged 50.4 mm and k_{CF} averaged 12.6% x min^{-1}, with a coefficient of variation of 16% and 45%, respectively. The average estimated RCT was 21.4 min, which was similar to that obtained from the lactodynamographs.

The sources of variation included in the statistical model are shown in Table 2. HTD affected all of the tested milk-quality traits (except for the lactose content), but was not significant for the milk yield, the traditional MCPs, or the model parameters describing the curd firmness of individual milk samples (Table 2). The stage of lactation was a major source of variation for all studied traits. Parity exerted a smaller effect than DIM; it was significant for the milk yield and quality traits (except for the fat content), but did not affect the traditional MCPs or the CF parameters (except for CF_p). The breed combination was less important than DIM and parity, but nevertheless affected most of the studied traits.

Table 2. Results from ANOVA (*F*-value and significance) for milk yield, quality, coagulation traits and individual parameters of curd firmness modeling

	HTD	DIM	Parity	Breed	Pendulum	Animal	RMSE[1]
Milk yield, kg/d	0.41^{ns}	91.59^{***}	30.71^{***}	4.80^{***}			6.99
Milk quality							
Fat, %	2.56^{*}	8.69^{***}	1.64^{ns}	3.53^{**}			0.81
Protein, %	3.07^{**}	99.10^{***}	4.06^{*}	3.01^{**}			0.21
Casein, %	4.94^{***}	51.43^{***}	6.29^{**}	$1.82^{\dagger}$			0.21
Lactose, %	1.78^{ns}	26.47^{***}	21.26^{***}	3.59^{**}			0.22
SCS, units	4.75^{***}	12.92^{***}	6.54^{**}	1.34^{ns}			1.72
pH	2.31^{***}	21.32^{***}	4.72^{**}	2.31^{*}			0.07
Milk coagulation traits[2]							
RCT, min	0.83^{ns}	31.06^{***}	1.31^{ns}	1.47^{ns}	3.59^{***}	6.07	1.61
k_{20}, min	0.78^{ns}	5.72^{***}	2.00^{ns}	2.35^{*}	3.66^{***}	2.12	0.97
a_{30}, mm	0.67^{ns}	5.81^{***}	1.11^{ns}	2.99^{**}	3.40^{***}	12.38	7.94
a_{45}, mm	0.40^{ns}	6.52^{***}	0.75^{ns}	1.27^{ns}	1.72^{*}	9.22	8.11
Curd firmness modeling[3]							
RCT_{eq}, min	0.95^{ns}	7.20^{***}	1.25^{ns}	2.04†	3.88^{***}	5.94	2.54
CF_P, mm	1.48^{ns}	3.33^{*}	3.14^{*}	1.65^{ns}	1.07^{ns}	0.01	8.03
k_{CF}, % × min^{-1}	0.72^{ns}	6.84^{***}	1.03^{ns}	2.39^{*}	2.07^{*}	3.08	4.51

[1]RMSE = root mean square error.
[2]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition; a_{45} = curd firmness 45 min after rennet addition.
[3]CF_P = asymptotical potential value of curd-firmness; k_{CF} = curd-firming instant rate constant; RCT_{eq} = rennet coagulation time estimated using the equation.
† P <0.10; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The duplicate analyses for the MCPs and CF parameters allowed us to discriminate between the effects of the animals and residuals on phenotypic variability, with the prevalence of the first for all MCP and RCT_{eq}, and of the second for the remaining traits (Table 2). Within each animal, the individual pendulum of the lactodynamograph exerted an effect on all traits except for the potential asymptotical curd firmness.

Effects of Parity and DIM on Milk Traits

Primiparous cows produced high-quality milk in lesser amounts compared to multiparous cows, and second-lactation cows had higher milk protein contents than cows with three or more lactations (-0.05%; $P < 0.05$; Table 3).

Table 3. Least squares means of milk yield and quality across parities and days in milk

	Cows n	Milk yield kg×d^{-1}	Milk quality traits					pH
			Fat %	Protein %	Casein %	Lactose %	SCS	
Parity (P):								
1	189	26.47	4.29	3.84	3.01	5.07	2.32	6.46
2	152	31.75	4.16	3.81	2.95	4.93	2.92	6.48
≥3	165	33.10	4.11	3.75	2.91	4.90	3.06	6.48
Contrasts, P-value								
P1 vs P2+≥P3		<0.001	0.08	<0.05	<0.001	<0.001	<0.001	<0.01
P2 vs ≥P3		0.12	0.57	<0.05	0.12	0.21	0.50	0.56
Days in milk:								
< 60 d	110	39.66	3.94	3.46	2.75	5.11	2.01	6.41
60 - 120 d	139	35.17	3.99	3.62	2.85	5.06	2.20	6.47
121 - 180 d	102	30.00	4.14	3.82	2.97	4.98	2.94	6.49
181 - 240 d	78	25.11	4.29	3.97	3.07	4.91	3.14	6.50
> 240 d	77	22.27	4.58	4.11	3.16	4.79	3.54	6.50
Contrasts, P-value								
Linear contrast		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic contrast		0.19	0.14	0.23	0.61	0.13	0.87	<0.001

Regarding milk coagulation, the incidence of NC samples was higher for second-lactation cows than for all other cows. Among the traditional MCPs, milk produced after the second calving presented an increase of k_{20} ($P < 0.10$) while, for CF modeling, parity was associated with a slightly higher CF_P of second-lactation cows relative to older cows (-1.65 mm, $P < 0.05$) (Table 4).

Table 4. Least squares means of milk coagulation properties measured by Formagraph and on estimated parameters of individual curd firmness equations across parities and days in milk

	NC[1]	Milk coagulation traits[2]				Curd firmness model[3]		
	%	RCT min	k_{20} min	a_{30} min	a_{45} min	RCT_{eq} min	CF_P mm	k_{CF} %×min^{-1}
Parity (P):								
1	10.3	20.2	4.5	36.3	45.7	20.3	50.5	13.4
2	17.1	21.3	5.0	35.5	45.4	21.3	51.7	13.2
>3	9.4	21.2	4.9	33.5	44.0	21.4	50.1	12.5
Contrasts, P-value								
P1 vs P2+P3		0.12	0.06	0.26	0.43	0.12	0.51	0.30
P2 vs P3		0.88	0.74	0.26	0.30	0.87	<0.05	0.26
Days in milk:								
<60 d	0.0	15.1	3.8	40.6	49.4	18.0	51.8	14.9
60-120 d	6.5	19.6	4.7	36.3	45.6	20.9	51.1	13.3
121-18 0d	17.2	22.2	5.0	34.3	45.4	21.6	51.6	13.0
181-240 d	23.7	23.8	5.2	31.2	41.1	22.5	48.8	11.5
>240 d	20.8	23.7	5.2	33.0	43.7	21.9	50.6	12.3
Contrasts, P-value								
Linear contrast		<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001
Quadratic contrast		<0.001	<0.05	<0.05	<0.05	<0.01	0.51	<0.05

[1]NC = non-coagulating samples within 30 min from rennet addition.
[2]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition; a_{45} = curd firmness 45 min after rennet addition.
[3]RCT_{eq} = rennet coagulation time estimated using the model; CF_P = asymptotical potential value of curd-firmess; k_{CF} = curd-firming instant constant rate.

In contrast, the effects of DIM were highly significant (P < 0.001) for all parameters that showed a linear increase during lactation, except for milk yield and lactose content (which decreased linearly), and pH (which showed a quadratic increase). The incidence of NC samples (i.e., those that failed to coagulate within 30 min) increased during the first half of lactation and remained high during the second half. Furthermore, all coagulation traits and curd-firming model parameters worsened during lactation (as reflected by increased coagulation- and curd-firming time, accompanied by decreases in curd firmness and the curd-firming instant rate constant), especially during the first half of lactation, when a quadratic pattern was seen. The exception here was CF_P, which showed a slight linear decrease during lactation (Table 4).

Effects of Breed Combination on Milk Traits

Compared with purebred H, crossbred cows produced less milk (*P* < 0.001) with lower lactose content (*P* < 0.01), higher fat and protein contents (*P* < 0.01 and *P* < 0.05, respectively), and a tendency for higher casein content (*P* = 0.06; Table 5). Milk from secondd-generation cows exhibited higher pH than first-generation animals (P < 0.05), and M × (B × H) crossbred cows showed higher fat content than M × (S × H) crossbreds (+0.39%; *P* < 0.05). Somatic cell score was not affected by breed combination, although values tended to be lower for S × H compared with M × H and B × H (*P* = 0.08).

Table 5. Least squares means of milk yield and quality across breed combinations[1]

	Cows n	Milk yield kg×d^{-1}	Milk quality traits					
			Fat %	Protein %	Casein %	Lactose %	SCS	pH
Purebreds:								
Holsteins, H×H	159	33.02	3.96	3.74	2.92	5.03	2.88	6.47
First generation crossbreds:								
Swedish Red×H, S×H	140	29.89	4.34	3.86	3.00	4.91	2.35	6.47
Montbeliarde×H, M×H	42	32.29	4.24	3.82	3.00	5.01	2.90	6.47
Brown Swiss×H, B×H	16	28.49	4.17	3.79	2.96	4.91	2.88	6.44
Second generation crossbreds:								
S×(M×H)	20	31.26	4.30	3.82	2.97	4.96	2.66	6.48
M×(S×H)	103	29.24	3.95	3.81	2.97	4.97	2.74	6.50
M×(B×H)	26	28.90	4.34	3.74	2.91	4.99	2.96	6.49
Contrasts, P-value								
Purebred vs Crossbreds		<0.001	<0.01	<0.05	0.06	<0.01	0.48	0.74
First vs Second generation		0.72	0.70	0.44	0.37	0.50	0.79	<0.05
S×H *vs* [M×H + B×H]		0.69	0.33	0.23	0.63	0.26	0.08	0.27
B×H *vs* M×H		0.07	0.77	0.69	0.56	0.15	0.97	0.31
S×(M×H) vs [M×(S×H)+ M×(B×H)]		0.21	0.45	0.41	0.60	0.70	0.66	0.29
M×(S×H) *vs* M×(B×H)		0.83	<0.05	0.21	0.20	0.70	0.58	0.49

[1]H: Holstein; B: Brown Swiss; S: Swedish Red; M: Montbèliarde.

The incidence of NC samples did not clearly differ between purebred H and crossbred cows, but the latter generally produced milk with shorter k_{20} and $RCT_{eq,}$ and higher a_{30}, CF_P, and k_{CF} ($P < 0.05$; Table 6). Among crossbred cows, fatS × H presented a higher incidence of NC samples and longer RCT compared with M × H and B × H animals ($P < 0.05$), and M × H showed shorter k_{20} compared with B × H cows ($P < 0.05$). Among second-generation cows, those sired by S bulls showed a lower incidence of NC, higher a_{30} and CF_P, and faster k_{CF} compared with animals sired by M bulls ($P < 0.05$).

Table 6. Least squares means of milk coagulation properties measured by Formagraph and on parameters of individual curd firmness equations across breed combinations[1]

		Milk coagulation traits[3]				Curd firmness model[4]		
	NC[2] %	RCT min	k_{20} min	a_{30} min	a_{45} min	RCT_{eq} min	CF_P mm	k_{CF} %×min^{-1}
Purebreds:								
Holstein, H×H	11.0	21.4	5.3	30.4	43.4	22.4	49.6	12.1
First generation crossbreds:								
Swedish Red×H, S×H	15.7	22.3	5.2	33.4	43.2	22.4	50.7	11.9
Montbeliarde×H, M×H	9.5	19.7	3.9	38.6	46.9	19.2	50.9	13.6
Brown Swiss×H, B×H	12.5	20.1	5.3	35.9	44.2	21.3	51.1	12.0
Second generation crossbreds:								
S×(M×H)	5.0	19.5	4.2	41.3	49.2	19.2	53.6	15.6
M×(S×H)	12.1	22.2	5.0	33.4	44.1	21.6	49.8	12.6
M×(B×H)	7.7	20.9	4.8	32.9	44.3	20.8	49.6	13.3
Contrasts, P-value								
Purebred vs Crossbreds		0.37	<0.05	<0.001	0.13	<0.05	<0.05	<0.05
First vs Second generation		0.88	0.76	0.97	0.56	0.67	0.94	0.09
S×H *vs* [M×H + B×H]		<0.05	0.16	0.13	0.23	0.06	0.78	0.23
B×H *vs* M×H		0.82	<0.05	0.53	0.41	0.25	0.93	0.23
S×(M×H) vs [M×(S×H) + M×(B×H)]		0.21	0.28	<0.05	0.07	0.23	<0.05	<0.05
M×(S×H) *vs* M×(B×H)		0.38	0.70	0.87	0.93	0.58	0.09	0.47

[1]H: Holstein; B: Brown Swiss; S: Swedish Red; M: Montbèliarde.
[2]NC = non-coagulating samples within 30 min from rennet addition.
[3]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition; a_{45} = curd firmness 45 min after rennet addition.
[4]RCT_{eq} = rennet coagulation time estimated using the model; CF_P = asymptotical potential value of curd-firmess; k_{CF} = curd-firming constant rate.

DISCUSSION

Effect of Breed Combination on Milk Yield and Quality Traits

The design of the contrasts used in the present study allowed us to specifically compare traits of the pure H cows versus all of the examined crossbreeds, and to evaluate differences within the crossbreeds. Our results suggest that the crossbred cows were fairly similar in terms of milk yield, milk-quality traits and SCS, as most of the observed variation was found when we compared pure H cows with all of the crossbreeds.

This study was based on a single milk sample per cow and was not designed to study milk production, but rather to examine its technological properties for cheese-making. However, the decreased milk yield of crossbred cows compared to H cows, which has generally been reported in the literature (Heins and Hansen, 2012), was also observed in the current study.

On average, crossbred cows produced 9.1% less milk per day when compared to H cows (30 vs. 33 kg/d). This average figures take into account the effect of HTD, parity and DIM, but do not consider differences in terms of age at parturition Malchiodi et al., 2011; (Hazel et al., 2013) and pregnancy stage (Blöttner et al., 2011a; Brown et al., 2012; Malchiodi et al., 2012) that also in the present study penalize crossbreds because of their higher fertility. Also body size was not taken into account, but the breeds considered in the present study are not characterized by large differences in body weight (Blöttner e al., 2011a; Hazel et al., 2013). The average figures include the negative effect of inbreeding (for purebreds) and the positive effect of heterosis (for crossbreds), but neither of these effects could be estimated due to the limited number of animals considered and the absence of purebred cows of the crossing breeds. However, it appears that heterosis among these breeds is very limited for milk yield and quality traits (Freyer et al., 2008; Sørensen et al., 2008; Walsh et al., 2008). On the contrary, the crossbred cows showed higher milk fat, protein and casein contents relative to pure H cows (+6.6%, +1.7% and +1.8%, respectively)

In the present study, the first and second crossbred generations did not differ with respect to any of the considered traits, with the limited exception of pH, which differed between the two-way and three-way crosses (i.e., between the 50% H and 25% H crosses). Heins and Hansen (2012) reported similar results when they compared M × H and Scandinavian Red × H crossbreds with purebred H cows, finding that the crossbreds had a lower milk yield (-6.4%), higher mean fat and protein contents (+3.3% and +3.4%, respectively), and a slightly lower fat content and protein yield (-3.3% and -

3.4%, respectively). Similar results were also reported for B × H crossbred cows (Brandt et al., 1974), which had higher fat and protein contents compared with pure H (+0.4% and +0.12%, respectively). Hazel et al. (2013) compared first-calving M × H with purebred H and found that the crossbreds produced 3.2% less milk than the purebreds, but did not differ significantly in terms of fat (-0.6%) or protein (-1.4%). Consistent with our results, Malchiodi et al. (2011) reported higher protein contents for S × H and second-generation M × (SR × H) crossbred cows (+0.09% and +0.11%, respectively) compared to pure H.

Our finding that SCS was not significantly affected by breed composition agreed with previous studies comparing B × H crossbred cows with pure H (Dechow et al., 2007; Blöttner et al., 2011b). Another study also failed to find any significant difference in milk SCS when comparing Jersey × H crossbreds with pure H (Prendiville et al., 2010). Heins and Hansen (2012) found significantly lower SCS in M × H or Scandinavian Red × H crossbred cows compared to pure H, with M × H crossbred cows showing the lowest milk SCS values over the entirety of lactation, while the Scadinavian Red cows showed intermediate values between those of pure H and M × H crossbred cows. In our study, S × H crossbreds showed the lowest milk SCS values; they tended to be lower compared to those of B × H and M × H cows.

Effect of HTD, Parity and DIM on MCPs and the Parameters Modeled for Curd Firming

While the herd and test date effects strongly affected milk quality traits, they did not influence MCP and CF parameter. These results are in accordance with other studies that reported a small effect of herd on the variation of MCPs (Ikonen et al., 2004; Tyrisevä et al., 2004). A recent review on the genetics and modeling of milk-coagulation properties (Bittante et al., 2012) indicated that the herd effects of RCT and a_{30} accounted on average for only about 5% of the total variance of these traits, while the effects of herd varied from about 10% to 30% for other milk traits.Cipolat-Gotet et al. (2012) found that the herd had a significant effect on MCPs; however, the number and heterogeneity of herds was high in their study, and the actual herd effect was similar to that of parity (and thus much smaller than that of DIM). Our study, in contrast, examined three herds that had similar management conditions and farm sizes, and used a very short sampling period.

Consistent with the results from others studies (Ikonen et al., 2004; Tyrisevä et al., 2004; Cipolat-Gotet et al., 2013), we found that the MCPs changed during lactation (i.e., they showed a quadratic response to DIM): they exhibited rapid worsening (delayed gelation time, slowing/reduction of curd firming) during the early stage of lactation, followed by a more stable pattern, and eventually recovered somewhat during the last part of lactation. The number of NC samples also increased rapidly during the first part of lactation and then stabilized thereafter, in agreement with the findings of Tyrisevä et al. (2004). This reflects that the milk pH, which showed a significant quadratic increase during lactation, is (together with titratable acidity) highly correlated to MCP but opposes the trends of protein and casein, which are positively correlated to curd firmness (Bittante et al., 2012; Macciotta et al., 2012).

Similar to previous reports (Ikonen et al., 2004; Tyrisevä et al. 2004), we found that parity did not affect MCP. Ikonen et al. (2004) reported that this only occurs when coagulated samples are analyzed, and that primiparous cows had more NC samples than multiparous cows(17% vs. 9%, respectively). However, our present results indicate that the number of NC samples increased from the first to second parity and decreased in multiparous cows. Cipolat-Gotet et al. (2012) found a similar trend for RCT in Brown Swiss cows, with this parameter increasing from the first to second lactations and decreasing thereafter. In the same study, the authors found curd firmness at 45 minutes to decrease as parity increased, in agreement with Tyrisevä et al. (2003), who showed that MCP deteriorated as parity increased. However, the contradictory effects of parity on MCPs could be explained by the use of different breeds across studies, suggesting that the effect of parity likely differs between breeds.

Comparison of the present results to those from the Brown Swiss and Holstein milk samples used to evaluate the curd-firmness model (Bittante, 2011) revealed that (on average) milk from the crossbred cows examined in the present study showed a longer RCT (19.0, 20.1, and 21.4 min for B, H and crossbred cows, respectively), a higher CF_P (43.6, 33.8 and 50.4 mm, respectively) and an intermediate k_{CF} (13.8, 11.3 and 12.6 %×min^{-1}, respectively). All of the abovementioned samples were analyzed in the same laboratory using the same instruments and conditions (except for test length); therefore, the observed differences can be attributed to the inherent characteristics of the analyzed milk samples. As expected, RCT_{eq} showed patterns similar to that of the single-point RCT.Unlike the curd firmness values measured after 30 or 45 min from enzyme addition, CF_P showed very modest (but significant) effects of parity and DIM. This suggests that the endpoint of curd differences in curd firmness registered in the early phase of the curd-firming process. In fact, k_{CF} was strongly affected by DIM (i.e., there was a quadratic decrease during lactation) but not by parity, which was similar to our observations for the traditional MCPs.

Effect of Crossbreeding and Breed Combinations on MCP and CF Model Parameters

In general, crossbred cows showed favorable MCP values compared with pure H cows, in terms of both milk coagulation traits directly measured by lactodynamograph and the curd firmness parameters obtained from instrument output modeling. The milk of crossbreds showed better curd-firming values in terms of both the k_{20} and k_{CF} coefficients, and CF_P and a_{30} were both positive for crossbred cows compared to pure H. The RCT showed some differences when they were measured by the instrument as single-point values (not significant) and estimated by our modeling of all CF measures obtained by the lactodynamographs (RCT_{eq} was improved by crossbreeding). The a_{45} was not significantly affected by crossbreeding. However, this could also reflect that curd firmness peaks at later coagulation times and decreases thereafter, in a process called micro-syneresis (McMahon et al., 1984). Since samples that coagulate earlier could show a decrease in curd firmness before the end of the analysis, the curd firmness at 45 min could be similar to that of later coagulating samples in which curd firmness is still in the incremental phase, as demonstrated by Bittante et al. (2013b) in Brown Swiss cows. We did not observe such an effect overall in the present study because our samples had a late coagulation time on average; however, some samples showed signs of undergoing this process. The use of a curd firmness model allowed us to account for this phenomenon, and CF_P was not affected by the decrease in curd firmness and thus by gelation time. The MCP differences that we observed among the crossbreds could reflect differences inherent to the pure breeds represented within the crossbred cows. Bittante et al. (2012) compared various breeds based on the results from numerous studies, and found that pure B had much more favorable MCPs than pure H. The M breed (which was grouped with Simmental in the study) was also favorable for all the parameters compared with pure H, but to a slightly lesser degree than pure B. In contrast, the Scandinavian breeds, particularly the Finnish Ayrshire, had the least favorable MCPs compared to pure H. In agreement with these results, our study suggests that B and M sires can improve RCTs but do not appear to affect the curd-firming time or curd firmness, as reported in previous studies on purebred B (Malossini et al., 1996; Malacarne et al., 2006; Cecchinato et al., 2009 and 2011) and M (Macheboeuf et al., 1993). Conversely, S is not currently considered a desirable breed for improving MCPs. For example, Poulsen et al. (2013) reported that S had a longer RCT and more NC samples relative to Danish H, although the two breeds did not differ in their curd-firming rates. Here, we report that the first-generation M × H and B × H crossbreds had faster coagulation times than the S × H crossbreds, whereas there was no significant difference in curd firmness among these crossbreds.

Figure 2. Least squares means for rennet coagulation time (RCT) and curd firmness 30 min after rennet addition to milk (a_{30}) across breed combinations (H: Holstein; S: Swedish Red; M: Montbeliarde; B: Brown Swiss).

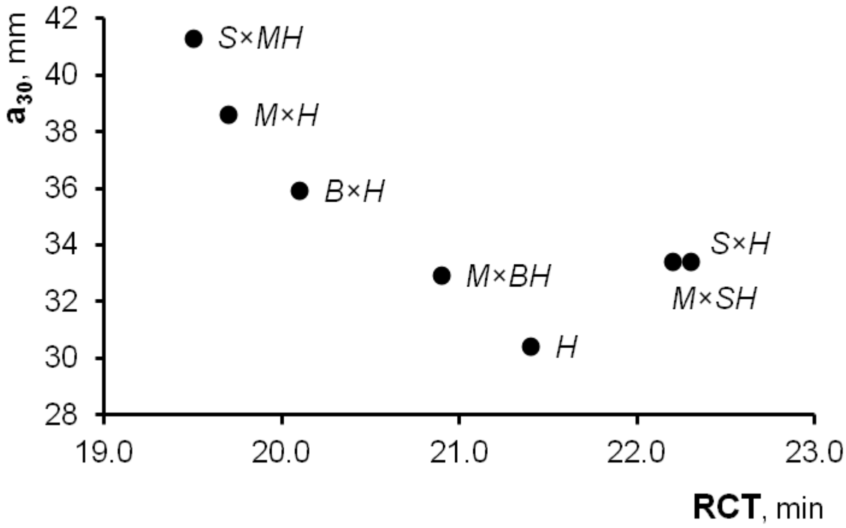
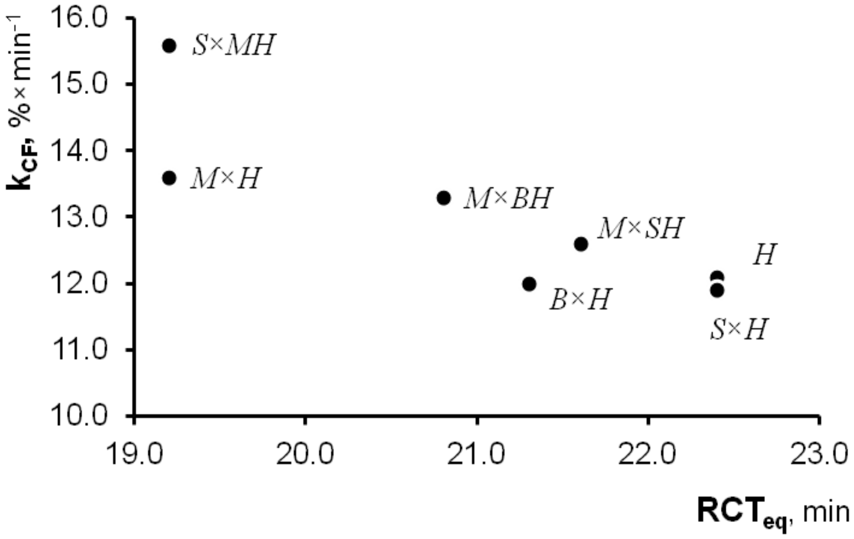


Figure 3. Least squares means for rennet coagulation time obtained through individual modeling (RCT_{eq}) and curd firming instant rate constant of milk (k_{CF}) across breed combinations (H: Holstein; S: Swedish Red; M: Montbeliarde; B: Brown Swiss).

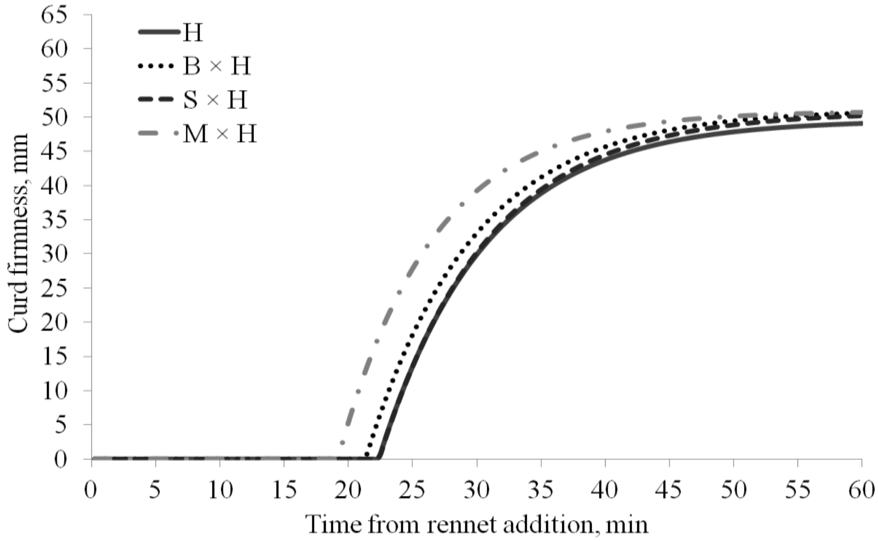


The comparison becomes more complex when we also consider three-way crosses. Figure 2 shows that the averages of the traditional MCPs for three-way crossbred cows are more similar to those of their maternal two-way breed combination than the two-way combination having the same sire breed. This explains why the S × H crossbreds have the longest RCT, while the S × (M × H) crossbreds have the shortest RCT. A possible maternal genetic effect on MCP may also be inferred from the results of the only two previous studies that compared crossbred cows and the purebred cows of both parental breeds. Kreuzer et al. (1996) and Tyrisevä et al. (2004) compared Jersey × H and H × Finnish Ayrshire crossbreds, respectively, with their parental breeds and found that the MCPs of the crossbred cows were more similar to those of the Jersey and Ayrshire purebreds, respectively, than to those of purebred H cows. Moreover, Figure 2 shows that the majority of breed combinations exhibited a strong negative correlation between RCT and a_{30}, as is often found in the literature (Bittante et al., 2012). A clear exception to this rule, however, may be seen in the S × H cows and their daughters [M × (S × H)], which had the longest RCT but not the lowest a_{30}. This finding is consistent with the comparison between S and H purebreds reported by Poulsen et al. (2013

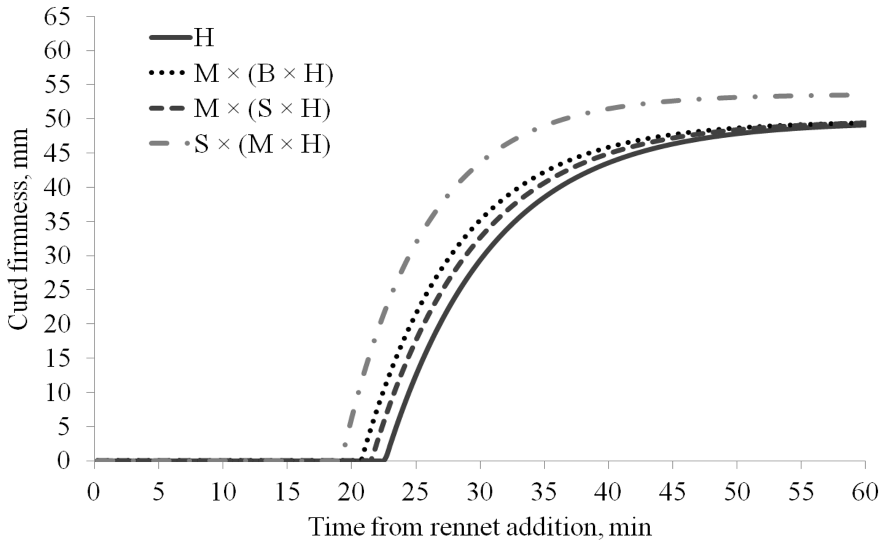
Comparisons based on individual CF model parameters (Figure 3) showed that the correlation between RCT_{eq} and k_{CF} was broader than that observed for the traditional MCPs, and the relative positions of the different breed combinations were rearranged compared to those seen in Figure 2. The estimation of the CF model parameters also allowed us to map curd firmness over time for each breed combination. Pure H cows exhibited the lowest curd firmness at any time relative to the three first-generation crossbreds, largely due to their longer RCTs. The most favorable pattern was exhibited by M × H cows followed by B × H cows, Whereas the S × H cows nearly overlapped with the H curve (Figure 4a). The second-generation crossbreds showed more favorable patterns of curd firming compared to pure H cows. Among the different crosses, it is evident that the more favorable outcomes are found, not in first-generation cows from M sires, but rather in their daughters obtained from S bulls. This more favorable pattern is not only due to a shorter RCT, but also to a steeper k_{CF} and a higher CF_P asymptotical value. The difference found between breed groups could also be explained by protein composition. In fact, studies on purebreds have suggested that differences in the proportion of different protein fractions (Jõudu et al., 2008), differences in the frequency of their genetic variants (Macheboeuf et al., 1993; Hallén et al., 2007), or both (Bonfatti et al., 2010) could help explain the differences in their MCPs. Ikonen et al. (1999), Penasa et al. (2010), and Bonfatti et al. (2011) evaluated pure Finnish Ayrshire, H and

Figure 4. Curd firmness modeling of (a) purebred Holsteins (H) and first generation Swedish Red (S) × H, Montbeliarde (M) × H and Brown Swiss (B) × H crossbred cows, and of (b) purebred Holsteins (H) and second generation S × (M × H), M × (S × H) and M × (B × H) crossbred cows

(a)



(b)



Simmental cows, respectively, and reported that an important fraction of the within-breed genetic variance of MCP depends on the genetic variants of milk proteins. Other studies have also shown that the MCP differences between H and M (Auldist et al., 2002) and between H and Finnish Ayrshire (Ikonen et al., 1999) were strongly reduced after the models were adjusted for the k-casein genotype. Therefore, further studies on milk protein profiles and the genetic variants of different protein fractions could help explain the differences found among breed groups.Notably, increasing attention is being paid to the effect of candidate genes beyond those encoding milk proteins (Glantz et al., 2011; Cecchinato et al., 2012) and the potential value of genomic approaches (Tyrisevä et al., 2008; Glantz et al., 2012). Thus, we may soon have new insights that could help us understand breed differences and the effect of crossbreeding on the technological properties of milk.

CONCLUSIONS

Previous studies have shown that H-based crossbred cows can compensate for their lower milk production by superior fertility, longevity, and milk quality. Beyond confirming these results, the present study also shows that crossbreeding can be a viable method for improving the technological properties of milk, particularly the coagulation time and curd-firming process. Different sire breeds are characterized by specific technological aptitudes that may not be strictly related to other milk-quality traits. We herein report that the favorable characteristics of the H breed (in terms of the quality and technological properties of milk) can be maintained in the third generation of three-way crosses without negative effects on milk yield, despite the further reduction of the H heritage from 50% to 25%. These findings suggest that, depending on the intended use of the milk, different types of sires can be chosen to assure the optimization of farm crossbreeding programs. However, further studies are needed on the genetic and technological bases of the milk properties of different breeds and on the possible maternal/dominance effects of different breed combinations.

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Chapter 6

General Conclusions

GENERAL CONLCUSIONS

The results obtained from this set of analyses confirmed results from previous studies. Pure Holstein cows were superior in terms of milk production, showing higher milk yield than crossbred cows. The amount of protein and fat was also higher for pure Holstein than crossbreds cows. However, milk quality, in terms of protein a fat content, was showed favorable value for crossbreds cows then for pure Holstein cows. These results were similar for the parameters related to coagulation properties of milk. Results show that crossbreeding can be a viable method for improving the technological properties of milk, particularly the coagulation time and curd-firming process.

As expected, pure Holstein cows showed lower reproductive performance than crossbreds. Crossbred animals showed an earlier onset first heat after calving and also became pregnant before pure Holstein cows with higher probability to reach the conception at the first service. These results suggest that the lower production of crossbred animals might be at lkeast partially compensated by the benefits from increased fat and protein content, and the better milk coagulation properties, depending on specific pay systems, and from the decrease of cost due to problems related to poor fertility. Reaching the insemination time before and with a lower number of inseminations, can reduce veterinary costs, and save economic losses derived from the non voluntary prolongation of the lactation. Heifer fertility was also analyzed in our study. Although also evidenced, the favorable effect of crossbreeding in heifers was less pronounced than in primiparous cows. This might be due to a higher differences in nutrients demands that purebred HO experience in order to support higher milk yields during early lactation. However, this differences could also be due to a different response of cows to the demands of high milk production. More studies are needed to help clarify this process.

Crossbreed difference between HO and crossbred for characters not considered before, such as the lactation curves parameter were evaluated. Crossbreds showed a different shape of the lactation curve, particularly in regards to persistency. Crossbreds showed also differences in effect of pregnancy on milk production, relative to pure HO. These include differences in the moment in which the effect of gestation on the production started to be significant, as well as the amount of milk lost because of the effect of pregnancy.

Finally, results showed differences across crossbreed types for the multiple traits evaluated in this set of studies. These findings suggest that, depending on the intended use of the milk, and the type of improvement needed, dairy farmers may choose between different types of sires, in order to ensure the optimization of farm crossbreeding programs and the capitalization of their benefits.