UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE CIÊNCIAS AGROVETERINÁRIAS – CAV
PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

EVALUATION OF FEED EFFICIENCY, BIOCHEMICAL PARAMETERS AND MAMMARY GLAND HEALTH OF CROSSBRED HOLSTEIN X SIMMENTAL COWS IN COMPARISON WITH PUREBRED HOLSTEIN COWS DURING LACTATION AND TRANSITION PERIODS

(Avaliação da eficiência alimentar, parâmetros bioquímicos e sanidade da glândula mamária de vacas mestiças Holandês x Simental em relação as vacas Holandês durante a lactação e período de transição)

DEISE ALINE KNOB

LAGES, SC,

FEVEREIRO, 2020

DEISE ALINE KNOB

EVALUATION OF FEED EFFICIENCY, BIOCHEMICAL PARAMETERS AND MAMMARY GLAND HEALTH OF CROSSBRED HOLSTEIN X SIMMENTAL COWS IN COMPARISON WITH PUREBRED HOLSTEIN COWS DURING LACTATION AND TRANSITION PERIODS

(Avaliação da eficiência alimentar, parâmetros bioquímicos e sanidade da glândula mamária de vacas mestiças Holandês x Simental em relação as vacas Holandês durante a lactação e período de transição)

Tese de doutorado apresentada ao Curso de Pós-Graduação em Ciência Animal na Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do grau de Doutor em Ciência Animal.

Orientador: André Thaler Neto
Co-orientador: Armin Manfred Scholz

LAGES, SC,
FEVEREIRO, 2020

Ficha catalográfica elaborada pelo programa de geração automática da Biblioteca Setorial do CAV/UDESC, com os dados fornecidos pelo(a) autor(a)

Knob, Deise Aline
EVALUATION OF FEED EFFICIENCY, BIOCHEMICAL PARAMETERS AND MAMMARY GLAND HEALTH OF CROSSBRED HOLSTEIN X SIMMENTAL COWS IN COMPARISON WITH PUREBRED HOLSTEIN COWS DURING LACTATION AND TRANSITION PERIODS (Avaliação da eficiência alimentar Holandês x Simental em relação as vacas Holandês durante a lactação e período de transição), parâmetros bioquímicos e sanidade da glândula mamária de vacas mestiças / Deise Aline Knob. -- 2020.
152 p.

Orientador: Andre Thaler Neto
Coorientador: Armin Manfred Scholz
Tese (doutorado) -- Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Programa de Pós-Graduação em Ciência Animal, Lages, 2020.

1. BHB. 2. dry matter intake. 3. glucose. 4. NEFA. 5. transition period. I. Thaler Neto, Andre. II. Scholz, Armin Manfred. III.

EVALUATION OF FEED EFFICIENCY, BIOCHEMICAL PARAMETERS AND MAMMARY GLAND HEALTH OF CROSSBRED HOLSTEIN X SIMMENTAL COWS IN COMPARISON WITH PUREBRED HOLSTEIN COWS DURING LACTATION AND TRANSITION PERIOD

(Avaliação da eficiência alimentar, parâmetros bioquímicos e sanidade da glândula mamária de vacas mestiças Holandês x Simental em relação as vacas Holandês durante a lactação e período de transição)

Tese de doutorado apresentada ao Curso de Pós – Graduação em Ciência Animal na Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do grau de Doutor em Ciência Animal.

Banca Examinadora:

Co-Orientador:______________________________
Prof. Dr. Armin Manfred Scholz
Ludwig Maximilians Universität München (LMU – Munich/Germany)

Membro: Daniele C. S. Kazama
Profa. Dra Daniele Cristina da Silva Kazama
Universidade Federal de Santa Catarina (UFSC – Florianópolis)

Membro:______________________________
Prof. Dr. André Ostrensky
Pontifícia Universidade Católica do Paraná (PUC – Paraná)

Membro: AnaBachmannSchogor
Profa. Dra. Ana Luiza Bachmann Schogor
Universidade do Estado de Santa Catarina (CEO/UDESC – Chapecó)

Membro:______________________________
Prof. Dr. Nilson Bröring
Universidade do Estado de Santa Catarina (CAV/UDESC – Lages)

Lages, 27 de fevereiro de 2020

AGRADECIMENTOS

A Deus pela vida, pelas bênçãos de cada dia, pela certeza de sua presença me protegendo e iluminando meu caminho.

Á minha família, aos meus pais Elmo e Lori, às minhas irmãs Elizângela, Leila e Joseane e ao meu sobrinho Gabriel, que mesmo distantes sempre estiveram me apoiando e incentivando. Obrigado pelo amor, pelo apoio e pela confiança.

Ao meu orientador, Professor André Thaler, pela orientação, apoio, confiança, amizade, ajuda, sugestões, críticas e horas de estudos para a realização deste trabalho. Agradecer principalmente por todo o incentivo ao longo desta caminhada.

Ao meu coorientador, Professor Armin Scholz, que desde nosso primeiro contato a respeito do doutorado sanduíche sempre se mostrou muito solicito. Agradecer pela oportunidade de trabalharmos juntos, por viabilizar este sonho de desenvolver parte da pesquisa na Alemanha. Pelo apoio na realização do projeto, pela ajuda críticas e sugestões.

A família Kessel, proprietários da fazenda Gato do Mato, por viabilizar a realização deste trabalho em sua propriedade. Ao senhor Carlos Eduardo Lepkaln, gerente da fazenda, e a todos os funcionários da fazenda, em especial ao Sidnei e Airton que viabilizaram a realização deste trabalho ao atender e adaptar a rotina da fazenda à rotina do experimento. Aos funcionários encarregados pela ordenha, Luciana, Josiane, Rudinei, pelas parceria e longas conversas na sala de ordenha. Agradecer também aos responsáveis pela alimentação dos animais, Nivaldo e Juninho, por estarem sempre dispostos a preparar a alimentação das vacas do experimento, mesmo que isso implicasse em horas extras de trabalho durante a madrugada. Da mesma forma agradecer os senhores Alberto Los, proprietário da fazenda Bela Vista em Carambeí – PR e ao senhor Manfred Epp, proprietário da fazenda "Eichhörnchen" em Colonia Wittmarsun – PR por participarem deste estudo.

Aos meus amigos Dileta Regina Moro Alessio e João Costa Filho, por terem dividido comigo as descobertas, o aprendizado e as vivências deste período de estudo. Agradecimento especial a Dileta pela paciência, amizade e carinho na convivência diária e pela ajuda na coleta e na análise estatística dos dados. Agradecer ao meu amigo Roberto Kappes, por toda ajuda na realização do experimento, nas análises de laboratório e na discussão dos resultados. Agradecer ao estagiário/amigo Wagner Biachin pela ajuda na execução do experimento. Agradecer também aos demais colegas de mestrado e doutorado do grupo de pesquisa do professor André

Thaler Neto, pelas experiências vivenciadas, pelo apoio e incentivo para a realização do trabalho.

Agradecer as bolsistas/amigas Bruna Bergamaschi e Laiz Perazolli pelo auxílio na realização dos experimentos, por estarem sempre disponíveis mesmo em condições adversas. Por compartilhar ótimos momentos de conversa e aprendizado e pelo apoio na realização deste trabalho.

As colegas/amigas da faculdade de medicina veterinária da LMU – Munique, Helen Schweizer e Anna Weigand, pela ajuda nas coletas de dados, incentivo, amizade e pelas longas conversas e parceira ao longo do doutorado sanduíche. Da mesma forma agradecer a todos os funcionários do instituto “Tierärztlichen Fakultät, Lehr- und Versuchsgut Oberschleißheim,” que sempre estiveram dispostos a ajudar e pacientes para adaptar a rotina do trabalho a rotina do experimento. Da mesma forma agradecer ao referido instituto e também a Ludwig Maximilians Universität München (LMU) pela oportunidade de realizar esta pesquisa.

Ao Programa de Pós-Graduação em Ciência Animal (Produção Animal) da Universidade do Estado de Santa Catarina - UDESC, Centro de Ciências Agroveterinárias - CAV, a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES pela bolsa concedida.

A todos, meu Muito Obrigada.

ABSTRACT

KNOB, Deise Aline. **Evaluation of feed efficiency, biochemical parameters and mammary gland health of crossbred Holstein x Simmental cows in comparison with purebred Holstein cows during lactation and transition periods**. 2020. 152 p. Tese (Doutorado em Ciência Animal) – Universidade do Estado de Santa Catarina. Centro de Ciências Agroveterinárias (CAV/UDESC), Lages, SC, 2020.

Crossbreeding in dairy cattle has been used to improve functional traits and milk composition of Holstein herds. The first generation of crossbred cows, which is the one with the best results because of the maximal heterosis, is the most studied. What to do or how to proceed with following generations in a crossbreeding program is always a big question. The objective of our study was to compare milk yield, and milk composition, feed efficiency (FE), physiological parameters, udder conformation traits and indicators of energy balance (beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, body condition score (BCS), backfat thickness (BFT)) of Holstein, Simmental cows and their crosses (F1, R1 – second generation, and following generation of crossbred cows). Four different experiments were carried out, three of them in South Brazil, and one in Germany. We performed the studies in Brazil in three commercial farms. The farms had Holstein, crossbred F1 and R1 (second generation – ¾ Holstein or ¾ Simmental) cows. We accessed the retrospective reproduction records of the farms to evaluate reproductive performance. We performed four visits to each farm to evaluate udder conformation traits and collect milk sample to determine milk yield, composition and somatic cell count. We performed two experiments to compare feed efficiency (on winter and summer) and, the transition period of Holstein and crossbred Holstein x Simmental cows. We also evaluate the psychological parameters rectal temperature (RT) and respiratory frequency (RF). The research carried out in Germany had Holstein, Simmental cows and their crosses. Weekly we accessed BCS, BFT, NEFA, glucose and body weight to evaluate the energetic metabolism. We used the MIXED procedure of the SAS software to perform the statistical analyses. Holstein and crossbred Holstein x Simmental cows have similar feed efficiency (1.75 x 1.81 kg dry matter intake/Kg milk yield, respectively). In winter the cows present better FE as on summer (1.91 x 1.66 kg DMI/Kg milk yield, respectively). For physiological parameters crossbred Holstein x Simmental cows have higher RF on summer and Holstein cows have higher RT on summer in the afternoon. The F1 crossbred Holstein × Simmental cows and ¾ Simmental cows had a shorter calving interval and calving to first service interval in comparison to the Holstein cows. Milk yield did not differ among the genetic groups except for R1 (¾ Simmental) that produced approximately 10% less milk than the other groups. Fat plus protein yield and somatic cell score did not differ among the genetic groups.

Holstein cows had shallower udders and a higher udder clearance than the other groups. For BCS the higher the Simmental proportion, higher the BCS, the same is observed for BFT. Crossbreds F1 and Holstein cows have the higher BCS – BFT loss after calving. Simmental cows present the lower NEFA value, F1 – Crossbred cows have the higher value. For the variables BHB and glucose we do not found difference between the genetic groups. In conclusion, the results our study show that crossbred Holstein x Simmental cows are an alternative to use in high productive systems. In comparison to Holstein cows the crossbred ones shown to reach the same feed efficiency for milk yield and composition. They produce similar amounts of milk with better reproductive performance. As higher the Simmental genes proportion the better the cows deal with the negative energy balance after calving. These genetic groups loss less BCS and BFT. Cows with at least 50% Holstein genes have the higher milk yield which impact on higher NEFA and BHB values, as indicators of higher body tissue mobilization to attempt the energy requirements.

Key-words: BHB; dry matter intake; glucose; NEFA; transition period

RESUMO

KNOB, Deise Aline. **Avaliação da eficiência alimentar, parâmetros bioquímicos e sanidade da glândula mamária de vacas mestiças Holandês x Simental em relação as vacas Holandês durante a lactação e período de transição.** 2020. 147 p. Tese (Doutorado em Ciência Animal) – Universidade do Estado de Santa Catarina. Centro de Ciências Agroveterinárias (CAV/UDESC), Lages, SC, 2020.

O cruzamento em bovinos leiteiros tem sido utilizado para melhorar as características funcionais e a composição do leite em rebanhos da raça Holandês. A primeira geração de vacas mestiças, que apresenta os melhores resultados devido à máxima heterose, é a mais estudada. O que fazer ou como proceder com as gerações seguintes em um programa de cruzamento é sempre uma grande questão. O objetivo com nosso estudo foi comparar a produção e composição do leite, a eficiência alimentar, os parâmetros fisiológicos, as características de conformação de glândula mamária e os indicadores de balanço energético (beta-hidroxibutirato (BHBA), ácidos graxos não esterificados (NEFA), glicose, escore de condição corporal (ECC), espessura de gordura subcutânea na garupa (EGG)) de vacas da raça Holandês, Simental e seus cruzamentos (F1, R1 - segunda geração e gerações subsequentes de vacas mestiças). Realizamos quatro experimentos diferentes, três no sul do Brasil e um na Alemanha. Realizamos os estudos no Brasil em três fazendas comerciais. As fazendas possuíam vacas holandesas, mestiças F1 e R1 (segunda geração – ¾ Holandês ou ¾ Simental). Acessamos os dados históricos de reprodução das fazendas para avaliar o desempenho reprodutivo. Realizamos 4 visitas a cada fazenda para avaliar as características de conformação do úbere e coletar amostras de leite para determinar a produção, composição e contagem de células somáticas. Realizamos dois experimentos para comparar a eficiência alimentar (EA) (no inverno e no verão) e no período de transição de vacas Holandês e mestiças Holandês x Simental. Também avaliamos os parâmetros fisiológicos: temperatura retal (TR) e frequência respiratória (FR). A pesquisa realizada na Alemanha teve vacas Holandês, Simental e seus cruzamentos. Semanalmente, realizamos avaliações de ECC, EGG, BHB, NEFA, glicose e peso corporal para avaliar o metabolismo energético. Utilizamos o procedimento MIXED do software SAS para realizar as análises estatísticas. Vacas Holandês e vacas mestiças Holandês x Simental têm EA similar (1,75 x 1,81 kg/leite/kg de matéria seca ingerida (MSI), respectivamente). No inverno, as vacas apresentam melhor EA em relação ao verão (1,91 x 1,66 kg/leite/ Kg de MSI, respectivamente). Vacas mestiças Holandês x Simental têm maior FR no verão. Vacas Holandês apresentam maior TR no verão à tarde. As vacas mestiças F1 Holandês × Simental e ¾ da raça Simental tiveram um menor intervalo entre partos e menor intervalo

parto primeira cobertura em comparação às vacas Holandês. A produção de leite não diferiu entre os grupos genéticos, exceto para vacas R1 (¾ Simental) que produziram aproximadamente 10% menos leite que os outros grupos. A produção de gordura mais proteína e o escore de células somáticas não diferiram entre os grupos genéticos. As vacas da raça Holandês apresentaram úbere mais raso e maior *udder clearance* em relação aos demais grupos grupamentos genéticos. Para o ECC, quanto maior a proporção de genes Simental, maior o ECC, o mesmo é observado para EGG. Vacas mestiças F1 e Holandês apresentam maior perda de ECC/EGG após o parto. Vacas Simental apresentam o menor valor de NEFA. Vacas mestiças F1 apresentam o maior valor. Para as variáveis BHBA e glicose, não foram encontradas diferenças entre os grupos genéticos. Em conclusão, os resultados de nosso estudo mostram que vacas mestiças Holandês x Simental são uma alternativa para uso em sistemas de alta produtividade. Em comparação com as vacas Holandês as mestiças demonstraram atingir a mesma eficiência alimentar para produção e composição do leite. Elas produzem quantidades semelhantes de leite com melhor desempenho reprodutivo. Quanto maior a proporção de genes Simental, melhor as vacas respondem ao balanço energético negativo após o parto. Esses grupos genéticos perdem menos ECC e EGG. Vacas com pelo menos 50% de genes da raça Holandês têm a maior produção de leite, com impacto nos valores mais altos de NEFA e BHBA, como indicadores de maior mobilização de tecido corporal para tentar atender às necessidades de energia.

Palavras-chave: BHBA; Consumo de matéria seca; Glicose; NEFA; Período de transição

LIST OF FIGURES

CHAPTER IV

CHAPTER V

TABLES LIST

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

ABBREVIATIONS LIST

AMS - Automatic milk system
BCS - Body condition score
BW - Body Weight
BHB - Beta hydroxybutyrate
BFT - Backfat thickness
CBP - Compost bedded pack barn confinement system
CFSI - Calving to first service interval
CI - Calving interval
DIM - Days in milk
DMI - Dry matter intake
DO - Days open
ECM - Energy corrected milk yield
F - Fat
F1 - First generation crossbred
FCM - Fat-corrected milk yield
FE - Feed efficiency
GG - Genetic group
LS - Lameness score
HO - Holstein
MY - Milk yield
NEB - Negative energy balance
NEFA - Non-esterified fatty acids
P - Protein
R1 - Second generation crossbred
RT - Rectal temperature
RF - Respiratory frequency
SCC - Somatic cell count
SCS - Somatic cell score
SIM - Simmental
TMR - Total mixed ration

SUMMARY

INTRODUCTION

Crossbreeding in dairy cattle has been used to improve functional traits and milk composition of Holstein herds (MALCHIODI et al., 2011; HAZEL et al., 2014; MENDONÇA et al., 2014; KNOB et al., 2016, 2018, 2019). The first generation of crossbred cows, which is the one with the best results because of the maximal heterosis, is the most studied. What to do or how to proceed with following generations in a crossbreeding program is always a big question. Three crossbreeding programs are the most used: Backcross - where one of the parental breeds is always used, and so after a few generations there will be a pure breed. Three - breed rotational cross, after the F1 generation a third breed is used, and so the maximum heterosis will be kept for one generation more. With the reintroduction of sires from the same three breeds again in subsequent generations heterosis averages will stabilize at 85.7%. The third system that can be used is the two breed rotational cross, which entails mating the F1 cow to a high genetic merit sire of one of the parent breeds used initially. In the short term, heterosis will be reduced but over time settles at 66.6% (GAMA, 2002; BUCKLEY et al., 2014). A simulation study showed that terminal and rotational crossbreeding strategies using Swedish Red and Swedish Holstein cows can improve profitability in average Swedish organic and conventional dairy herds with purebred Swedish Holstein cows. The largest economic benefits were shown for rotational crossbreeding, in which all animals in the herd were crossbreds and expressed 67% of the full heterosis (CLASEN et al., 2019).

Most of the studies that evaluate the performance of crossbred cows are focused on the results of the F1, the first generation crossbred cows. Just a few evaluate the following generation like the F2 (F1 x F1) or the R1 (F1 x third breed, or F1 x one of the parental breeds) generations of cows. Lopez-Villalobos et al. (2000) reported, in a study evaluating performance with cows in grazing systems, that when backcrossing crossbred Holstein x Jersey cows with the Jersey breed showed a 5% decrease in milk yield. In contrast there was an increase of 16% in fat and 27% in protein yield, with a decrease of 0.4% in the stocking rate/ha. When using the Holstein breed as the backcross, the stocking rate dropped 11%, but increased the production of milk, fat and protein by 10, 8 and 21% respectively, in comparison to the F1 crossbred Holstein x Jersey cows. Nemes et al. (2012) in their study in Serbia, estimated a positive heterosis effect (+ 3.84%) for milk yield in crossbred F1 Holstein x Simmental cows, representing an increase of 185.8 kg/cow/year. However, for R1 cows (¾ Holstein x ¼ Simmental) the heterosis was negative, with less 21 kg milk/cow/year. By evaluating reproductive performance of F1 crossbred Holstein x Simmental cows, Brähmig (2011) reported a 10% heterosis for days open and calving to first service calving interval, representing approximately 13 days less than Holstein cows. The R1 – ¾ Simmental cows, differed from the R1 – ¾ Holstein cows for the variable days open, with an average of less 26 days (111 x 137 days, respectively), while the calving interval did not differ between the two R1 generations.

The positive effects of heterosis can also improve the feeding efficiency of the cows, due to the different energy partitions for milk production and deposition of body tissues (OLSON et al., 2009; PRENDIVILLE; PIERCE; BUCKLEY, 2009; MENDONÇA et al., 2014). In the works of Anderson et al. (2007) and Prendiville; Pierce; Buckley, (2009), crossbred Holstein x Jersey cows had higher dry matter intake (DMI) per kg of live weight, thus showing higher efficiency in the production of solids, with no difference for efficiency in milk production. Gruber et al., (2014), for example, by comparing DMI and efficiency of two different breeds, Holstein and Simmental cows, reported that the latest had lower DMI intake than the Holstein cows, 17.59 vs. 19.49 kg/d, respectively. For milk yield, they observed a corresponding difference with values of 24.8 and 30.1 kg/day for Simmental and Holstein cows, respectively.

Crossbred Holstein x Simmental cows present positive results in terms of improved milk quality, body condition score, fertility and longevity in comparison with pure Holstein dairy herds (BRÄHMIG, 2011; KNOB et al., 2016, 2018; DIEPOLD, 2019; NOLTE, 2019). There are, however, no studies that compare the feed efficiency (FE) of Holstein and crossbred Holstein x Simmental cows. In an evaluation of the first 150 days of the lactation of Holstein and crossbred Holstein x Montbeliarde cows, there was no difference for milk yield and dry matter intake (DMI) between the genetic groups (Hazel et al., 2013). Crossing Holstein with the dual purpose breed Simmental may lead to a modified partition of the feed energy between milk synthesis and deposition of body fat and/or body protein that consequently may reduce the FE for milk synthesis.

The transition from dry period to lactation is a critical period for all the cows. It is a period with several metabolic and physiological changes, and the way the cow go through the period will affect the entire lactation (ŠAMANC et al., 2015; BARLETTA et al., 2017; DJOKOVIĆ et al., 2017). During this period, one of the main changes is the reduction in the DMI, due to the inability to rumen physical filling, first by the occupation of space by the fetus (WANKHADE et al., 2017), and second, after calving, by uterine involution, aggravated by the high energy demand for milk production (DJOKOVIĆ et al., 2017).With this, a period of mobilization of body reserves to supply energy demand begins (YOUSSEF; EL-ASHKER,

2017) well known as and negative energy balance period (MANN et al., 2016; BARLETTA et al., 2017; DJOKOVIĆ et al., 2017). With the lipomobilization, the blood levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) increase, which serves as an energy source for tissues (OSPINA et al., 2010; YOUSSEF; EL-ASHKER, 2017). Accompanied by this, there is a decrease in serum glucose concentrations, which is diverted to the mammary gland for the milk synthesis (GONZÁLEZ; RIVERO, 2016; YOUSSEF; EL-ASHKER, 2017). This mechanism normally occurs during the transition period, however, several factors will determine the impact of this period on productive and reproductive performance throughout the entire lactation (WANKHADE et al., 2017).

Crossbreeding of specialized dairy breeds like Holstein and Simmental has positive results by improving fertility, longevity, milk composition and mammary gland health. All these traits have a positive impact on the suitability of a dairy farm by reducing costs with replacement animals or with higher payment for milk due to the better quality. So, to perform studies evaluating animals from the generations next to the F1 are necessary to better understand the performance of these in a rotational crossbreeding system. Since the importance of the FE and the transition period for the productive performance and the efficiency of the productive system, evaluating and comparing crossbred animals and the parental breeds can bring knowledge about the topic and help farmers to make better decisions in their productive systems. So, the objective with our study was to compare milk yield, and milk composition, feed efficiency, physiological parameters, conformation traits and indicators of energy balance (BHB, NEFA, Glucose, BCS, BFT) of Holstein, Simmental cows and their crosses (F1, R1 – second generation, and following generation of crossbred cows).

The thesis is being presented in the form of chapters. The first chapter is composed by the bibliographic review. The second one by the paper “Feed efficiency and physiological parameters of Holstein and crossbred Holstein x Simmental cows”. The third chapter by the paper “Reproductive and productive performance, udder health and conformation traits of purebred Holstein, F1 and R1 crossbred Holstein x Simmental cows”. The fourth one by the paper “Dry matter intake, body condition score, beta-hydroxy-butyrate concentration, milk yield and composition of Holstein and crossbred Holstein x Simmental cows during the transition period”. The last chapter is represented by the paper “Energy balance during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses”.

CHAPTER I

1. BIBLIOGRAPHIC REVIEW

1.1 Crossbreeding between Holstein, Simmental and Montbeliarde

Based on consanguinity negative effects, through selection pressure and heterosis positive effects, it is advantageous to adopt crossbreeding as a way to use the maximum of the beneficial characteristics of both specialized breeds used on crossbreeding.

Crossbreeding between specialized breeds in dairy production is being used for years. One of the motivating factors to utilize this practice is the increase in milk solids contents, fertility, calving ease, longevity and consanguinity reduction (CASSELL; MCALLISTER, 2009). The same was described by Weigel; Barlass, (2003), in a research where the farmers nominate the benefits of the use of crossbred cows. By comparing the performance of crossbred Holstein x Jersey cows, Holstein Brown x Swiss Brown cows and Holstein cows, the farmers reported as the main benefits for the crossbred ones the longevity, high conception rates, high milks solids contents, health improvement and higher rentability on crossbreed herd.

The classic studies that report the use of crossbreeds evaluated mainly the cross between Holstein and Jersey breeds (LOPEZ-VILLALOBOS et al., 2000; PRENDIVILLE; PIERCE; BUCKLEY, 2009, 2010), always using Holstein as a base, with the aim of elevate yield of the crossbreed products. The country that uses these crossbreeding the most is the New Zeeland, where more than 40% of the cows are crossbred. In Europe, other breeds are used to cross with the Holstein cows, especially dairy linages of Simmental and Montbeliarde breeds. In Brazil, this practice has been growing in the last years, as a way to improve productive and reproductive performance (KNOB et al., 2016, 2018, 2019).

The crossbreeding of these breeds has been demonstrating some positive effects. Schichtl, (2007) in a study made in Germany, reported that Holstein x Simmental cows did not differ for milk yield (7.934 x 8.189 kg) and fat content (3,71 x 3,54%) in comparison to pure Holstein cows, respectively, but showed a higher protein content (3,53 x 3,39, respectively). In other studies, also in Germany, Holstein cows had higher milk yield (BRÄHMIG, 2011; NOLTE, 2019). In Serbia, Nemes et al., (2012) also did not observe differences in milk yield between genetic groups, with superiority for crossbreeds Holstein x Simmental in fat content.

Knob et al., (2018) in their study in South of Brazil, observed higher milk yield for F1 crossbred Holstein x Simmental in comparison with pure Holstein (31,95 x 30,55 liters/day, respectively), standing out in terms of protein content and somatic cell count.

Other similar studies were made by crossbreeding Holstein with Montbeliarde, showing good results as well. Hazel et al., (2014), in their study, comparing first-generation Holstein x Montbeliarde cows with Holstein in the 5 first lactations. They did not observe differences either in the first (7,561 x 7,901 kg), second (9,142 x 9,179 kg) nor from the third lactation (9,949 x 10,012 kg) for the respective genetic groups. Also, it was not found differences for fat content (P=0,30). In Walsh et al., (2008) study in Ireland and Saha et al., (2017) in Italy, there were no differences between genetic groups for milk yield, fat and protein contents.

Yet, other studies diverge these results. Heins; Hansen, (2012), in the United States, observed a higher milk yield for Holstein cows in comparison with Holstein x Montbeliarde for the entire lactation (11.417 x 10.744, respectively), but with lower fat contents (3.58 x 3.69%, respectively) and protein (3.08 x 3.17%, respectively) for the crossbred. Hazel; Heins; Hansen, (2017) also in the United States, found a higher milk yield throughout lactation for Holstein cows in relation to Holstein x Montbeliarde cows (9,200 x 8,905, respectively), but with lower fat contents (3,54 x 3,63%, respectively) and protein (3,08 x 3,14%, respectively). Other studies like the one made by Dezetter et al., (2015) in France, Mendonça et al., (2014) in the United States, Puppel; Kuczyńska, (2016) in Poland and Malchiodi; Cecchinato; Bittante, (2014) in Italy, found the same relation, in which Holstein cows showed higher milk yield in relation to crossbreed Holstein x Montbeliarde, but with lower fat and protein contents.

Besides the advantage of elevating milk solids contents, Holstein x Simmental or Holstein x Montbeliarde crossbreed brings as a benefit the milk somatic cells count (SCC) reduction. In Brähmig (2011) study in Germany, Holstein x Simmental crossbred cows had lower SCC in comparison with pure Holstein (250.000 x 104.000, respectively). In Brazil, Knob et al., (2018) observed lower somatic cells score (SCS) for crossbreed animals in comparison with Holstein (2.81 x 4.46, respectively). Nolte, (2019) in Germany also observed the same relation (2.78 x 3.03). In Italy, Malchiodi; Cecchinato; Bittante, (2014) did not observe a difference for SCS between crossbred Holstein x Montbeliarde and Holstein cows (2.90 x 2.88, respectively). Puppel; Kuczyńska, (2016) in Poland, also did not observe differences comparing SCS between Holstein, Holstein x Simmental and Holstein x Montbeliarde cows (192,730, 158,290 e 136,850, respectively).

Another advantage of Holstein and Simmental or Montbeliarde crossbred use is the better reproductive performance. In Germany were reported lower calving interval (CI) (393 x

422 days) and a higher conception rate in the second calving 27,5 x 23,8% for Holstein x Simmental cows when compared with pure Holstein cows (SCHICHTL, 20017). Remarkably similar results were found by De Haas et al., (2013) in their study in the Netherlands, were crossbred Holstein x Simmental had a CI of 392 days, while for pure Holstein was 422 days. In Brazil was reported a CI of 445±5,7 days for Holstein and 381±8,7 for crossbred Holstein x Simmental cows, with calving to first service interval of 89±2,5 and 65±3,2 days for Holstein and Holstein x Simmental cows, respectively. In the same study, the conception rate in first and second inseminations was 31,2 and 35,4% for Holstein, 34 and 40,1% for Holstein x Simmental crossbred cows (KNOB et al., 2016). The heterosis effect for CI in the second lactation represents 20 days less (SCHICHTL, 20017). This result corroborates with the ones found by Brähmig, (2011).

The same way the crossbreeds presented before, Holstein x Montbeliarde cows showed superior reproductive performance over pure Holstein cows. In a study carried out in Ireland, Walsh et al., (2008) found a lower calving to first service interval in favour of Holstein x Montbeliarde in comparison with Holstein cows ($P<0,05$; 68,2 x 73,3, respectively). Similarly, Hazel et al., (2014) observed fewer days open ($P<0,05$; 128 x 167 days) and higher conception rates ($P<0,05$; 45,1 x 26,9) for crossbreed Holstein x Montbeliarde cows in comparison to Holstein cows.

1.2 Transition period (energetic metabolism indicators)

The transition period comprehends three weeks before and three weeks after calving. This period is the most critical for the cow because of several physiological changes on this phase, leaving the animal more susceptible to metabolic disorders and health issues (ŠAMANC et al., 2015; BARLETTA et al., 2017; DJOKOVIĆ et al., 2017). These occurrences in the transition period will determine the animal's productive and reproductive performance over lactation.

In the period before calving, an increase in energetic demand for colostrum formation is required, concomitantly the end of the fetal growing, which occupies a big part of the abdominal cavity, reducing the feed ingestion capacity (WANKHADE et al., 2017). From that, a negative energy balance (NEB) begins, extending and aggravating even more after calving, on behalf of energetic demand for milk yield fast increases (DJOKOVIC et al., 2011), not accompanied by dry matter intake (DMI) (MANN et al., 2016; BARLETTA et al., 2017; DJOKOVIĆ et al., 2017).

To compensate DMI decrease, a body reserve tissues mobilization begins, principally adipose tissue, for energy formation from hepatic gluconeogenesis (YOUSSEF; EL-ASHKER, 2017). This adipose tissue mobilization can be observed through serum concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) increase (OSPINA et al., 2010), or visually by body condition score (BCS) loss.

The NEFA from reserve triglycerides are directly used by peripheral tissues as an alternative energy source. In the liver, NEFA is oxidized and generates metabolites like Acetyl-CoA, that can be used for energy generation through Krebs Cycle or tricarboxylic acids cycle. When this oxidation occurs incompletely, ketone bodies are generated, principally acetoacetate and BHB to compensate glucose precursor ingestion deficit (ADEWUYI; GRUYSI; EERDENBURG, 2005). BHB can be used as an energy source by the liver itself or released into the blood circulation to be used as an energy source to other tissues. Another possible way is NEFA re-esterification as triglycerides, which they will be stored in adipose tissue again (BARLETTA et al., 2017; DJOKOVIĆ et al., 2017; YOUSSEF; EL-ASHKER, 2017). Besides that, NEFA can suffer a gluconeogenesis process in the liver, by insulin stimuli, which will reduce the ketogenesis process (HAYIRLI, 2006).

During the transition period, glucose demand is exceeded by hepatic gluconeogenesis. Gluconeogenic pathways in the liver are maximally stimulated, but, for lack of glucose sources, remains low. With a low glucose serum concentration and consequent low insulin, a glucagon stimulus occurs to increase reserve tissues catabolism, especially fat, with this, higher ketonic bodies formation to compensate the energetic deficit (GONZÁLEZ; RIVERO, 2016; YOUSSEF; EL-ASHKER, 2017). With the disordered increase of these ketonic bodies, a triglycerides accumulation on liver occurs, given that ruminants have low capacity to synthesise and secrete very low-density lipoproteins, which will carry these triglycerides via blood flow, facilitating the fatty acids disponibility in fat tissue for lipogenesis and storage in fat tissue (GONZÁLEZ; RIVERO, 2016; BARLETTA et al., 2017; DJOKOVIĆ et al., 2017). Lipidic infiltration in hepatocytes will implicate morphological and functional integrity of these cells, resulting in total proteins, glucose, albumin, globulins, triglycerides, cholesterol and urea synthesis reduction (ADEWUYI; GRUYSI; EERDENBURG, 2005; DJOKOVIĆ et al., 2017; YOUSSEF; EL-ASHKER, 2017).

Lipidic infiltration in hepatocytes in large scales may develop a condition known as insulin resistance (HAYIRLI, 2006) in which, insulin receptors lose or reduce sensibility to this hormone, demanding higher concentrations to obtain the expected response (DE KOSTER; OPSOMER, 2013). In this process, a great part of serum glucose deviates for the mammary gland to lactose synthesis, around 50 to 85%, increasing 2.5 times the demand in the third week of lactation. This occurs because the glucose captation by the mammary gland is regulated by GLUT1, GLUT8, GLUT 12, SGLT1 and SGLT2 receptors. They are independent of insulin to carry glucose inside mammary gland alveoli epithelial cells to lactose synthesis (DE KOSTER; OPSOMER, 2013). The insulin independence to capture glucose by the mammary gland is demonstrated by GLUT4 receptors absence, which are insulin-dependent (HAYIRLI, 2006).

Glycemia may vary according to age. Hypoglycaemic states in dairy cows are associated with ketosis and severe energy deficiencies or, to a lesser extent, elevated milk production. The glucose level tends to decrease with productions above 30 kg of milk/day. In lactation, the glucose supply in the cow is important, especially when it reaches maximum milk yield, as the mammary gland needs glucose for the synthesis of lactose. When hypoglycemia occurs during lactation (glycemia <35 mg/dl), milk yield decreases as a form of compensation. In extreme cases, ketosis can occur (GONZÁLEZ; RIVERO, 2016).

Variations in glucose levels occur mainly depending on the stage of lactation. At the beginning, due to the high milk yield, glucose levels are lower, due to their captation into the mammary gland for lactose synthesis, or due to fatty infiltration due to NEB, which will reduce hepatic gluconeogenesis capacity and glycogen reserves decrease (GONZÁLEZ; RIVERO, 2016; DJOKOVIĆ et al., 2017). González et al., (2011) in their study, observed values of 3.37 ± 0.74 (mmol/L) at the beginning of lactation and 3.82 ± 0.41 (mmol/L) in the lactation middle third. Djoković et al., (2017), observed values of 2.29 ± 0.48 mmol/L at the beginning of lactation, while in half, values ranged from 2.5 to 4.2 (mmol/L). In another study by Djokovic et al., 2011) the animal's glycemia averages ranged from 2.2 to 4.0 mmol / L, however for cows in the puerperal period the glycemia was significantly lower in relation to pregnant cows.

Glucose levels variations also occur according to production level, by the same mechanism mentioned above, as seen in Blum et al., (1983), comparing low and high milk yield cows between days 0-40, 40-150 and 150-305 of lactation, found values of 3.24 ± 0.02 - 2.74 ± 0.11; 3.22 ± 0.08 - 2.96 ± 0.06 and 3.17 ± 0.06 - 3.02 ± 0.02 (mmol/L), respectively. Concomitantly to this study, blood glucose levels were compared between the Holstein, Brown Swiss, Simmental and crossbreed Holstein x Simmental breeds, however, they found no differences. The same was found by Shaffer; Roussel; Koonce, (1981), when comparing the serum glucose concentrations between the Holstein, Guernsey, Jersey and Brown Swiss breeds, with an average of 53.27 ± 0.37 (mg/100 mL).

NEFA and BHBA levels also vary during lactation, but with different glucose behaviour. At the beginning of lactation, NEFA and BHBA values present higher values, which decrease with the progress of lactation. González et al., (2011), evaluated cows at the beginning and middle of lactation, found values of 536.60 ± 260.8 and 237.20 ± 178.50 (µmol/L) and 1.45 ± 1, 55 and 1.00 ± 0.39 (mmol/L), for NEFA and BHBA, respectively. Blum et al., (1983), evaluating NEFA concentrations throughout lactation (0-40; 40-150 and 150-305) in low and high yield cows found values of 0.30 ± 0.06 - 0,35 ± 0.04; 0.16 ± 0.01 - 0.19 ± 0.02 and 0.15 ± 0.01 - 0.15 ± 0.01 (mmol/L), respectively, with no difference between low and high milk yield cows.

The way to measure all the metabolic changes that occurs with the cows during this period is by evaluating BCS, and especially the BCS loss. BCS is usually accessed in a visual score between 1 (extremely thin) and 5 (very fat) (FERGUSON; GALLIGAN; THOMSEN, 1994). The higher the BCS loss, especially after calving the higher the NEFA and BHB values.

1.3 Feed efficiency

Feed efficiency (FE) is the ability of cows to transform nutrients into products, in this case into milk and its components (VANSAUN; WHITE, 2018). FE is calculated by dividing the average milk production by the dry matter intake (DMI) for each cow or herd in a given period of time (PAIVA et al., 2013). A common standardization measure for the calculation of FE is the use of ECM (milk corrected for energy and protein), using the equation: ECM = (0.327 * milk yield) + (12.95*% fat*milk yield/100) + (7.65 *% protein*milk yield 100) (TYRRELL; REID, 1965). The DMI is divided by the ECM.

Another method of assessing FE for dairy cows is to evaluate the DMI and milk yield corrected to 4% fat content, in order to standardize yield by comparing different diets, genetic groups or even between animals. In this case the formula used is as follows: Milk yield 4% fat = [(0.4*milk yield) + 15*(Fat*milk /100)]/DMI (DUARTE et al., 2005). Araújo Teixeira et al., (2010) used 3.5% fat correction, with the equation milk yield 3.5% fat content = (0.432*milk yield) + (0.1625 *milk yield *fat).

Another way to calculate FE is to estimate DMI /100 kg of body weight and adjust milk yield for this measure. This efficiency measure is widely used to compare the FE of different genetic groups, to adjust to the weight (size) of the animals, usually when the Jersey breed is used, and/or their crosses, which are more efficient for the production of milk constituents. in comparison with Holstein cows, for example (PRENDIVILLE; PIERCE; BUCKLEY, 2009).

Recently, the use of estimating the efficiency of a specific nutrient in the diet has grown, for example, estimating protein yield by the amount of crude protein (CP) in the diet. And also, mathematical models have been used to evaluate FE. In this model, FE is calculated by the difference between the DMI (or energy intake) and its predicted DMI (or energy intake), based on the mathematical model that considers the energy requirement for maintenance and production in a specific period of time (CONNOR, 2015).

In a meta-analysis study evaluating the diets used for cows in experimental conditions in Brazil, Alessio, (2017) presented the differences in diet that reflect better FE according to the genetic group, Holstein or crossbred Holstein x Zebu cows. Crossbred cows present better FE with diets with higher crude protein content and ether extract, less amount of non-fibrous carbohydrate, intermediate neutral detergent fiber and acid detergent fiber values resulting in a diet with higher total digestible nutrients. These factors associated with higher apparent digestibility of dry matter, crude protein and neutral detergent fiber have a higher impact on the FE for milk yield in crossbred Holstein x Zebu cows. A similar situation was observed for Holstein cows, but with a higher production level.

In view of the high costs of dairy production, where maximizing the production efficiency is always a goal, FE becomes an important tool for optimizing production. In this sense, more and more the genomic selection of animals more efficient for this trait, the ability to transform food consumed into milk, or constituents of milk (taking into account the environment to which they are inserted) is in evidence (HARDIE et al., 2017).

About the FE of crossbred animals, some have been done in different parts of the world, comparing the performance of crossbred and Holstein cows. Olson; Cassell; Hanigan, (2010) comparing the FE of pure Holstein and crossbred Holstein x Jersey cows concluded that crossbred cows consumed the same or slightly less energy than Holstein cows, which need less energy for maintenance and need the same amount of energy for growth. However, crossbred cows produce the same amount of energy in milk, that means, crossbred cows have been shown to be more efficient in relation to energy use. The same authors also demonstrate that the heterosis effect for the maintenance energy is negative and positive for the energy used for production.

Hazel et al., (2013) reported no difference for DMI in the first 150 lactation days by comparing crossbred Holstein x Montbeliarde, Montbeliarde x Holstein/Jersey and Holstein cows with values of 2,904, 2,906 and 2,999 kg/DMI respectively. Still evaluating Holstein and crossbred Holstein x Montbeliarde cows in the first 6 weeks postpartum, Mendonça et al., (2014) reported a tendency for higher DMI by Holstein cows, with this difference appearing mainly in weeks 5 and 6 postpartum where Holstein cows intake approximately 23 kg of dry matter per day while crossbred Holstein x Montbeliarde cows intake approximately 21 kg day.

1.4 References (Introduction and Bibliographical review)

ADEWUYI, A. A.; GRUYSI, E.; EERDENBURG, F. J. C. M. V. Non esterified fatty acids (NEFA) in dairy cattle. A review. **Veterinary Quarterly**, v. 27, n. 3, p. 117–126, 2005.

ALESSIO, D. R. M.,2017 Produção e composição do leite em função da alimentação de vacas em condições experimentais no Brasil. **Thesis**, Universidade do Estado de Santa Catarina, Udesc. Lages – SC. Brasil.

ARAÚJO TEIXEIRA, R. M. et al. Desempenho produtivo de vacas da raça Gir leiteira em confinamento alimentadas com níveis de concentrado e proteína bruta nas dietas. **Revista Brasileira de Zootecnia**, v. 39, n. 11, p. 2527–2534, 2010.

BARLETTA, R. V. et al. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. **Theriogenology**, v. 104, p. 30–36, 2017.

BLUM, J. W. et al. Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. **Animal Production**, v. 36, n. 1, p. 93–104, 1983.

BRÄHMIG, J. Einfluss der Wechselkreuzung von Deutschen Holsteins und Deutschem Fleckvieh auf Milchleistung und Milchqualität in einem automatischen Melksystem. **Thesis.** Veterinary Medicine Faculty, Ludwig Maximilians University, Munich, Germany. 2011.

BUCKLEY, F.; LOPEZ-VILLALOBOS, N.; HEINS, B. J. Crossbreeding : implications for dairy cow fertility and survival. **Animal**, v. 8, p. 122–133, 2014.

CASSELL, B. G.; MCALLISTER, J. Dairy Crossbreeding Research : Results from Current Projects. **Virginia Cooperative Extension**, p. 1–6, 2009.

CLASEN, J. B. et al. Economic consequences of dairy crossbreeding in conventional and organic herds in Sweden. **Journal of Dairy Science**, n. 2012, p. 514–528, 2019.

CONNOR, E. E. Invited review: Improving feed efficiency in dairy production: Challenges and possibilities. **Animal**, v. 9, n. 3, p. 395–408, 2015.

DE HAAS, Y. et al. Suitability of cross-bred cows for organic farms based on cross-breeding effects on production and functional traits. **Animal**, v. 7, n. 4, p. 655–664, 2013.

DE KOSTER, J. D.; OPSOMER, G. Insulin resistance in dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 299–322, 2013.

DEZETTER, D. et al. Inbreeding and crossbreeding parameters for production and fertility traits in Holstein , montbéliarde , and normande cows. **Journal of Dairy Science**, v. 98, p. 4904–4913, 2015.

DJOKOVIC, R. et al. Functional and morphological state of the liver in Simmental dairy cows during transitional period. **Revue de Medecine Veterinaire**, v. 162, n. 12, p. 574–579, 2011.

DJOKOVIĆ, R. et al. Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid lactation. **Advances in Diabetes and Metabolism**, v. 5, n. 2, p. 26–30, 2017.

DUARTE, L. M. D. et al. Effects of different dietary fat sources on intake and milk yield and composition of Jersey cows. **Revista Brasileira de Zootecnia**, v. 34, n. 6, p. 2020–2028, 2005.

FERGUSON, J. D.; GALLIGAN, D. T.; THOMSEN, N. Principal descriptors of body condition score in Holstein cows. **Journal of Dairy Science**, v. 77, n. 9, p. 2695–703, set. 1994.

GONZÁLEZ, F. D. et al. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. **Journal of Veterinary Science**, v. 12, n. 3, p. 251–255, 2011.

GRUBER, L. et al. Influence of energy and nutrient supply pre and post partum on performance of multiparous Simmental, Brown Swiss and Holstein cows in early lactation. **Animal**, v. 8, n. 1, p. 58–71, 2014.

HARDIE, L. C. et al. The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. **Journal of Dairy Science**, v. 100, n. 11, p. 9061–9075, 2017.

HAYIRLI, A. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. **Veterinary Research Communications**, v. 30, n. 7, p. 749–774, 2006.

HAZEL, a R. et al. Montbéliarde-sired crossbreds compared with pure Holsteins for dry matter intake, production, and body traits during the first 150 days of first lactation. **Journal of Dairy Science**, v. 96, n. 3, p. 1915–23, mar. 2013.

HAZEL, A. R. et al. Production, fertility, survival, and body measurements of Montbéliarde-sired crossbreds compared with pure Holsteins during their first 5 lactations. **Journal of Dairy Science**, p. 1–14, 2014.

HAZEL, A. R.; HEINS, B. J.; HANSEN, L. B. Fertility , survival , and conformation of

Montbéliarde × Holstein and Viking Red × Holstein crossbred cows compared with pure Holstein cows during first lactation in 8 commercial dairy farms. **Journal of Dairy Science**, v. 100, n. 11, p. 9447–9458, 2017.

HEINS, B. J.; HANSEN, L. B. Short communication: Fertility, somatic cell score, and production of Normande×Holstein, Montbéliarde×Holstein, and Scandinavian Red × Holstein crossbreds versus pure Holsteins during their first 5 lactations. **Journal of Dairy Science**, v. 95, n. 2, p. 918–24, fev. 2012.

KNOB, D. A. et al. Reproductive performance and survival of Holstein and Holstein x Simmental crossbred cows. **Tropical Animal Health and Production**, v. 48, n. 7, p. 1409–1413, 2016.

KNOB, D. A. et al. Growth, productive performance, and udder health of crossbred Holstein x Simmental cows and purebred Holstein cows. **Semina: Ciências Agrárias**, p. 2597–2606, 2018.

KNOB, D. A. et al. Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows. **Tropical Animal Health and Production**, 2019.

LOPEZ-VILLALOBOS, N. et al. Profitabilities of some mating systems for dairy herds in New Zealand. **Journal of Dairy Science**, v. 83, n. 1, p. 144–53. 2000

MALCHIODI, F. et al. Milk yield traits, Somatic cell score, milking time and age at calving of pure Holstein versus crossbred cows. **Agriculturae Conspectus Scientificus**, v. 76, n. 3, p. 259–261, 2011.

MALCHIODI, F.; CECCHINATO, A.; BITTANTE, G. Fertility traits of purebred Holsteins and 2-and 3-breed crossbred heifers and cows obtained from Swedish Red, Montbéliarde, and Brown Swiss sires. **Journal of Dairy Science**, n. 2012, p. 7916–7926, 2014.

MANN, S. et al. Short communication: Association of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids. **Journal of Dairy Science**, v. 99, n. 7, p. 5851–5857, 2016.

MENDONÇA, L. G. D. et al. Comparison of peripartum metabolic status and postpartum health of Holstein and Montbéliarde-sired crossbred dairy cows. **Journal of Dairy Science**, v. 97, n. 2, p. 805–18, fev. 2014.

NEMES, Z. et al. An Example for the “ Transit-Heterosis ” in the Non-Return Rate of Upgraded Dairy Genotypes. **Animal Science and Biotechnologies**, v. 45, n. 1, p. 215–219, 2012.

NOLTE, O. Effekte der Rückkreuzung auf Fleckvieh aus einer Kreuzungsherde mit

Deutschen Holstein-Genanteilen auf Milchleistung und Milchqualität. **Thesis**. Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München, 2019.

OLSON, K. M. et al. Dystocia, stillbirth, gestation length, and birth weight in Holstein, Jersey, and reciprocal crosses from a planned experiment. **Journal of Dairy Science**, v. 92, n. 12, p. 6167–75, 2009.

OLSON, K. M.; CASSELL, B. G.; HANIGAN, M. D. Energy balance in first-lactation Holstein , Jersey , and reciprocal F 1 crossbred cows in a planned crossbreeding experiment. **Journal of Dairy Science**, v. 93, n. 9, p. 4374–4385, 2010.

OSPINA, P. A. et al. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. **Journal of Dairy Science**, v. 93, n. 2, p. 546–554, 2010.

PAIVA, V. R. et al. Teores proteicos em dietas para vacas Holandesas leiteiras em confinamento. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 65, n. 4, p. 1183–1191, 2013.

PRENDIVILLE, R.; PIERCE, K. M.; BUCKLEY, F. An evaluation of production efficiencies among lactating Holstein-Friesian , Jersey , and Jersey × Holstein-Friesian cows at pasture. **Journal of Dairy Science**, v. 92, n. 12, p. 6176–6185, 2009.

PRENDIVILLE, R.; PIERCE, K. M.; BUCKLEY, F. A comparison between Holstein-Friesian and Jersey dairy cows and their F 1 cross with regard to milk yield , somatic cell score , mastitis , and milking characteristics under grazing conditions. **Journal of Dairy Science**, v. 93, n. 6, p. 2741–2750, 2010.

PUPPEL, K.; KUCZYŃSKA, B. Metabolic profiles of cow's blood; a review. **Journal of the science of food and agriculture**, v. 96, n. 13, p. 4321–4328, 2016.

SAHA, S. et al. Effects of cossbreeding of holsteins cows with Montbéliarde and Swedish red in first and second generation on cheese yield traits. **Agriculturae Conspectus Scientificus**, v. 82, n. 3 Special Issue 2, p. 241–244, 2017.

ŠAMANC, H. et al. Body condition score loss, hepatic lipidosis and selected blood metabolites in holstein cows during transition period. **Animal Science Papers and Reports**, v. 33, n. 1, p. 35–47, 2015.

SCHICHTL, V. Kreuzungszucht beim Milchvieh - ein Ausblick Vor und Nachteile der Kreuzungszucht zwischen Deutschem Fleckvieh und Deutschen Holstein.**Thesis.** Veterinary Medicine Faculty, Ludwig Maximilians University, Munich, Germany. 2007.

SHAFFER, L.; ROUSSEL, J. D.; KOONCE, K. L. Effects of Age, Temperature-Season, and Breed on Blood Characteristics of Dairy Cattle. **Journal of Dairy Science**, v. 64, n. 1, p.

62–70, 1981.

TYRRELL, H. F.; REID, J. T. Prediction of the energy value of cow’s milk. **Journal of dairy science**, v. 48, n. 9, p. 1215–1223, 1965.

VANSAUN, R.; WHITE, R. **Feed efficiency in lactating cows and relationship to income over feed costs**, 2018. . Disponível em: <http://extension.psu.edu/animals/camelids>.

WALSH, S. et al. Effects of breed and feeding system on milk production, body weight, body condition score, reproductive performance, and postpartum ovarian function. **Journal of Dairy Science**, v. 91, n. 11, p. 4401–13, nov. 2008.

WANKHADE, P. R. et al. Metabolic and immunological changes in transition dairy cows: A review. **Veterinary World**, v. 10, n. 11, p. 1367–1377, 2017.

WEIGEL, K. a; BARLASS, K. a. Results of a producer survey regarding crossbreeding on US dairy farms. **Journal of Dairy Science**, v. 86, n. 12, p. 4148–54, dez. 2003.

YOUSSEF, M.; EL-ASHKER, M. Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period. **Tropical Animal Health and Production**, v. 49, n. 2, p. 239–244, 2017.

CHAPTER II

Feed efficiency and physiological parameters of Holstein and crossbred Holstein x Simmental cows

Feed efficiency and physiological parameters of Holstein and crossbred Holstein x Simmental cows

Deise Aline Knob[12]*, Armin Manfred Scholz[2], Laiz Perazzoli[1], Bruna Paula Bergamaschi Mendes[1], Roberto Kappes[1], Dileta Regina Moro Alessio[3], Andre Thaler Neto[1]

[1]Universidade do Estado de Santa Catarina (UDESC), Centro de Ciências Agroveterinárias (CAV), Avenida Luis de Camões, 2090 Cep: 88520-000, Lages, Santa Catarina, Brasil.

[2]Ludwig Maximilians Universität München (LMU), Tierärztlichen Fakultät, Lehr- und Versuchsgut Oberschleißheim, St-Hubertus Straße, 12, 85764, Oberschleißheim, Deutschland.

[3]Centro Universitário Leonardo da Vinci, Rua Marechal Deodoro da Fonseca, 252 Cep- 89130-000, Indaial, Santa Catarina, Brasil.

Abstract: The aim with this study was to compare feed efficiency (FE) and physiological parameters of Holstein and crossbred Holstein x Simmental cows in a confinement system during a winter and a summer period. The research was carried out in a dairy farm in south Brazil. A total number of 48 multiparous cows with 25 Holstein and 23 first generation crossbred Holstein x Simmental cows, respectively, entered the study. The research was performed in 2 periods in the year 2017; a) during summer (February) with 22 cows and b) during winter (July) with 26 cows. Each study period lasted 21 days. Daily, the dry matter intake (DMI) and milk yield were recorded. Two times a day, rectal temperature (RT) and respiratory frequency (RF) were measured. At the end of each experimental period blood was sampled. On the first and last day the body condition score and body weight was evaluated. A variance analysis was performed using the MIXED procedure of SAS statistical package The REML model included the variable fixed effects genetic group, period, day, and days in milk, as well as the interaction between them. Holstein and crossbred Holstein x Simmental cows have similar FE (1.75 x 1.81 kg DMI/Kg milk yield, respectively). Similar amounts of DMI and milk yield were observed. In winter the cows present better FE as in summer (1.91 x 1.66 kg DMI/Kg milk yield, respectively). For physiological parameters crossbred Holstein x Simmental cows have higher RF in summer and Holstein cows have higher RT in summer in the afternoon. Crossbred Holstein x Simmental cows have a similar feed efficiency as the Holstein cows in a high productive system. They can also reach the same production level as purebred Holstein. Therefore, the use of crossbred Holstein x Simmental cows is an alternative to use in high productive systems. We found some evidence that F1 crossbred cows can better dissipate the body heat in a heat stress situation.

Key-words: Dry matter intake, milk yield, rumination time

Introduction

Optimization of feed efficiency (FE) in order to obtain higher productivity in dairy herds with better use of the energy is one of the most important themes in the dairy industry. It allows the better use of the energy and reduces costs, given that the feed of the dairy herd is often the main cost in the production system. It is becoming so important that a breeding value for FE has been included in some breeding programs like in Australian (Pryce et al., 2015) and in United States of America (VanRaden et al., 2017) herds.

FE for dairy herds is usually estimated by calculating the milk yield or fat + protein yield for each Kg of dry matter intake (DMI) (Beever; Doyle 2007). There is a high variability between farms and numerous factors can influence the FE of dairy herds, like milk yield and milk solids content, days in milk, diet composition and digestibility, body weight and body condition score (BCS), herd management, environmental conditions (especially heat stress) as well as cow breed and genetic value of each cow (Beever; Doyle 2007). Gruber et al., (2014), for example, by comparing DMI and efficiency of two different breeds, Holstein and Simmental cows, reported that the latest had lower DMI intake than the Holstein cows, 17.59 vs. 19.49

kg/d, respectively. For milk yield, they observed a corresponding difference with values of 24.8 and 30.1 kg/day for Simmental and Holstein cows, respectively.

Crossbred Holstein x Simmental cows can improve milk quality, body condition score, fertility and longevity in comparison with pure Holstein dairy herds (Brähmig, 2011; Knob et al., 2016, 2018; Diepold, 2019; Nolte, 2019). There are, however, no studies that compare the DMI and FE of Holstein and crossbred Holstein x Simmental cows. In an evaluation of the first 150 days of the lactation of Holstein and crossbred Holstein x Montbeliarde cows, there was no difference for milk yield and dry matter intake (DMI) between the genetic groups (Hazel et al., 2013). Since BCS is one important factor that affects DMI and FE as well as milk yield and crossbred Holstein x Simmental cows present higher BCS scores than Holstein cows (Knob et al., 2016), it is necessary to mention that crossbred cows usually yield similar or only slightly lower amounts of milk than purebred Holstein cows (Brähmig, 2011; Nemes, 2016; Knob et al., 2018, 2019; Puppel et al., 2018). Therefore, a further important factor that affects FE for milk synthesis is the genetic composition of the cows. Crossing Holstein with the dual purpose breed Simmental may lead to a modified partition of the feed energy between milk synthesis and deposition of body fat and/or body protein that consequently may reduce the FE for milk synthesis. Based on these metabolic connections, our hypothesis is that the crossbred Holstein x Simmental cows would show a higher dry matter intake, a similar milk yield and a lower FE in comparison with the Holstein cows. Therefore, the aim with this study was to compare FE and physiological parameters of Holstein and crossbred Holstein x Simmental cows in a confinement system during winter and summer periods.

Methodology

All procedures used in this research were approved by the Santa Catarina State University Ethical Committee, protocol n° 6330030517.

The research was carried out in a dairy farm located in Santa Catarina State, South Brazil. The region belongs to a sub-tropical humid climate zone, type Cfb according to the Köppen classification (Alvares et al., 2013). Cows were maintained in a compost bedded pack barn confinement system. A total number of 48 multiparous cows with 25 Holstein and 23 first generation crossbred Holstein x Simmental cows, respectively, entered the study. The research was performed in 2 periods in the year 2017; a) during summer (February) with 22 cows and b) during winter (July) with 26 cows. Each study period lasted 25 days, including 4 days for adaptation of the cows to the experimental routine and the new group composition, and 21 for data collection. Cows received the same diet during the research period as before the study started. In order to standardize the experimental groups, cows were always selected from the same farm management group (group 1= high yielding group). During the summer period, the average days in milk (DIM) of the experimental group at the start of the research was 144 ± 75.8 days, while during the winter period, the average DIM at the start of the experiment was 96 ± 49.9 days. For each period, cows were also standardized for parity. Only multiparous cows were used for the research.

The diet offered to the cows was a total mixed ration (TMR) based on the use of corn silage, ryegrass (fresh and silage) and concentrates. The composition of the diet during winter and during summer can be seen in Table 1. The diet offered to the cows was calculated to provide 100% of nutritional requirement (NRC, 2001). Cows were mechanically milked 3 times a day (05:00 h, 13:30 h and 20:30 h), and the individual milk yield (MY) was electronically recorded (DeLaval®).

Individual milk samples were taken every 7 days in 40-mL bottles containing Bronopol. Each sample consisted of an average mixture of the 3 daily milking and was sent to the laboratory of milk analysis from UDESC/Lages, SC, Brazil, where the samples were analyzed for milk composition by the infrared method with a DairySpec (Bentley®) equipment. For each cow, on the same day, an additional composite milk sample was collected in 40 ml bottles, containing Bronopol® as the preserving additive and then sent to a laboratory participating in the Brazilian Quality Milk Network of the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) to perform the analysis of somatic cell count (SCC) using automated equipment (SomaCount FC and Dairy Spec FT Bentley®). After having been milked, cows had access to the feed parlor for about 2 hours and 30 minutes. The feed parlor had an individual self-locking feed front, where each cow stayed for the duration of each meal. The offered TMR was weighed and offered individually, to allow individual feed intake measurements. The base feed mix was offered ad libitum for each cow, allowing for 5-10% residuals, and was prepared using a horizontal forage mixer. After each meal, non-consumed feed was weighed. Samples of the TMR offered and the residual of each cow as well as the individual ingredients of the diet were collected and then dried in a forced air oven at 55 °C for 72 h. After this procedure, the samples were minced through a 1-mm screen for subsequent chemical analyses. The dry matter content was determined by drying the samples at 105 °C for 24 h. The ash was quantified by combustion in a muffle furnace at 550°C for 4 h, and the organic material was quantified by mass difference. The total nitrogen was assayed using the Kjeldahl method (method 984.13; AOAC International, 1998). The neutral detergent fiber (NDF) concentration was assessed according to Mertens (2002), except that the samples were weighed in filter bags and treated with neutral detergent in Ankom A220 equipment (Ankom Technology, Macedon, NY). The concentrations of acid detergent fiber (ADF) and ADL were analyzed according to AOAC International (1998). Body condition score (BCS) was assessed and cows were weighed on the first day and on the last day of each period. The evaluation of the BCS was carried out using the scale from 1 (extremely thin) to 5 (very fat) (Ferguson et. al., 1994). Cows were weighed in the morning after milking before the first meal. The daily rumination data were collected through the Heatime® (SCR/Allflex) system, an automatic system composed of a neck collar with a tag that records the rumination time (in minutes) of each cow.

Physiological measurements and blood collection

Every day, from experimental day one until experimental day fifteen, in the morning and in the afternoon, we registered the temperature in the feed parlor with a digital thermometer (Kasvi®). In the morning, around 6:00 a.m. and in the afternoon, around 3:00 p.m, we measured the respiratory frequency (RF) of the cows by observing costal movements for one minute. After RF measurements, we recorded manually the rectal temperature of each cow with a clinical thermometer (G Tech®) carefully inserted into the rectum with a minimal animal disturbance.

On the last day of each experimental period, we collected blood samples of each cow through jugular venipuncture using a vacuum system with plastic tubes. Every tube contained a clot activator. After 3 hours, the blood samples were centrifuged with 3000 rpm for the serum separation for 10 minutes. The serum samples were then frozen at -20 °C for posterior analyses. For the blood parameters analyses, we sent the serum samples to the Laboratório de Patologia Clínica da UCEFF - Unidade Central de Educação FAEM Faculdade, Itapiranga, SC (Clinical Pathology Laboratory of UCEFF). The determination of total protein, albumin and gamma glutamyl transferase (GGT) were made through colorimetry with specific commercial test kits (Gold Analisa Diagnóstica®, Belo Horizonte, Minas Gerais, Brasil). The colorimetric evaluations were made with a biochemical semi automatic analyzer (Bioplus 200S, Barueri, São Paulo, Brasil). To determine the ketone bodies in blood (beta hydroxybutyrate - BHB), we used the portable BHB measurement device Optium Xceed (Abott, São Paulo, Brasil).

Statistical analysis

To obtain normality of data, SCC was transformed to somatic cell score (SCS) by the logarithmic scale applying the following equation: SCS = log2 (SCC/100) + 3 (Ali and Shookg, 1980). To calculate the milk yield corrected to 3.5% fat, we used the following equation: fat-corrected milk yield (FCM) = (0.432*milk yield) + (0.1625*milk yield*fat). The energy corrected milk yield (ECM) was obtained by the equation: ECM = (0.327 * MY) + (12.95% * F* MY / 100) + (7.65 % P * MY / 100), where, MY = milk yield in l/day, F = fat percentage, and P = protein percentage.

A variance analysis was performed using the MIXED procedure of SAS statistical package (SAS 2002) after having the data tested for normality of residuals by Kolmogorov–Smirnov test. The REML model included the fixed effects genetic group (Holstein, Holstein x Simmental), period (winter, summer), day, and DIM (nested in period), as well as the interaction between genetic group and day nested in period and interaction between genetic group and period. The significance level was set to $P<0.05$.

Results

Crossbred Holstein x Simmental cows present similar feed efficiency (FE) (kg of 3.5% fat-corrected milk/kg of DMI) as Holstein cows (Table 2). The effect of the period was significant with a higher FE during winter. Both genetic groups presented similar milk yields including ECM. There was, however, a difference between periods, with higher milk yield and FCM during winter. Like for milk yield, DMI did not show a difference between genetic groups. During winter, cows consumed approximately 2 kg/day more feed (DMI) than during summer. For the variables fat and protein content, there was no difference between genetic groups and between periods. The same was observed for SCS; with both genetic groups presenting similar values. During the experimental period, cows had a daily rumination time of about 9 hours, which represents approximately 37% of the day, with no difference between genetic groups.

Crossbred Holstein x Simmental cows were heavier and had higher BCS than Holstein Cows. This difference was observed during both periods. It has to be highlighted that despite high milk yield of both genetic groups, cows did not lose weight or BCS from the beginning until the end of each experimental period (Table 2).

For all the blood parameters evaluated, we do not find any difference between the genetic groups (Table 3). The observed values for total protein, albumin, globulin, albumin/globulin ratio and GGT for both genetic groups were close. Though not significant (P=0.083), the period seems to have a small effect on total protein, with higher values in winter in comparison to the summer time (8.37 ± 0.07 g/dl and 8.16 ± 0.09 g/dl, respectively).

For the physiological parameters, we found only a slightly higher rectal temperature (RT) in Holstein cows (P=0.0624, Table 4), while the rectal temperature differed significantly between periods with higher values during summer (P<0.0001). The interaction effects were not significant. As we consider the evaluation made in the afternoon as being the hottest time of the day, we observed that during winter there was no difference between the genetic groups, but during summer, Holstein cows had 0.3 °C higher RT than crossbred Holstein x Simmental cows. The averages values for respiratory frequency (RF) show that crossbred Holstein x Simmental cows had higher values than purebred Holstein cows. There was a significant difference between periods (P<0.0001). During summer, cows doubled their RF. We could also observe a tendency to an interaction between genetic groups and periods. During winter, there was no difference between genetic groups, but during summer, crossbred cows had a higher RF (P=0.057).

Discussion

The results presented in Table 2 demonstrate the high productive performance and FE of crossbred Holstein x Simmental cows and Holstein cows under a confinement system in South Brazil. These results are important to show that crossbred Holstein x Simmental cows have the potential to reach the same productive performance as Holstein cows with no difference for FE, milk yield, ECM and DMI. Therefore, with our results we can demonstrate that crossbred Holstein x Simmental cows are a suitable alternative to be used in confinement systems, where the cows should reach a high productive performance to stay competitive in the system. In a study comparing the efficiency of crossbred cows in 3 different productive levels, Clasen et al., (2018) have shown that the crossbred cows can be as competitive as the Holstein cows in all production levels. The Nordic Red × Holstein crosses produce the same or higher fat yield, for example, combined with a better reproductive performance than the Holstein cows.

By comparing DMI, milk yield, and FE of purebred Holstein and Simmental cows throughout the entire lactation, Harder et al., (2019) reported that Simmental cows have lower values for DMI (20.2 vs. 21.8 Kg/day), milk yield (27.4 vs. 35.5 Kg/day), and FE (1.35 vs 1.62 Kg/day) respectively. Purebred Simmental cows produced approximately 77% of the milk yield of the Holstein cows, while the DMI reached 92% leading to a reduction of the FE. Since we evaluated crossbred cows in comparison to purebred Holstein cows, we could demonstrate that, due to positive heterosis effects and complementarity between these two breeds, the difference that could be seen for the purebred cows are not present anymore for high yielding crossbred Holstein x Simmental cows kept in a confinement system. Crossbred Holstein x Simmental cows reach a similar FE for milk synthesis as the Holstein cows independent of the season of the year. Nasrollahi et al., (2017), by evaluating FE of Holstein cows, reported that cows that present ruminal pH in accordance with the recommended values ($pH \geq 6.0$) had a DMI of 26.8 kg/day, while the FE for milk synthesis was 1.94 (kg/kg), which are very similar values that we also found in our study during the winter season for both genetic groups. Therefore, the diet offered to the cows seems to be adequate to maintain a stable ruminal health and to meet the energy requirements of the cows to express their full productive potential. In another study evaluating the FE of purebred Simmental cows at the beginning of the lactation, Münnich et al., (2018) reported that the cows achieved a FE of 1.56 kg/kg, while they produced 36.1 Kg milk/day and a DMI of 23.1 (Kg/day) on a lower efficiency level than the Holstein and crossbred Holstein x Simmental cows in the present study.

Further authors reported no difference for DMI and milk yield by comparing Holstein cows with crossbred cows. Dong et al., (2015), for example, reported no difference for DMI (16.6 vs. 16.5 kg/day) and milk yield (21.8 vs 21.1 Kg/day) by comparing Holstein and a group of crossbred cows (Holstein x Jersey and crossbred Holstein x Norwegian red cows) respectively. Ledinek et al., (2018) evaluating Austrian Holstein and crossbred Simmental x Holstein cows (with Holstein gene proportion between 50 to 75%) found no difference between the genetic groups for DMI (20.86 vs 20.82 kg/day, respectively) and ECM (29.20 vs 29.30 kg/day, respectively). Generally, DMI and ECM reached lower levels than in the present study possibly because the cows were kept on a pasture system, while the performance data represent the average of the entire lactation.

Although the differences between the genetic groups for FE, milk yield, and DMI were not significant, we observed a significant difference between periods. During winter, cows produced 12 liters more milk per day and had approximately 2 kg/day higher DMI resulting in a better feed efficiency during winter (1.9 vs. 1.65 kg/kg. respectively). The differences, however, may be mainly related to the lactation stage of the cows during each period. DIM reached an average 96 days during winter and 144 days during summer. The average DIM during winter was close to the time when cows have the lactation peak, about 50-60 days after calving (Knob et al., 2018). At this point of time, cows have the highest milk yield and have the best FE of the entire lactation. Even during summer with an average DIM of 144 days, when cows are in the middle of lactation, they still show a high FE. Another important factor is that the DMI as a percentage of body weight was approximately 3.0 and 3.3 % for both genetic groups with no difference between them. These values are in accordance with the recommendation for high milk yielding cows (NRC. 2001).

Besides lactation stage, a rising temperature may negatively affect the production performance during summer. Rising temperatures stimulate the satiety center that causes cows to reduce their DMI as a tool to decrease the metabolic heat generated by the ruminal fermentation (Tapki and Şahin, 2006). This homeostatic mechanism generates physiological changes, which affect milk yield and consequently FE for milk synthesis (Kadzere et al., 2002). Könyves et al., (2017) have shown that the performance of Holstein cows was lower during summer than during winter regarding to milk yield (23.32 vs. 24.59 kg/day), forage intake (37.67 vs. 38.92 kg/day) and FE (1.61 vs. 1.58 kg/kg), respectively. High yielding cows are strongly affected by higher temperatures (Tapki and Şahin, 2006), because they started to lose the capacity of regulating the body temperature (Gantner et al., 2017). By comparing high and low yielding purebred Holstein and Simmental cows, both genetic groups of high yielding cows demonstrate negative effects of high temperature-humidity-index (THI) on milk yield. Simmental cows, however, lost less milk yield than Holstein cows indicating a better heat stress tolerance for Simmental cows (Gantner et al., 2017).

Still related to higher milk yield and FE of the cows during the winter is the fact that the diets offered to the cows were different in these two periods. In summer cows received maize silage as a roughage source while in winter they also received fresh grass as a roughage source. In the process of conserving a forage, like silage for example, there are some quality losses and this impact on the content and nutrients provided by the silage (Borreani et al., 2018) and can indirectly affect the milk yield of the cows.

Crossbred cows present higher BCS during the entire lactation which can lead to a better reproductive performance of these cows (Knob et al.. 2016). In order to maintain a higher BCS in comparison with Holstein cows, however, crossbred cows do not have a higher DMI. The better BCS seems to be closely related with the complementarity effect of the breeds used in the crossbreeding programs. Simmental - as a dual purpose breed - has a higher BCS than the Holstein breed (Ledinek et al., 2018; Schweizer et al., 2018). Because of having the same FE, milk yield, and DMI like Holstein cows, it seems likely that crossbred Holstein x Simmental cows are more efficient in terms of energy use, since, based on our results, they can maintain a high production level and have still a better (higher) BCS than the Holstein cows. Olson et al. (2010) evaluating the energy efficiency of primiparous Holstein and crossbred Holstein x Jersey cows concluded that the crossbred cows had similar or a slightly smaller energy intake. They need less energy for maintenance and use the same amount of energy for growth as the Holstein cows. Crossbred cows, however, produce the same amount of energy in milk, that means that they are more efficient regarding energy use for the milk (solids) synthesis.

The BHB is commonly used as an indicator for the mobilization of fat/body reserves. Usually, the higher values occur at the start of the lactation, the time when cows go through the negative energy balance and have to mobilize body reserves in order to supply the energy requirements for the milk synthesis (Barletta et al., 2017; Youssef and El-Ashker, 2017). In our study, no difference between the genetic groups for BHB is possibly related to the fact that the experiment was carried out after the lactation peak. Even with the high milk yield, especially during winter, both genetic groups seem to fully receive the energy they require from the diet offered and are not forced to mobilize body energy reserves. Ferris et al., (2018), by comparing the BHB from Holstein and a triple-crossbred line Swedish Red × Jersey/Holstein crossbred, found a difference between the genetic groups (0.45 vs 0.51 mmol/l). They evaluated the entire lactation and compared additionally low and medium production systems. Our records for BHB are greater than those reported by Ferris et al., (2018), possibly, because of the higher production level of the cows and the associated larger energy requirements for both genetic groups. Młynek et al., (2018), by evaluating Simmental and Holstein cows divided into high and low yielding cows, reported a difference for BHB between the genetic groups with greater values for Holstein cows. Another interesting factor is that for the Simmental breed the values for high and low yielding cows are very close (0.765 vs 0.611 mmol/l), while for Holstein cows the values for high yielding cows are much higher than for the low yielding cows (1.435 vs 0.867 mmol/l).

The total rumination time as well as the rumination time for Kg of DMI was similar for both genetic groups; possibly, due to the fact that the DMI was also similar for Holstein and crossbred Holstein x Simmental cows. The total DMI is one of the factors that have a relation with rumination time. Other authors found also no difference for the rumination time between Holstein and crossbred cows. For example, Stone et al., (2017) have shown that there is no difference for rumination time between Holstein and Jersey cows. The same authors reported that rumination time has a positive correlation with milk yield (0.30). Generally, cows with a higher milk yield have also a greater DMI leading to a negative correlation with lying time (-0.14) (Stone et al., 2017). Prendiville et al. (2010), by comparing Holstein and crossbred Holstein x Jersey cows, found no difference between the genetic groups for the daily total rumination time with 426 and 383 minutes/day, respectively, and for rumination time for each kg of DMI (25.4 x 23.6 minutes, respectively).

With the objective of investigating indicators of the immunity status related to the mammary gland health, we evaluated some serum proteins in blood described by serum proteins, albumin, globulin and ratio albumin:globulin (Bobbo et al., 2017a). In a previous study crossbred Holstein x Simmental have shown a lower SCS than the Holstein cows (2.81 vs 4.46, respectively) (Knob et al., 2018). In the present study, however, we did not find any difference for this trait between the genetic groups. This could possibly explain why we did not find a difference between the genetic groups for the serum proteins. (Bobbo et al., 2017b) have shown that an increase of SCS leads also to an increase of the total protein and albumin level.

The average values for total serum proteins found in our study are in accordance to the reference values for multiparous dairy cows for booth genetic groups (7.4-9.2 g/dl) (Cozzi et al., 2011). Bobbo et al., (2017b) reported variations among specialized dairy breeds and dual purpose breeds. We, however, could not confirm this difference by comparing Holstein and crossbred Holstein x Simmental cows possibly due to the high milk yield and similar SCS for both genetic groups.

Holstein cows have shown slightly higher rectal temperature (RT) than crossbred Holstein x Simmental cows (P=0.0624), while the crossbred cows reached a higher respiratory frequency (RF) (P=0.0310). This might be an indicator for the observation that crossbred Holstein x Simmental cows are more efficient in dissipating the body heat to maintain body homeothermy (Dalcin et al., 2016; de Vasconcelos et al., 2019). A similar observation was made by Kadzere et al., (2002), where Jersey cows showed a higher RF than the Holstein cows, and the higher RF was related to a compensatory mechanism for dissipate body heat (Martello et al., 2010). Umphrey et al., (2001) observed a negative correlation between RF and RT in Holstein cows, that led to the conclusion that the increase of the RF is associated with the decrease of RT. The higher RT for Holstein cows during summer, especially in the afternoon during the highest temperatures of the day (Martello et al., (2010), can be an indicator, that this genetic group has more difficulties to dissipate body heat than the crossbred Holstein x Simmental cows. The Simmental as a dual-purpose breed has a different energy storage. Usually, the meat (and possible the dual-purpose) breeds convert more energy into muscle (protein) deposition, while dairy breeds store surplus energy mainly as subcutaneous and visceral fat (Pfuhl et al., 2007). These fat compartments can act as thermal insulation making endogenous heat exchange more difficult (Blackshaw and Blackshaw, 1994). Kadzere et al., (2002) also reported subcutaneous fat deposition as a fact that negatively affected the thermoneutrality zone. Due to genetic complementarity, the dual-purpose breed Simmental may have an advantage for favorable heat dissipation in comparison with the Holstein cows.

By comparing the two seasons, we observed that both genetic groups reach higher RF and RT during summer in comparison with the winter season ($P<0.0001$). Martello et al., (2010) reported similar results with higher values for RF (54.6 and 30 RF/minute), and RT (38.6 and 37.8 C°) during summer than during winter, respectively. The thermoregulatory strategy of the animals is to maintain a body temperature higher than the environment to allow the body heat flow (Collier et al., 2006). If the environmental temperature reaches values close to the body temperature, the efficiency of dissipating the body heat decreases (Kadzere et al., 2002; Collier et al., 2006). That leads to a greater accumulation of body heat (Badakhshan and Mohammadabadi, 2015), and the only effective way to dissipate the heat is by increasing the RF (Kadzere et al., 2002; Collier et al., 2006; de Vasconcelos et al., 2019).

Conclusion

Crossbred Holstein x Simmental cows have a similar feed efficiency as the Holstein cows in a high productive system. They can also reach the same production level as purebred Holstein. Therefore, the use of crossbred Holstein x Simmental cows is an alternative to use in high productive systems. We found some evidence that F1 crossbred cows can better dissipate the body heat in a heat stress situation, but more studies are required.

Acknowledgements

We would like to thank the Ordemilk and Allfex® that kindly donate the cervical collar monitoring system for the realizations of this research. We would also thank the dairy farm that kindly collaborated on this study. This study was financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001.

References

Alvares, C.A., J.L. Stape, P.C. Sentelhas, J.L. De Moraes Gonçalves, and G. Sparovek. 2013. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22:711–728. doi:10.1127/0941-2948/2013/0507.

Ali, A. K. A. and Shook, G. E. 1980. An optimum transformation for somatic cell concentration in milk. J. Dairy Sci. 63:487-490.

Badakhshan, Y., and M.R. Mohammadabadi. 2015. Thermoregulatory mechanisms of jersey adult cattle and calves based on different body sites temperature. Iran. J. Appl. Anim. Sci. 5:793–798.

Barletta, R. V., M. Maturana Filho, P.D. Carvalho, T.A. Del Valle, A.S. Netto, F.P. Rennó, R.D. Mingoti, J.R. Gandra, G.B. Mourão, P.M. Fricke, R. Sartori, E.H. Madureira, and M.C. Wiltbank. 2017. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. Theriogenology 104:30–36. doi:10.1016/j.theriogenology.2017.07.030.

Beever, D.E., and P.T. Doyle. 2007. Feed conversion efficiency as a key determinant of dairy herd performance: A review. Aust. J. Exp. Agric. 47:645–657. doi:10.1071/EA06048.

Blackshaw, J.K., and A.W. Blackshaw. 1994. Heat stress in cattle and the effect of shade on production and behaviour: A review. Aust. J. Exp. Agric. 34:285–295. doi:10.1071/EA9940285.

Bobbo, T., P.L. Ruegg, E. Fiore, M. Gianesella, M. Morgante, D. Pasotto, L. Gallo, G. Bittante, and A. Cecchinato. 2017a. Short communication: Association between udder health status and blood serum proteins in dairy cows. J. Dairy Sci. 100:9775–9780. doi:10.3168/jds.2017-13111.

Bobbo, T., P.L. Ruegg, G. Stocco, E. Fiore, M. Gianesella, M. Morgante, D. Pasotto, G. Bittante, and A. Cecchinato. 2017b. Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows. J. Dairy Sci. 100:4868–4883. doi:10.3168/jds.2016-12353.

Borreani, G., E. Tabacco, R.J. Schmidt, B.J. Holmes, and R.E. Muck. 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101:3952–3979. doi:10.3168/jds.2017-1383

Brähmig, J. 2011. Einfluss der Wechselkreuzung von Deutschen Holsteins und Deutschem Fleckvieh auf Milchleistung und Milchqualität in einem automatischen Melksystem 162.

Clasen, J.B., A. Fogh, and M. Kargo. 2018. Differences between performance of F 1 crossbreds and Holsteins at different production levels. J. Dairy Sci. 102:436–441. doi:10.3168/jds.2018-14975.

Collier, R.J., G.E. Dahl, and M.J. Vanbaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89:1244–1253. doi:10.3168/jds.S0022-0302(06)72193-2.

Cozzi, G., L. Ravarotto, F. Gottardo, A.L. Stefani, B. Contiero, L. Moro, M. Brscic, and P. Dalvit. 2011. Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. J. Dairy Sci. 94:3895–3901. doi:10.3168/jds.2010-3687.

Dalcin, V.C., V. Fischer, D. dos S. Daltro, E.P.M. Alfonzo, M.T. Stumpf, G.J. Kolling, M.V.G.B. Da Silva, and C. McManus. 2016. Physiological parameters for thermal stress in dairy cattle. Rev. Bras. Zootec. 45:458–465. doi:10.1590/S1806-92902016000800006.

Diepold, D.A. 2019. Effekte der Fleckvieh - Rückkreuzung auf Fruchtbarkeit und Gesundheit. Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München,.

Dong, L.F., T. Yan, C.P. Ferris, and D.A. Mcdowell. 2015. Comparison of maintenance energy requirement and energetic efficiency between lactating Holstein-Friesian and other groups of dairy cows. J. Dairy Sci. 1136–1144.

Ferris, C.P., P.J. Purcell, A.W. Gordon, T. Larsen, and M. Vestergaard. 2018. Performance of Holstein and Swedish-Red × Jersey/Holstein crossbred dairy cows within low- and medium-concentrate grassland-based systems. J. Dairy Sci. 101:7258–7273. doi:10.3168/jds.2017-14107.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows.. J. Dairy Sci. 77:2695–703. doi:10.3168/jds.S0022-0302(94)77212-X.

Gantner, V., T. Bobic, R. Gantner, M. Gregic, K. Kuterovac, J. Novakovic, and K. Potocnik. 2017. Differences in response to heat stress due to production level and breed of dairy cows. Int. J. Biometeorol. 61:1675–1685. doi:10.1007/s00484-017-1348-7.

Gruber, L., M. Urdl, W. Obritzhauser, A. Schauer, J. Häusler, and B. Steiner. 2014. Influence of energy and nutrient supply pre and post partum on performance of multiparous Simmental, Brown Swiss and Holstein cows in early lactation. Animal 8:58–71. doi:10.1017/S1751731113001894.

Harder, I., E. Stamer, W. Junge, and G. Thaller. 2019. Lactation curves and model evaluation for feed intake and energy balance in dairy cows. J. Dairy Sci. 102:7204–7216. doi:10.3168/jds.2018-15300.

Hazel, a R., B.J. Heins, a J. Seykora, and L.B. Hansen. 2013. Montbéliarde-sired crossbreds compared with pure Holsteins for dry matter intake, production, and body traits during the first 150 days of first lactation.. J. Dairy Sci. 96:1915–23. doi:10.3168/jds.2012-5667.

Kadzere, C.T., M.R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. Livest. Prod. Sci. 77:59–91. doi:10.1016/S0301-6226(01)00330-X.

Knob, D.A., D.R.M. Alessio, A.T. Neto, F.D. Mozzaquatro, A. Thaler Neto, and F.D. Mozzaquatro. 2018. Growth , productive performance , and udder health of crossbred Holstein x Simmental cows and purebred Holstein cows. Semin. Ciências Agrárias 2597–

2606. doi:10.5433/1679-0359.2018v39n6p2597.

Knob, D.A., D.R.M. Alessio, A. Thaler Neto, and F.D. Mozzaquatro. 2016. Reproductive performance and survival of Holstein and Holstein x— Simmental crossbred cows. Trop. Anim. Health Prod. 48:1409–1413. doi:10.1007/s11250-016-1103-9.

Knob, D.A., A.M. Scholz, D.R.M. Alessio, B.P.B. Mendes, L. Perazzoli, R. Kappes, and A. Thaler Neto. 2019. Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows. Trop. Anim. Health Prod.. doi:10.1007/s11250-019-02174-9.

Könyves, T., N. Zlatković, N. Memiši, D. Lukač, N. Puvača, M. Stojšin, A. Halász, and B. Miščević. 2017. Relationship of temperature-humidity index with milk production and feed intake of holstein-frisian cows in different year seasons. Thai J. Vet. Med. 47:15–23.

Ledinek, M., L. Gruber, F. Steininger, K. Zottl, M. Royer, K. Krimberger, M. Mayerhofer, C. Egger-Danner, and B. Fuerst-Waltl. 2018. Analysis of lactating cows in commercial Austrian dairy farms: diet composition, and influence of genotype, parity and stage of lactation on nutrient intake, body weight and body condition score. Ital. J. Anim. Sci. 18:202–214. doi:10.1080/1828051X.2018.1504632.

Martello, L.S., H.S. Junior, S.L. Silva, and J.C.C. Balieiro. 2010. Alternative body sites for heat stress measurement in milking cows under tropical conditions and their relationship to the thermal discomfort of the animals. Int. J. Biometeorol. 54:647–652. doi:10.1007/s00484-009-0268-6.

Mertens, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International, v. 85, n. 6, p. 1217–1240, 2002.

Młynek, K., B. Głowińska, E. Salomończyk, J. tkaczuk, and W. Styś. 2018. The effect of daily milk production on the milk composition and energy management indicators in Holstein–

Friesian and Simmental cows. Turkish J. Vet. Anim. Sci. 42:223–229. doi:10.3906/vet-1711-31.

Münnich, M., F. Klevenhusen, and Q. Zebeli. 2018. Feeding of molassed sugar beet pulp instead of maize enhances net food production of high-producing Simmental cows without impairing metabolic health. Anim. Feed Sci. Technol. 241:75–83. doi:10.1016/j.anifeedsci.2018.04.018.

Nasrollahi, S.M., A. Zali, G.R. Ghorbani, M. Moradi Shahrbabak, and M. Heydari Soltan Abadi. 2017. Variability in Susceptibility to Acidosis among High Producing Mid-Lactation Dairy Cows Is Associated with Rumen PH, Fermentation, Feed Intake, Sorting Activity, and Milk Fat Percentage. Elsevier B.V.

Nemes, Z. 2016. Estimation of nonadditive genetic influences on standard lactation production (305 days) in upgrading of Serbian-Fleckvieh to Holstein-Friesian cattle breed. Mljekarstvo 66:330–336. doi:10.15567/mljekarstvo.2016.0409.

Nolte, O. 2019. Effekte der Rückkreuzung auf Fleckvieh aus einer Kreuzungsherde mit Deutschen Holstein-Genanteilen auf Milchleistung und Milchqualität. Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München,.

Olson, K.M., B.G. Cassell, and M.D. Hanigan. 2010. Energy balance in first-lactation Holstein , Jersey , and reciprocal F 1 crossbred cows in a planned crossbreeding experiment. J. Dairy Sci. 93:4374–4385. doi:10.3168/jds.2010-3195.

Pfuhl, R., O. Bellmann, C. Kühn, F. Teuscher, K. Ender, and J. Wegner. 2007. Beef versus dairy cattle: A comparison of feed conversion, carcass composition, and meat quality. Arch. Anim. Breed. 50:59–70. doi:10.5194/aab-50-59-2007.

Prendiville, R., E. Lewis, K.M. Pierce, and F. Buckley. 2010. Comparative grazing behavior of lactating Holstein-Friesian, Jersey, and Jersey × Holstein-Friesian dairy cows and its association with intake capacity and production efficiency. J. Dairy Sci. 93:764–774.

doi:10.3168/jds.2009-2659.

Pryce, J.E., O. Gonzalez-Recio, G. Nieuwhof, W.J. Wales, M.P. Coffey, B.J. Hayes, and M.E. Goddard. 2015. Hot topic: Definition and implementation of a breeding value for feed efficiency in dairy cows. J. Dairy Sci. 98:7340–7350. doi:10.3168/jds.2015-9621.

Puppel, K., E. Bogusz, M. Gołębiewski, T. Nałęcz-Tarwacka, B. Kuczyńska, J. Slósarz, A. Budziński, P. Solarczyk, M. Kunowska-Slósarz, and T. Przysucha. 2018. Effect of Dairy Cow Crossbreeding on Selected Performance Traits and Quality of Milk in First Generation Crossbreds. J. Food Sci. 83:229–236. doi:10.1111/1750-3841.13988.

Schweizer, H., M. Bernau, and A.M. Scholz. 2018. Comparison of performance and health parameters of German and New Zealand dairy cows. Page in Proceedings of the World Congress on Genetics Applied to Livestock Production. vol. Electronic Poster Session - Biology & Species - Bovine (dairy) 2, p. 669, 2018.

Stone, A.E., B.W. Jones, C.A. Becker, and J.M. Bewley. 2017. Influence of breed, milk yield, and temperature-humidity index on dairy cow lying time, neck activity, reticulorumen temperature, and rumination behavior. J. Dairy Sci. 1–9. doi:10.3168/jds.2016-11607.

Tapki, I., and A. Şahin. 2006. Comparison of the thermoregulatory behaviours of low and high producing dairy cows in a hot environment. Appl. Anim. Behav. Sci. 99:1–11. doi:10.1016/j.applanim.2005.10.003.

Umphrey, J.E., B.R. Moss, C.J. Wilcox, and H.H. Van Horn. 2001. Interrelationships in lactating Holsteins of rectal and skin temperatures, milk yield and composition, dry matter intake, body weight, and feed efficiency in summer in Alabama. J. Dairy Sci. 84:2680–2685. doi:10.3168/jds.S0022-0302(01)74722-4.

VanRaden, P. M., J. R. Wright, E. E. Connor, M. J. VandeHaar, R. J. Tempelman, J. S. Liesman, L. E. Armentano, and K. A. Weigel. 2017. Preliminary genomic predictions of feed saved for 1.4 million Holsteins. J. Dairy Sci. 100(Suppl. 2):200–201. (Abstr.)

de Vasconcelos, A.M., C.C. de Albuquerque, J.F. de Carvalho, D.A.E. Façanha, F.R.G. Lima, R.M.F. Silveira, and J. Ferreira. 2019. Adaptive profile of dairy cows in a tropical region. Int. J. Biometeorol.. doi:10.1007/s00484-019-01797-9.

Youssef, M., and M. El-Ashker. 2017. Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period. Trop. Anim. Health Prod. 49:239–244. doi:10.1007/s11250-016-1211-6.

Table 1. Ingredients and composition of TMR[1] (% of dry matter) offered to the dairy cows during the research.

Ingredients	Summer period	Winter period
Corn silage	37.19	11.76
Ryegrass fresh	-	16.97
Ryegrass silage	-	9.40
Commercial concentrate	35.18	36.15
Brewery waste	8.99	9.15
Soybean hull	10.55	4.52
Ground corn	7.04	10.54
Mineral mix (commercial)	1.06	1.51
Chemical composition		
Dry Matter %	-	34.13
Organic material	95.24	91.81
Ash	4.76	8.19
Crude Protein	15.91	15.44
Ether Extract	3.50	5.04
NDF (neutral detergent fiber)	-	43.37
ADF (Acid detergent fiber)	-	20.74
NFC (non fiber carbohydrates)	-	28.5

[1] Values were obtained from chemical analysis of feed samples. NFC = 100 – (% CP + % NDF + % fat + % ash)

Table 2. Means adjusted to the model ± standard error (SEM) for feed efficiency, dry matter intake (DMI), milk yield (MY), fat and protein content, rumination time (RT), DMI % of body weight (BW) intake, BW and body condition score (BCS) for Holstein and crossbreds Holstein x Simmental (Hol x Sim) cows

Variables	Genetic Group		P	Period		P
	Holstein	Hol x Sim		Winter	Summer	
Feed Efficiency [1]	1.75±0.03	1.81±0.03	0.130	1.91±0.03	1.66±0.03	<0.0001
DMI (Kg/day)	24.44±0.45	24.10±0.50	0.582	25.20±0.51	23.34±0.54	0.012
DMI % of BW	3.25±0.07	3.11±0.07	0.153	3.32±0.07	3.04±0.07	0.006
MY (liters/day)	44.4±0,95	44.0±1,01	0.739	50.13±1,02	38.29±1,11	<0.0001
ECM[2]	43.53±0.66	43.43±0.69	0.9118	48.54±0.69	38.42±0.75	0.9118
FCM[3]	43.17±1.06	42.67±1.13	0.7129	48.16±1.15	37.69±1.26	0.5937
Fat %	3.30±0.07	3.33±0.08	0.824	3.24±0.08	3.39±0.09	0.235
Protein %	3.11±0.03	3.07±0.03	0.318	3.08±0.03	3.10±0.03	0.764
SCS	2.80±0.30	2.16±0.31	0.1091	2.21±0.32	2.74±0.36	0.1091
BHB (mmol/l)	0.74±0.05	0.84±0.05	0.2404	0.70±0.05	0.87±0.06	0.0509
RT (minutes/day)	539±11	543±12	0.619	558±12	524±13	0.347
RT kg DMI (Minutes/Kg DMI)	22.4±0.6	22.7±0.7	0.8072	22.5±0.7	22.7±0.7	0.7552
BW (Kg) first day[4]	688±12	729±12	0.019	697±11	720±12	0.192
BW (Kg) last day[5]	699±11	739±11	0.017	722±11	716±12	0.754
BCS first day[4]	2.79±0.08	3.62±0.09	<0.0001	3.31±0.08	3.10±0.09	0.101
BCS last day[5]	2.90±0.09	3.66±0.10	<0.0001	3.35±0.09	3.21±0.10	0.334

[1] Feed efficiency kg of 3.5% fat-corrected milk/kg of Dry matter intake

[2] Fat-corrected milk yield

[3] Energy corrected milk yield

[4] Body weight (BW) and body condition score (BCS) on the first day of booth periods

[5] Body weight (BW) and body condition score (BCS) on the last day of booth periods

Table 3. Means adjusted to the model ± standard error (SEM) of blood parameters for Holstein and crossbreds Holstein x Simmental cows.

Variable	Genetic Group (GG)		P Value		
	Holstein	Holstein x Simmental	GG	Period	GG*Per
Total Protein (g/dl)	8.25±0.07	8.27±0.08	0.8475	0.0838	0.7980
Albumin (g/dl)	2.94±0.07	2.99±0.08	0.6627	0.8252	0.2821
Globulin (g/dl)	5.31±0.12	5.27±0.12	0.8402	0.5187	0.9080
Albumin:Globulin	0.56±0.02	0.57±0.02	0.6716	0.7757	0.3773
GGT (U/L)	34.86±1.35	34.32±1.32	0.7793	0.6127	0.6430

Table 4. Means adjusted to the model ± standard error (SEM) of the rectal temperature and respiratory frequency according to genetic group (GG) (Holstein or crossbred F1 Holstein x Simmental cows) and period (summer or winter)

Variable		Rectal temperature		Respiratory frequency	
	Category	Means± SEM	P	Means± SEM	P
GG	Hol	38.43±0.04	0.0624	45.66±1.07	0.0310
	F1	38.29±0.05		49.02±1.12	
Period	Winter	38.08±0.04	<0.0001	30.14±1.06	<0.0001
	Summer	38.64±0.05		64.54±1.14	
GG Period	Hol Winter	38.10±0.06	0.1487	29.93±1.51	0.0571
	F1 Winter	38.07±0.06		30.33±1.49	
	Hol Summer	38.75±0.07		61.38±1.52	
	F1 Summer	38.52±0.07		67.69±1.67	
GG Period Time*	Hol Winter pm	38.18±0.07	0.0154	32.90±1.63	0.6143
	F1 Winter pm	38.17±0.07		33.76±1.60	
	Hol Summer pm	38.92±0.07		64.02±1.59	
	F1 Summer pm	38.64±0.07		70.43±1.74	

CHAPTER III

Paper published in the Journal: *Tropical Animal Health and Production*
https://doi.org/10.1007/s11250-019-02174-9

Reproductive and productive performance, udder health and conformation traits of purebred Holstein, F1 and R1 crossbred Holstein x Simmental cows

Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows

Deise Aline Knob[1,2]*, Armin Manfred Scholz[2], Dileta Regina Moro Alessio[3], Bruna Paula Bergamaschi Mendes[1], Laiz Perazzoli[1], Roberto Kappes[1], Andre Thaler Neto[1]

[1]Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Avenida Luis de Camões, 2090 Cep: 88520-000, Lages, Santa Catarina, Brasil.

[2]Ludwig Maximilians Universität München, Tierärztlichen Fakultät, Lehr- und Versuchsgut Oberschleißheim, St-Hubertus Straße, 12, 85764, Oberschleißheim, Deutschland.

[3]Centro Universitário Leonardo da Vinci, Rua Marechal Deodoro da Fonseca, 252 Cep- 89130-000, Indaial, Santa Catarina, Brasil.

*Corresponding author: Deise Aline Knob. Email: deisealinek@gmail.com

ORCID: 0000-0003-3972-1094

Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows

Abstract: The objective of this study was to compare the reproductive performance, milk yield and composition, and udder health and conformation traits of Holstein (Ho), F1, and R1 crossbred Ho × Simmental (Sim) cows. Three commercial dairy farms in south Brazil were used as the research units. All farms held Ho, F1, and R1 crossbred Ho × Sim (¾ Ho × ¼ Sim and ¾ Sim × ¼ Ho) cows. The collection of milk samples and evaluation of udder conformations traits occurred during four visits to each farm. In addition to the actively collected data, retrospective reproduction records of the farms served as the basis for the statistical analysis using analysis of variance models using SAS. The F1 crossbred Ho × Sim cows and ¾ Sim (first rotational crossbreeding generation = R1 using Sim semen) cows had a shorter calving interval and calving to first service interval compared to the Ho cows ($P < 0.0001$). Milk yield did not differ among the genetic groups except for R1 (¾ Sim) that produced approximately 10% less milk than the other groups ($P = 0.0245$). Fat plus protein yield and somatic cell score did not differ among the genetic groups. Ho cows had shallower udders ($P < 0.0001$) and a higher udder clearance ($P < 0.0001$) than the other groups. F1 and R1 crossbred Ho × Sim cows had shorter reproduction intervals than purebred Ho cows. Although udder conformation traits lacked high-quality scores in crossbred cows, somatic cell scores reached the same level as in purebred Ho cows.

Key words: Days open, Heterosis, Milk Composition, Milk Yield, Somatic Cell Score

Introduction

The challenge of producing high-quality milk in a competitive dairy farming environment leads to the constant search and development of technologies that might improve the efficiency of the production process. One technology that could be used to achieve this objective is crossbreeding of dairy breeds, which is aimed at utilizing positive heterosis effects and complementarity among dairy breeds. According to Gregor Mendel (Buchanan 2010), the heterosis effect is expectedly highest in the (heterozygote) first filial (F1) generation and is higher the greater the genetic distance between the parental breeds or breeding lines (Puppel et al. 2018). Fiévet et al. (2018) explained heterosis as a "systemic property emerging from non-linear genotype-phenotype relationships" based on non-additive dominant and/or epistatic gene actions. The heterosis effect of the filial generation(s) is usually calculated as the difference (in absolute measurement units or as a percentage) from the so-called mid-parent value—the average performance of the (two) parental breeds or genetic lines (Andorf et al. 2009).

The use of crossbreeding in dairy cattle has provided favorable results (positive heterosis) for fertility traits, milk composition, and udder health of crossbred Holstein (Ho) × Jersey or Ho × Norwegian Red cows (Buckley et al. 2014; Dall Pizzol et al. 2017; Fellipe et al. 2017; Heins et al. 2008), Ho × Montbéliarde cows (Hazel et al. 2013, 2014; Saha et al. 2017), and Ho × Simmental (Sim) cows (Brähmig 2011; de Haas et al. 2013; Knob et al. 2016, 2018; Schichtl 2007). These studies, however, focused mainly on the performance of first generation crossbred cows (F1), although Ezra et al. (2016) compared the performance of purebred Ho with Ho × Norwegian Red crossbreds in first and second generations, as did Ledinek and Gruber (2015) for Ho × Sim crossbreds in comparison with purebred Ho or Sim cows (Fleckvieh). Freyer et al. (2008) reviewed—besides more recent crossbreeding studies—the history of the "crossbreeding" program for the creation of the synthetic breed "Black-Pied Dairy Cattle" in the former East Germany covering several crossbreeding generations by testing the crosses of different Ho, Jersey, and the German Black-Pied Lowland cattle. Only a few studies have examined the performance in crossbreeding generations following the F1, e.g., the F2 (F1 × F1), where only 50% of the maximum heterosis effect may be expected and the first rotational (R1) crossbreeding (or backcross) generation by crossing the F1 again with one of the (two) purebred parental lines. Therefore, scientific information regarding the performance of later crossbreeding generations in comparison with F1 crossbreds or purebreds is limited. In a study undertaken in Serbia, Nemes (2016) estimated heterosis for Ho × dairy Sim F1 and R1 crossbred cows. The heterosis for milk yield was positive in the F1 cows (+3.84%) with more than 185.8 kg/cow/year, but negative with less than 21 kg in the R1 cows (with ³/₄ Ho and ¼ Sim). Data originated from a herd with an average yield of 3.900 kg for Sim and 5.800 kg for Ho cows. Another study using comparable breeds in Germany estimated heterosis effects for reproductive traits. For days open and calving to first service interval (CFSI), a heterosis effect of 10% (approximately 13 days less than the average of the purebreds) was estimated for the average F1 generation (Ho × Sim or Sim × Ho) (Brähmig 2011). The R1 cows with ¾ Sim differed significantly from the R1 cows with ¾ Ho for days open by an average of 26 days (111 days vs. 137 days), whereas the CFSI did not differ between the two R1 generations. The shortest average period of days open was achieved by the pure Sim cows at 103 days.

The crossing of Ho and Sim breeds aims to utilize the positive characteristics of each breed, such as greater milk yield from Ho and a more favorable milk composition, lower number of somatic cells, and better reproductive performance from Sim (Kara and Koyuncu 2018; Piccand et al. 2013; Rehak et al. 2012). Sim is a dual-purpose breed and their dairy lineages are being crossed with Ho in many countries. In previous studies comparing the performance of F1 Ho × Sim with purebred Ho cows, crossbreeding improved fertility traits by creating shorter calving and CFSIs, prolonged longevity, improved udder health combined with lower somatic cell score (SCS), and greater amounts of milk solids (Brähmig 2011; Nemes et al. 2012; Knob et al. 2016, 2018). However, more studies regarding the performance of the generations following F1 are required. Thus, the objective of the present study was to compare reproductive performance, milk yield and composition, and udder health and conformation traits of purebred Ho, crossbred F1, and crossbred R1 Ho and Sim cows.

Materials and Methods

Animals and management

The study was undertaken at three dairy farms located in the southern region of Brazil with herd one located in Bom Retiro, Santa Catarina State (27°47′50″ S, 49°29′21″ W, altitude 890 m); herd two in Carambeí, Paraná State (24°55′04″ S, 50°05′50″ W, altitude 1120 m); and herd three in Palmeira, Paraná State (25°25′46″ S, 50°00′23″ W, altitude 890 m). The region belongs to a sub-tropical humid climate zone, type Cfb (C = humid sub-tropical, f = oceanic climate, without dry season, and b = with temperate summer) according to the Köppen classification (Alvares et al. 2013). The dairy herds consisted of purebred Ho and crossbred Ho × Sim cows. The first generation of crossbred cows (F1) originated from random mating between purebred Ho cows and Sim bulls (dairy lineage of Sim imported from Germany), which were selected for artificial insemination based on their dairy breeding values. Canadian and USA Ho bulls delivered the semen for crossing with the purebred Brazilian Ho cows. Randomly assigned Ho or Sim bulls that were mated artificially with F1 cows provided the basis for the first rotational crossbreeding generation, R1 (Table 1).

In herd one, cows were kept in a compost bedded pack barn confinement system. The animals had access to the feeding parlor three times a day, after each milking, where they remained for approximately 2.5 h. The diet offered to the cows was a total mixed ration (TMR) based on maize silage, ryegrass haylage, fresh grass according to the season, sorghum (*Sorghum bicolor*) in the summer, and oats (*Avena sativa*) plus ryegrass (*Lolium multiflorum*) in the winter—always supplemented by concentrates. The herd consisted of approximately 280 lactating cows with approximately 60% purebred Ho and 40% F1 or R1 crossbred Ho × Sim cows.

In herd two, the cows were kept in a freestall confinement system with constant access to the feeding parlor. Milking was performed twice a day in a tandem-milking parlor. The diet provided to the animals was a TMR based on maize silage, ryegrass haylage, and concentrates. The herd had approximately 80 lactating cows, of which approximately 60% were purebred Ho and 40% were F1 and R1 crossbred Ho × Sim cows.

In herd three, the production system was semi-confined, where cows were fed on pastures according to the season: sorghum (*S. bicolor*) in summer and oats (*A. sativa*) plus ryegrass (*L. multiflorum*) in winter, supplemented with maize silage, ryegrass haylage, and concentrate twice a day. Milking occurred twice a day in a herringbone-milking parlor. The herd consisted of approximately 140 lactating cows, of which approximately 15% were purebred Ho and 85% were F1 and R1 crossbred Ho × Sim cows. Table 2 contains the average values for milk yield and composition as well as the somatic cell count (SCC) of each farm.

In addition to the recommended cleaning and maintenance procedures of the milking system, all farms performed pre- and post-dipping as part of the milking routine to ensure retention of the milk quality standards and udder health.

Productive and udder conformation traits data

Four visits occurred to each farm from April 2017 to March 2018. Udder conformation traits of all lactating cows were recorded at each visit just before milking began. The evaluation of udder depth followed the methodology used by Coentrão et al. (2008) measuring the distance (in cm) from the udder floor to the hock line. Udder clearance is defined as the distance between the udder bottom and ground and was also measured in cm. Front and rear teat placement on a linear scale of 1 (open teats) to 5 (normal teats placement) to 9 (closed teats) and the individual teat length (in cm) were further udder traits measured. Individual milk yield, milk composition (fat, protein, and lactose content), and SCC provided further performance and quality data. For each cow, a composite milk sample was collected in 40 ml bottles, containing Bronopol® as the preserving additive, over a 24-h period. A laboratory participating in the Brazilian Quality Milk Network of the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) received the samples in an isothermal box and performed the analysis using automated equipment (SomaCount FC and Dairy Spec FT Bentley®). Flow cytometry, according to the ISO 13366-2 method, was used to measure the SCC. An infrared technique based on ISO 9622 guidelines provided the data for the milk composition parameters. INMETRO IEC 17025:2002 contains the details for both methods. The evaluation of the teat end hyperkeratosis score occurred immediately after milking and just before the application of the post-dipping. The classification of hyperkeratosis by visual scoring on a scale of 1 to 4 (1 – teat end without ring formation, 2 – teat end with small ring formation, 3 – teat end with rough ring formation, and 4 – teat end with a very rough ring) followed the methodology described by Mein et al. (2001). Farm records provided information regarding days in milk (DIM) and calving interval (CI) for each cow on the day of data collection. In total, 1464 individual data from 603 cows with 45% purebred Ho, 25.4% F1, and 37.8% R1 crossbred Ho × Sim origin were included in the statistical analysis.

Reproductive data

The farm management data from 2010 to 2017 (ProdapTech®, DairyPlan®, GEA Farm Technologies, and Excel spreadsheets) provided animal records containing information on genealogy, date of birth, insemination, and calving dates. Age at first calving, CFSI, CI, and conception response (yes or no) served as variables for the reproductive performance analysis. All farms adopted a voluntary waiting period after calving of 40 days and used artificial insemination. Artificial insemination of all heifers—independent of the genetic group—occurred at approximately 15 months of age and body weight of approximately 350 kg. Records from 1004 cows were included in the statistical analysis.

Statistical analyses

Outliers or potential errors from milk sampling were removed by data standardization. Records between 8 and 305 days of lactation, milk yields between 10 and 60 liters per day, fat contents between 1.5% and 5.6%, protein contents between 2.0% and 5.3%, and lactose contents between 3.0% and 5.2% remained in the data set. Parity was grouped as first, second, third, or more parturitions. To obtain normality of data, SCC was transformed to SCS by the logarithmic scale applying the following equation: SCS = log2 (SCC/100) + 3. The energy corrected milk yield (ECM) was obtained by the equation: ECM = (0.327 * MY) + (12.95% * F* MY / 100) + (7.65 % P * MY / 100), where, MY = milk yield in l/day, F = fat percentage, and P = protein percentage. An analysis of variance (ANOVA) using the MIXED model procedure of SAS 9.4 with a repeated measures test-day effect tested the impact of the fixed effects on the variables of milk yield and composition and SCS. The model contained the following fixed effects: genetic group, parity, herd, season, interaction between genetic group and parity, and interaction between genetic group and herd, as well as the linear and quadratic effect of the covariate DIM. Before the variance analysis, data were tested for normality of the residuals using the Kolmogorov–Smirnov test and for homogeneity of the variances using the Levene test.

The ANOVA of the reproductive data followed the same procedure as described above for “milk” data, but without the repeated measures effect of the test day. The MIXED model contained the following fixed effects: genetic group, parity, herd, calving year, calving season, and the interaction between parity and genetic group. Only the binary variable conception response (yes or no) resulting in the conception rate for the herd or population was analyzed with a generalized linear model for binomial distributed variables using the GENMOD procedure of SAS, with a statistical model analogous to that described above. In the case of a difference among genetic groups, a Chi-squared test comparing the genetic groups two by two was performed to determine statistically significant associations.

Results

Crossbred Ho × Sim cows had a higher fertility than purebred Ho cows. F1 Ho × Sim and ¾ Sim cows showed a 45 or 52 days, respectively, shorter CI than the purebred Ho cows ($P < 0.0001$; Table 3). The ¾ Ho cows had intermediate CI values, which did not differ from the other genetic groups. No differences were found between parities ($P = 0.3581$) and there were no interactions between genetic group and parity or parity and herd for CI. The F1 and ¾ Sim cows presented better performance for CFSI. Insemination of these cows occurred, on average, 12 and 8 days earlier than for Ho cows, respectively ($P < 0.001$). The ¾ Sim cows presented an average conception rate of approximately 40%, which significantly surpassed the conception rate of the pure Ho cows, with intermediate values for the other two genetic groups ($P < 0.0001$; Table 4). A better conception rate was observed for the first and the following inseminations after calving. We observed no differences among genetic groups for age at first calving ($P = 0.229$) with average values of approximately 28 months. Ho cows calved the first time at 28.3 ± 0.2 months of age, the F1 Ho × Sim cows at 28.8 ± 0.3 months, the ¾ Ho cows at 28.5 ± 0.6 months, and the ¾ Sim cows at 29.3 ± 0.6 months.

Ho, F1 Ho × Sim, and ¾ Ho cows yielded similar amounts of milk (Table 5), whereas ¾ Sim cows reached an approximately 10% lower milk yield than the other genetic groups ($P = 0.0245$). After correcting milk yield for energy and protein (ECM), differences among genetic groups ($P = 0.1411$) and interactions between genetic groups and parity vanished for these variables ($P > 0.72$).

Ho cows recorded lower fat and protein contents in their milk than the other genetic groups ($P < 0.0001$), although there was no significant difference among genetic groups for the lactose content and the daily fat + protein yield. Ho cows had a lower total solids content in milk ($P < 0.0001$) because of the lower fat and protein content. These characteristics of milk yield and composition show—despite a slightly lower yield in comparison with other genetic groups—that R1 (¾ Sim) cows were equivalent to the others for milk solids yield (Table 5). There was no difference among genetic groups for udder health represented by the SCS ($P = 0.7523$). All genetic groups achieved moderate SCS values ranging between 2.60 and 2.86 (mean SCC of 362,000cells/ml).

There was no interaction found between genetic groups and season for all variables related to milk yield and composition. The highest milk yield occurred during winter (28.7 ± 0.4 l/day)), an intermediate yield was observed in autumn (26.9 ± 0.4 l/day), and the lowest milk yield during spring and summer (24.7 ± 0.4 l/day and 24.2 ± 0.4 l/day, respectively; $P < 0.0001$). A higher fat content was recorded in spring (3.65 ± 0.03%), an intermediate one in winter and autumn (3.52 ± 0.03% and 3.49 ± 0.03%, respectively), and the lowest content in summer (3.39 ± 0.03%; $P < 0.0001$). The highest protein contents (%) were recorded in winter and autumn (3.28 ± 0.01 and 3.26 ± 0.01, respectively), and the lowest values in spring and summer (3.14 ± 0.01 and 3.18 ± 0.01, respectively). The lactose content, however, did not differ among seasons.

Ho cows presented the best results for udder conformation traits with shallower udders ($P < 0.0001$; Table 6) and a greater distance between the base of the udder and the ground (udder clearance) ($P < 0.0001$) than the other genetic groups. Ho cows, however, have shorter front teats compared with F1 crossbred Ho × Sim and ¾ Sim cows ($P = 0.0146$). The posterior teat length did not differ among genetic groups ($P = 0.3754$). Ho cows had a better front teat placement that is more favorably centered, whereas the other genetic groups had a front teat placement more open and distant (P = 0.0013). Teat end hyperkeratosis scores achieved highest values in Ho cows and the lowest ones in R1 ¾ Sim cows, while F1 crossbred Ho × Sim and ¾ Ho cows showed intermediate values between the other genetic groups (Table 6).

Discussion

Heterosis and complementarity effects might cause better reproductive performance of crossbred F1 Ho × Sim and ¾ Sim cows in comparison with purebred Ho cows. Maximum heterosis is expected in F1 crossbred cows and the heterosis effect is usually higher for traits characterized by a low heritability such as reproductive or fitness traits (Sørensen et al. 2008). The heterosis effect for reproductive traits can reach values between 5% and 25% (Sørensen et al. 2008), improving the reproductive performance of crossbred cows in comparison with the average of the purebred parent lines. In addition to the usage of positive heterosis effects, crossbreeding systems aim to optimize the complementarity between breeds. This aspect might help explain the better reproductive indexes of crossbred cows, especially the R1 (¾ Sim) crossbred cows in the present study (Tables 3 and 4). The Sim breed usually shows more favorable results for reproductive traits than the Ho breed (Piccand et al. 2013). Because Sim is a dual-purpose breed, the cows lose less body weight than Ho. The smaller loss of body weight is combined with a smaller decrease of the body condition score (BCS) after calving. Therefore, Sim presents a better energetic balance especially after calving. This aspect is positively correlated with a better reproductive performance in comparison with Ho cows (Rehak et al. 2012; Młynek et al. 2018). Under hot and humid climatic conditions, the reproductive performance of (Ho) cows may also be negatively affected by heat stress as described by Leyva-Corona et al. (2018). These authors discussed the fertility associated action of 12 molecular markers (single nucleotide polymorphisms)related to the genes of the bovine genome, which regulate prolactin, growth hormone, and insulin-like growth factor-1 in various metabolic pathways of thermoregulation, growth, lactation, and reproduction. Services per conception and days open may be affected positively or negatively depending on the marker haplotype occurrence.

Knob et al. (2016) showed that crossbred Ho × Sim cows had a higher BCS during the entire lactation period in comparison with purebred Ho cows. This finding was also reported by Janzekovic et al. (2015), although crossbreds and purebred Sim cows did not differ. Different aspects of the influence of negative energy balance after calving, represented by lower BCS for Ho cows, such as damage in follicular growth and development of the embryo, and reduction of oocyte quality and luteal function were described by Knob et al. (2016).

The F1 crossbred and R1 ¾ Ho cows produced the same amount of milk as the purebred Ho cows (Table 5), possibly due to the positive heterosis effect for this trait. The average milk yields shown in the present study, however, were lower than the milk yields reported by Knob et al. (2018), which originated from cow data recorded in south Brazil also. One reason for the observed differences might be the farming system itself as discussed by Abin et al. (2018) who compared low and high input dairy farming in South Africa. On average, Ho and F1 crossbred Ho × Sim cows yielded around 30 liters of milk per day in the study by Knob et al. (2018). In another study that included data of Ho cows from the entire country, the average milk yield was recorded at around 24 liters per day (Alessio et al., 2018). The lower milk yield of crossbred ¾ Sim cows might be related to their genetic composition because the Sim genes amount to 75% of the total. Purebred Sim cows have a milk production of approximately 70% to 80% of the yield reached by Ho cows (Brähmig 2011; Kara and Koyuncu 2018; Nolte 2019). The lower milk yield of this genetic group is also related to the better reproductive performance of ¾ Sim cows compared to Ho cows, as high milk yields may negatively affect the reproductive performance (Bedere et al. 2018). F1 crossbred Ho × Sim cows and ¾ Ho cows had the same milk yield as the purebred Ho cows in the present study, showing that the application of this crossbreeding system did not negatively influence the productive performance of these two genetic groups. These results are in agreement with the study outcome by Brähmig (2011) in a German crossbred herd. Despite having the same milk yield, crossbred cows had a better reproductive performance compared to Ho cows. Therefore, due to these positive heterosis effects related to reproductive performance and milk yield, the crossbreed cows had favorable results compared to Ho cows.

ECM did not differ among the genetic groups. Because, especially in ¾ Sim cows, a relatively low milk yield was combined with a greater content of milk solids, such as fat and protein, the differences among breeds got close to zero. Ho cows, however, had slightly lower fat and protein contents compared to crossbred F1 and R1 Ho × Sim cows. Purebred Sim cows usually have higher milk solid content than Ho cows, especially for fat content (Młynek et al. 2018). This may lead to higher fat and protein content (in %) in crossbred Ho × Sim cows. Generally, the fat and protein content (in %) will increase with an increasing proportion of Sim genes, although F1 Ho × Sim cows almost reached the level of purebred Sim cows (Nolte 2019). Daily fat and protein yields (in kg), however, did not differ among genetic groups. This information is important for farmers because milk payment programs are usually based on milk solid yields. Therefore, the use of crossbred Ho × Sim cows (F1 + R1) does not negatively affect their milk payments. The possible positive economic effect of the use of crossbred cows will not only be related to milk composition improvement but also to better reproductive performance, which could lead to lower replacement costs in the herd (Buckley et al., 2014).

Similarly, SCS showed no significant differences among the genetic groups. In contrast to a previous study by Knob et al. (2018), where F1 crossbred Ho × Sim cows showed lower SCS than the purebred Ho cows, the three herds involved in the present study achieved SCS values below the values established as the highest limit for milk quality in Brazil according to the “Normative Instruction 76/2018 of MAPA” (Brasil, 2018). All cows were maintained under good management conditions to guarantee favorable udder health. Sub-optimal management conditions such as the conditions described in Knob et al. (2018) heavily challenge cow udder health and often lead to elevated SCS independent of the genetic group. Multiparous crossbred cows, however, might be able to adapt better to an environment with a higher challenge to udder health. These environmental challenges for cows kept in a semi-confinement system can be related to a higher level of dirtiness, especially on rainy days or high heat stress during summer. Thus, the higher capacity by the crossbred cows to cope with challenges compared to the Ho cows results in comparably lower SCS as shown previously by Knob et al. (2018). As expected, udder conformation traits differed among genetic groups (Table 6). Ho cows presented shallower udders and higher udder clearance than the other genetic groups, which might be related to the genetic improvement program of the Ho breed, where yield and conformations traits always had a high breeding weight (Miglior et al. 2017). A similar result, with higher udder clearance for Ho cows, was found by Hazel et al. (2014) who compared Ho and crossbred Ho × Montbéliarde cows. In another study comparing udder conformations traits of crossbred Ho × Sim with Sim cows, Belkov and Panin (2015) showed that crossbred cows had a greater distance from the udder floor to the ground and slightly shorter teats than that of purebred Sim cows. Therefore, the addition of Ho genes improves these characteristics in Sim cows.

Shallow udders and better udder clearance are positively related with udder health. Cows with deeper udders and less udder clearance are more susceptible to high SCS (Carlström et al. 2016; Dadpasand et al. 2012; Stefani et al. 2018). Although crossbred F1 and R1 Ho × Sim cows were expected to have a higher risk of poor udder health caused by unfavorable udder conformation traits, they did not show an increased SCS. This observation might be caused by the positive heterosis effect and favorable complementarity between Ho and Sim, because the Sim breed has a lower SCS than the Ho breed (Brähmig 2011; Stocco et al. 2017; Toledo-Alvarado et al. 2018).

The differences among genetic groups for the teat end hyperkeratosis score are possibly related to milk yield and not directly to the cattle breed. Cows with a higher milk yield such as the Ho cows in the present study showed a higher teat end hyperkeratosis score in agreement with Cardozo et al. (2015) and Cerqueira et al. (2018), whereas the ¾ Sim cows yielded less milk and had the lowest teat end hyperkeratosis score. Based on these findings, the genetic group or breed has at least an indirect effect on hyperkeratosis because the breed has an influence on milk yield, which affects the risk of hyperkeratosis. The factors related to changes at the teat end canal causing hyperkeratosis could be related to individual cow characteristics, including milk yield, age, lactation stage, and parity; and to the milking procedure such as over milking time and milking machine attributes such as insufficient or excessive vacuum levels, which would affect the milking time and increase the risk of higher teat end hyperkeratosis (Cardozo et al. 2015; Cerqueira et al. 2018). Therefore, breed is not the primary factor associated with low or high hyperkeratosis score rates.

Generally, the variance in udder conformation traits among genetic groups might be seen as evidence of a more successful genetic improvement in the Ho breed compared to the Sim breed, possibly because the Ho breed is, worldwide, the most often used pure dairy breed with the greatest population size combined with a very high selection intensity (Miglior et al. 2005). However, some of the unfavorable characteristics of the Sim breed, such as deeper udder and longer front teats, are also expressed in the crossbred Ho × Sim cows. Sim as a dual-purpose breed with a smaller population size also showed positive selection responses for milk yield and udder health traits. From the breeding progress in the Austrian Sim dairy cow population, milk yield has increased from approximately 4000 kg/year in 1975 to 7300 kg/year in 2017 (ZuchtData, 2017). Specific udder conformation traits, however, still require improvement. Wufka and Willeke (2001) showed in a comparative study of udder conformation traits that Sim cows presented less favorable characteristics with a shorter distance from the teat end to the floor and longer rear teats in comparison with purebred Ho cows. Bobić et al. (2014) also showed that Sim cows had longer rear teats than Ho cows.

Results from our study showed that F1 and R1 crossbred Ho × Sim cows had a better reproductive performance than purebred Ho cows. The R1 ¾ Sim crossbred cows yielded lower amounts of milk than the other genetic groups, but there was no difference among genetic groups for milk solids. Ho, F1, and R1 crossbred Ho × Sim cows did not differ in SCS, although crossbred cows had the most unfavorable udder conformation. Therefore, the better reproductive performance combined with the same amount of milk solids and good udder health is the basis for the use of crossbred cows as an alternative breeding approach for the improvement of fertility parameters in Ho dairy herds without productivity losses in first- and second-generation crossbred cows.

Acknowledgments

We would like to thank the dairy farmers that kindly collaborated in this research. This study was financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001.

Statement of Animal Rights

The Santa Catarina State University Ethical Committee, protocol no. 6330030517, approved all procedures with the animals in this study.

Declaration of Interest

The authors declare no conflict of interests.

References

Abin, S., Visser, C. and Banga, C.B., 2018. Comparative performance of dairy cows in low-input smallholder and high-input production systems in South Africa, Tropical Animal Health and Production, 50, 1479–1484.

Alessio, D.R.M., Velho, J.P., Pires Silveira, V.C., Knob, D.A., Busanello, M., Cortiana Tambara, A.A. and Thaler Neto, A.T., 2018. Roughage sources for Holstein cows under experimental feeding conditions in Brazil - A meta-analysis. Semina, 39, 2749–2760.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L. and Sparovek, G., 2013. Köppen's climate classification map for Brazil, Meteorologische Zeitschrift, 22, 711–728.

Andorf, S., Gärtner, T., Steinfath, M., Witucka-Wall, H., Altmann, T. and Repsilber, D., 2009. Towards systems biology of heterosis: A hypothesis about molecular network structure applied for the *Arabidopsis* metabolome, EURASIP Journal on Bioinformatics and Systems Biology, Article ID 147157, 12 pages.

Bedere, N., Cutullic, E., Delaby, L., Garcia-Launay, F. and Disenhaus, C., 2018. Meta-analysis of the relationships between reproduction, milk yield and body condition score in dairy cows, Livestock. Science, 210, 73–84.

Belkov, G.I. and Panin, V., 2015. Milk productivity of the crossbreeds obtained from crossing Simmental cows with Holstein bulls of different populations, Russian Agricultural Sciences, 41, 262–265.

Bobić, T., Mijić, P., Vučković, P., Gregić, M., Baban, M. and Gantner, V., 2014. Morphological and milkability breed differences of dairy cows, Mljekarstvo, 64, 71–78.

Brähmig, J., 2011. Einfluss der Wechselkreuzung von Deutschen Holsteins und Deutschem Fleckvieh auf Milchleistung und Milchqualität in einem automatischen Melksystem. Thesis. Veterinary Medicine Faculty, Ludwig Maximilians University, Munich, Germany.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62 de 29 de dezembro de 2011. Diário Oficial [da] República Federativa do Brasil, Brasília, 29 dez. 2011, Seção 1, p. 8.

Buchanan, D.S., 2010. Genetics: Mendelian, Encyclopedia of Animal Science, Second Edition, Taylor & Francis, London, UK, 1: 1, 508–510.

Buckley, F., Lopez-Villalobos, N. and Heins B.J., 2014. Crossbreeding: Implications for dairy cow fertility and survival, Animal, 8, 122–133.

Cardozo, L.L., Thaler Neto, A., Souza, G.N., Picinin, L.C.A., Felipus, N.C., Reche, N.L.M., Schmidt, F.A., Werncke, D. and Simon, E.E., 2015. Risk factors for the occurrence of new and chronic cases of subclinical mastitis in dairy herds in southern Brazil, Journal of Dairy Science, 98, 7675–7685.

Carlström, C., Strandberg, E., Johansson, K., Pettersson, G., Stålhammar, H. and Philipsson, J., 2016. Genetic associations of in-line recorded milkability traits and udder conformation with udder health. Acta Agriculturae Scandinavica, Section A—Animal Science, 66, 84–91.

Cerqueira, J.L., Araújo, J.P. and Cantalapiedra, J., 2018. How is the association of teat-end severe hyperkeratosis on udder health and dairy cow behavior ? Revue de Médecine Vétérinaire, 169, 30–37.

Coentrão, C.M., Souza, G.N., Brito, J.R.F., Paiva E Brito, M.A.V. and Lilenbaum, W., 2008. Fatores de risco para mastite subclínica em vacas leiteiras, Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 60, 283–288.

Dadpasand, M., Zamiri, M.J., Atashi, H. and Akhlaghi, A., 2012. Genetic relationship of conformation traits with average somatic cell score at 150 and 305 days in milk in Holstein cows of Iran, Journal of Dairy Science, 95, 7340–7345.

Dall Pizzol, J.G., Gomes, I.P.O., Braun, W., LIsboa, J.A.N., Flaiban, K.K.M.C. and Thaler Neto, A., 2017. Comparação Entre Vacas Da Raça Holandesa E Mestiças Das Raças Holandesa X Jersey Quanto À Sanidade, Imunidade E Facilidade De Parto, Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 69, 955–961.

de Haas, Y., Smolders, E. a a, Hoorneman, J.N., Nauta, W.J. and Veerkamp, R.F., 2013. Suitability of cross-bred cows for organic farms based on cross-breeding effects on production and functional traits, Animal, 7, 655–664.

Ezra, E., Van Straten, M., and Weller, J.L., 2016. Comparison of pure Holsteins to crossbred Holsteins with Norwegian Red cattle in first and second generations, Animal, 10, 1254–1262.

Fellipe, E.W., Gomes, I.P. de O. and Thaler Neto, A., 2017. Comparação de vacas mestiças Holandês x Jersey com vacas puras quanto à eficiência produtiva e reprodutiva, Archives of Veterinary Science, 22, 48–54.

Fiévet J.B., Nidelet, T., Dillmann, C. and de Vienne, D., 2018. Heterosis is a systemic property emerging from non-linear genotype-phenotype relationships: Evidence from in vitro genetics and computer simulations, Frontiers in Genetic 9:159.

Freyer, G., König, S., Fischer, B., Bergfeld, U. and Cassell., B.G., 2008. Invited review: Crossbreeding in dairy cattle from a german perspective of the past and today, Journal of Dairy Science, 91, 3725–3743.

Hazel, A. R., Heins, B.J., Seykora, A. J. and Hansen, L.B., 2013. Montbéliarde-sired crossbreds compared with pure Holsteins for dry matter intake, production, and body traits during the first 150 days of first lactation, Journal of Dairy Science, 96, 1915–23.

Hazel, A. R., Heins, B.J., Seykora, A. J. and Hansen, L.B., 2014. Production, fertility, survival, and body measurements of Montbéliarde-sired crossbreds compared with pure Holsteins during their first 5 lactations, Journal of Dairy Science, 97, 2512–2525.

Heins, B.J., Hansen, L.B., Seykora, A.J., Johnson, D.G., Linn, J.G., Romano, J.E. and Hazel, A.R., 2008. Crossbreds of Jersey × Holstein compared with pure Holsteins for production, fertility, and body and udder measurements during first lactation, Journal of Dairy Science, 91, 1270–1278.

Janzekovic, M., Mocnik, U. and Brus, M., 2015. Ultrasound measurements for body condition score assessment of dairy cows, DAAAM International Scientific Book 2015, 51–58.

Kara, N. and Koyuncu, M., 2018. A research on longevity, culling reasons and milk yield traits in between Holstein and Simmental cows, Mediterranean Agricultural Sciences, 31, 325–328.

Knob, D.A., Alessio, D.R.M., Thaler Neto, A. and Mozzaquatro, F.D., 2016. Reproductive performance and survival of Holstein and Holstein × Simmental crossbred cows, Tropical Animal Health and Production, 48, 1409–1413.

Knob, D.A., Alessio, D.R.M., Neto, A.T. and Mozzaquatro, F.D., 2018. Growth , productive performance, and udder health of crossbred Holstein x Simmental cows and purebred Holstein cows, Semina. Ciências Agrárias, 39, 2597–2606.

Ledinek, M. and Gruber, L., 2015. Körpermaße und BCS im Laufe der Laktation und ihre Beziehungen zu Lebendmasse und Energiebilanz, Züchtungskunde, 87, 181–200.

Leyva-Corona, J.C., Reyna-Granados, J.R., Zamorano-Algandar, R., Sanchez-Castro, M.A., Thomas, M.G., Enns, R.M., Speidel, S.E., Medrano, J.F., Rincon, G., Luna-Nevarez, P., 2018. Polymorphisms within the prolactin and growth hormone/insulin-like growth factor-1 functional pathways associated with fertility traits in Holstein cows raised in a hot-humid climate. Tropical Animal Health and Production. 50, 1913–1920.

Mein, G.A., Neijenhuis, F., Morgan, W.F., Reinemann, D.J., Hillerton, J.E., Baines, J.R., Ohnstad, I., Rasmussen, M.D., Timms, L., Britt, J.S., Farnsworth, R., Cook, N. and Hemling, T., 2001. Evaluation of bovine teat condition in commercial dairy herds: 1.

Noninfectious factors. Pages 347–351 in Proc. 2nd Int. Symp. Mastitis Milk Qual., Vancouver, BC, Canada. Natl. Mastitis Counc., Madison, WI.

Miglior, F., Muir, B.L. and Van Doormaal, B.J., 2005. Selection indices in Holstein cattle of various countries, Journal of Dairy Science, 88, 1255–1263.

Miglior, F., Fleming, A., Malchiodi, F., Brito, L.F., Martin, P. and Baes, C.F. 2017. A 100-year review: Identification and genetic selection of economically important traits in dairy cattle, Journal of Dairy Science, 100, 10251–10271.

Młynek, K., GŁowińska, B., Salomończyk, E., Tkaczuk, J. and Styś, W., 2018. The effect of daily milk production on the milk composition and energy management indicators in Holstein–Friesian and Simmental cows, Turkish Journal of Veterinary and Animal Sciences, 42, 223–229.

Nemes, Z., 2016. Estimation of nonadditive genetic influences on standard lactation production (305 days) in upgrading of Serbian-Fleckvieh to Holstein-Friesian cattle breed, Mljekarstvo, 66, 330–336.

Nemes, Z., Đedović, R., Vidović, V., Eszes, F. and Gáspárdy, A., 2012. An example for the “transit-heterosis” in the non-return rate of upgraded dairy genotypes, Animal Science and Biotechnologies, 45, 215–219.

Nolte, O., 2019. Effekte der Rückkreuzung auf Fleckvieh aus einer Kreuzungsherde mit Deutschen Holstein-Genanteilen auf Milchleistung und Milchqualität. Thesis. Veterinary Medicine Faculty Ludwig Maximilians University, Munich, Germany.

Piccand, V., Cutullic, E., Meier, S., Schori, F., Kunz, P.L., Roche, J.R. and Thomet, P., 2013. Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system, Journal of Dairy Science, 96, 5352–5363.

Puppel, K., Bogusz, E., Gołębiewski, M., Nałęcz-Tarwacka, T., Kuczyńska, B., Slósarz, J., Budziński, A., Solarczyk, P., Kunowska-Slósarz, M. and Przysucha, T., 2018. Effect of dairy cow crossbreeding on selected performance traits and quality of milk in first generation crossbreds, Journal of Food Science, 83, 229–236.

Rehak, D., Barton, L., Vodkova, Z., Kubešová, M., Volek, J. and Rajmon, R., 2012. Relationships among body condition score, milk yield and reproduction in Czech Fleckvieh cows, Journal of Animal Science, 57, 274–282.

Saha, S., Malchiodi, F., Cipolat-Gotet, C., Bittante, G., Gallo, L., Di Agronomia, D., Alimenti, A., Naturali, R. and Dafnae, A., 2017. Effects of crossbreeding of Holsteins cows with Montbéliarde and Swedish Red in first and second generation on cheese yield traits, Agriculturae Conspectus Scientificus, 82, 241–244.

Schichtl, V., 2007. Kreuzungszucht beim Milchvieh - ein Ausblick. Vor und Nachteile der Kreuzungszucht zwischen Deutschem Fleckvieh und Deutschen Holstein. Thesis. Veterinary Medicine Faculty Ludwig Maximilians University, Munich, Germany.

Sørensen, M.K., Norberg, E., Pedersen, J. and Christensen, L.G., 2008. Invited review : Crossbreeding in dairy cattle : A Danish perspective, Journal of Dairy Science, 91, 4116–4128.

Stefani, G., El Faro, L., Santana Júnior, M.L. and Tonhati, H., 2018. Association of longevity with type traits, milk yield and udder health in Holstein cows, Livestock Science, 218, 1–7.

Stocco, G., Bobbo, T., Cecchinato, A. and Bittante, G., 2017. Breed of cow and herd productivity affect milk composition and modeling of coagulation, curd firming, and syneresis, Journal of Dairy Science, 100, 129–145.

Toledo-Alvarado, H., Vazquez, A.I., Campos, G.D.L., Tempelman, R.J., Gabai, G., Cecchinato, A. and Bittante, G., 2018. Changes in milk characteristics and fatty acid profile during the estrous cycle in dairy cows, Journal of Dairy Science, 101, 9135–9153.

Wufka, M. and Willeke, H., 2001. Einfluss von Rasse und Laktationsnummer auf

Eutermerkmale bei Milchkühen, Archiv Tierzucht 44, 497-504.

ZuchtData 2019. Yearly report 2017, Vienna, Austria. Retrieved 13 May 2019, from http://zar.at/Downloads/Jahresberichte/ZuchtData-Jahresberichte.html

Table 1. Crossbreeding program used in the farms

Genetic group	Sire		Dam
Purebred Holstein	Holstein	×	Holstein
F1 - First Cross	Simmental	×	Holstein
R1 - First rotational cross (¾ Holstein , ¼ Simmental)	Holstein	×	F1
R1 - First rotational cross (¾ Simmental, ¼ Holstein)	Simmental	×	F1

Table 2. Descriptive statistics (average ± mean squares error) for milk yield and composition and somatic cell count of each farm in this study

Variable	Herd 1	Herd 2	Herd 3
Number of animals - Lactating cows	280	80	140
Milk yield (Liters/day)	32.6 ± 9.75	23.9 ± 7.03	22.8 ± 6.37
Fat %	3.48 ± 0.53	3.56 ± 0.64	3.40 ± 0.60
Protein %	3.16 ± 0.28	3.11 ± 0.32	3.25 ± 0.27
Somatic cell count (cells/ml)	392,000	341,000	195,000

Table 3. Least squares means ± mean squares error, P-value, and number of observations (N) for calving interval and calving to first service interval by genetic group, parity, and herd for purebred Holstein, and F1, and R1 Holstein × Simmental crossbred cows.

Variable	Category	Calving interval (days)		Calving to first service interval (days)	
		N	Mean±MSE	N	Mean±MSE
Genetic Group	Holstein	882	438.0 ±2.8^{a}	1340	87.9±0.9^{a}
	H x S*	420	393.9±4.0^{b}	650	75.1±1.4^{b}
	¾ H**	43	420.6±13.8^{ab}	101	80.2±4.1^{ab}
	¾ S***	98	386.0±12.7^{b}	181	79.8±3.8^{b}
Parity	1	-	-	790	83.7 ± 1.7^{a}
	2	571	411.6±5.2	586	77.4±1.8^{b}
	≥3	872	407.7±5.7	895	81.0±1.9^{a}
Herd	1	638	425.1±4.8^{a}	1033	77.6±1.5^{b}
	2	294	417.6±9.3^{a}	490	91.0±3.2^{a}
	3	511	386.3±10.0^{b}	749	73.6±2.6^{b}

Different superscript letters within columns describe significant differences at p≤ 5%.

* H x S = F1 Holstein x Simmental

** ¾ H = R1 ¾ Holstein ¼ Simmental.

*** ¾ S = R1- ¾ Simmental ¼ Holstein.

Table 4. Conception rate (%) by genetic group and insemination number for purebred Holstein, and F1 and R1 Holstein × Simmental crossbred cows.

Variable	Category	Holstein		F1 Holstein x Simmental*		¾ Holstein*		¾ Simmental**		Average
		N	%	N	%	N	%	N	%	
Insemination	First	1962	33.08b	871	38.69a	159	38.99ab	268	44.78a	35.83
	Others	3415	32.01b	1322	34.11ab	278	30.22b	349	37.25a	32.77
Average		5379	32.40c	2193	35.93b	437	33.41bc	617	40.52a	

Different superscript letters within rows describe significant associations at p≤ 5% by Chi^2-Test.

* R1, ¾ Holstein ¼ Simmental.

** R1, ¾ Simmental ¼ Holstein.

Table 5. Least squares mean ± mean squares error and P-value for milk yield and composition and somatic cell score for purebred Holstein and F1 and R1 Holstein × Simmental crossbred cows.

Variables	Holstein	F1 Holstein x Simmental	¾ HOL*	¾ SIM**	P-value
Number of observations	672	370	170	252	
Milk yield (Liters/day)	27.02±0.43^{a}	26.47±0.63^{a}	26.49±0.76^{ab}	24.56±0.68^{b}	0.0245
Energy corrected milk yield (ECM)	27.06±0.44	26.99±0.64	27.60±0.77	25.45±0.69	0.1411
Fat content (%)	3.39±0.03^{b}	3.64±0.05^{a}	3.72±0.06^{a}	3.61±0.06^{a}	<0.0001
Protein content (%)	3.11±0.01^{b}	3.23±0.02^{a}	3.30±0.03^{a}	3.28±0.02^{a}	<0.0001
Fat + protein yield (kg/day)	1.73±0.03	1.74±0.04	1.79±0.06	1.65±0.05	0.3735
Lactose content (%)	4.64±0.01	4.66±0.01	4.64±0.02	4.65±0.02	0.6586
Total dry extract content (%)	12.10±0.04^{b}	12.32±0.06^{a}	12.5±0.07^{a}	12.43±0.07^{a}	<0.0001
Somatic cell count (SCC)	2.69±0.12	2.60±0.18	2.86±0.21	2.79±0.19	0.7523

Different superscript letters within rows describe significant differences at p≤ 5%.

* R1, ¾ Holstein ¼ Simmental.

** R1, ¾ Simmental ¼ Holstein.

Table 6. Least squares mean ± mean squares error and P-value for udder conformation traits and teat end hyperkeratosis score for purebred Holstein and F1 and R1 Holstein × Simmental crossbred cows.

Variables	Holstein	F1 Holstein x Simmental	¾ HOL*	¾ SIM**	P
Udder depth (cm)	9.98±0.3^{a}	7.27±0.6^{b}	8.56±0.6^{b}	7.72±0.6^{b}	<0.0001
Udder Clearance (cm)	59.4±0.6^{a}	55.3±0.4^{b}	56.7±0.6^{b}	55.4±0.6^{b}	<0.0001
Front teat length (cm)	5.66±0.06^{b}	5.96±0.09^{a}	5.79±0.11^{ab}	5.99±0.10^{a}	0.0146
Rear teat length (cm)	4.73±0.05	4.86±0.07	4.77±0.09	4.88±0.08	0.3754
Front teat placement	5.12±0.11^{a}	4.74±0.15^{b}	4.55±0.19^{b}	4.39±0.17^{b}	0.0013
Rear teat placement	6.0±0.15^{a}	5.57±0.21^{ab}	5.67±0.25^{ab}	5.13±0.24^{b}	0.0198
Hyperkeratosis	1.83±0.05^{a}	1.73±0.07^{ab}	1.72±0.08^{ab}	1.56±0.08^{b}	0.0487

Different superscript letters within rows describe significant differences at p≤ 5%.

* R1, ¾ Holstein ¼ Simmental.

** R1, ¾ Simmental ¼ Holstein.

CHAPTER IV

Paper submitted to the Journal: Journal of Applied Animal Research

Dry matter intake, body condition score, beta-hydroxy-butyrate concentration, milk yield and composition of Holstein and crossbred Holstein x Simmental cows during the transition period

Dry matter intake, body condition score, beta-hydroxy-butyrate concentration, milk yield and composition of Holstein and crossbred Holstein x Simmental cows during the transition period

Deise A. Knob[12*], Armin M. Scholz[2], Roberto Kappes[1], Wagner R. Bianchin[3], Dileta R. M. Alessio[4], Laiz Perazzoli[1], Bruna P. B. Mendes[1], Andre Thaler Neto[1]

[1]Programa de Pós-Graduação em Ciência Animal - Centro de Ciências Agroveterinárias - Universidade do Estado de Santa Catarina - CAV/UDESC, Lages – Santa Catarina. Brasil.

[2]Ludwig Maximilians Universität München (LMU), Tierärztlichen Fakultät, Lehr- und Versuchsgut Oberschleißheim, Oberschleißheim, Deutschland.

[3]Instituto Federal Catarinense- Campus Santa Rosa do Sul. Santa Rosa do Sul – Santa Catarina, Brasil

[4]Centro Universitário Leonardo da Vinci, Indaial, Santa Catarina, Brasil.

*Correspondence: Deise Aline Knob
Avenida Luis de Camões, 1231, ap. 3.
Bairro Coral, Lages, Santa Catarina,
Brasil Cep: 88523-000
Phone +55 49 991046241
Email: deisealinek@gmail.com

Abstract

This research paper addresses the hypothesis that crossbred Holstein x Simmental cows reduce the DMI during the weeks before calving to a lower degree, while showing a higher DMI right after calving combined with a similar milk yield in comparison with purebred Holsteins. Therefore, we aimed at comparing DMI, BCS, body weight, BHB, milk yield, and milk composition during the transition period (3 weeks before until 3 weeks after calving) for both breeding groups. A commercial dairy farm served as the experimental unit for the research. A total number of 30 multiparous cows with 18 Holstein and 12 crossbred F1 Holstein x Simmental cows entered the study. Each cow entered the study 21 days before the expected calving day (prepartum) and stayed in the research group until day 21 after calving (postpartum) covering a six-week transition period. Pre and postpartum cows received a total mixed ration previously weighed and offered ad libitum for each cow allowing 5-10% residuals. Once a week, body weights and BCS were recorded. On the same day, blood was collected for the measurement of BHB. For the variance analysis, we used the MIXED procedure of the SAS software. Holstein and crossbred Holstein x Simmental cows have similar DMI with higher amounts after calving reaching around 18 kg/day. Crossbred Holstein x Simmental cows have a higher BCS than Holstein cows. For the variable BHB, we just observed a difference for the period with lower values pre partum for both genetic groups. There was no difference for rumination time, milk yield, protein and lactose content between the genetic groups. Crossbred Holstein x Simmental cows tend to have a higher fat content. The genetic groups also have similar milk yield and composition, so the use of the crossbreds does not negatively affect the milk and solids yield at the beginning of the lactation in Holstein herds.

Key-Words: BCS, BHB, body weight, milk composition, milk yield

Introduction

Crossbreeding between dairy breeds has been used as an alternative to pure breeding in order to improve phenotype characteristics related mainly to fitness or to reduce problems with high consanguinity in purebred herds (Cassell and McAllister, 2009; Weigel and Barlass, 2003) and to increase the genetic variability of the herds through heterosis and complementarity (Mendonça et al., 2014). Positive heterosis effects can improve the feed efficiency of the animals by reducing the energy requirements for milk production, maintenance and deposition of body tissues due to different partitioning of the consumed energy (Olson et al., 2010; Prendiville et al., 2009). Heins et al. (2008), for example, cited that crossbred Holstein x Jersey cows, because of having higher dry matter intake proportional of body weight and better feed efficiency, destine bigger part of the energy to body reserves, allowing quickly recuperation of the body condition score (BCS) after calving. A favorable BCS of the crossbred Holstein x Jersey cows has a positive impact on the reproductive performance for example, with less days open in comparison to Holstein cows (Heins et al., 2008).

The transition period of dairy cows is receiving more attention resulting in more studies regarding physiological and metabolic changes that happen at the end of the dry period and the beginning of a new lactation. One of the changes is the reduction of the dry matter intake (DMI) at the end of the dry period, which may amount up to around -30% about 24 hours before calving (Dewhurst et al., 2000). The DMI reduction can reach 3.8 kg of dry matter, leading consequently to a reduction of the rumination time - of up to 60 minutes/day (Schirmann et al., 2013). After calving, a gradual increase of DMI is expected (Youssef and El-Ashker, 2017), although the DMI does not increase as fast as the demand of nutrients for milk production rises. The animals go trough a period with a deficit of energy (Barletta et al., 2017; Djoković et al., 2017; Mann et al., 2016) leading to a situation of negative energy balance (NEB) (Esposito et al., 2014). A NEB may lead to an increase of the concentration of non-esterified fatty acids (NEFA), which is an indicator of body fat mobilization (Mendonça et al. 2014).

NEFA can be used as direct energy source for the tissues or may be completely oxidized in the liver in form of ketone bodies, especially as beta-hydroxy-butyrate (BHB), which again can be used as energy source by the liver or by other tissues (Barletta et al., 2017; Djoković et al., 2017). Situations when the NEFA and BHB levels are higher than the physiological limits (plasma or serum concentration >0.4 mmol/l or >1.2 mmol/l, respectively), have been associated with lower milk yields, a reduced reproductive performance, higher risks of clinical and metabolic diseases, and finally an increased culling rate of the animals (Leblanc, 2010; McCarthy et al., 2015).

The effects of NEB around the transition period have been studied frequently in high yielding herds, especially keeping Holstein cows. Just a few studies for this specific period, however, compare Holstein and crossbred cows. Crossbred cows may differ from Holsteins in DMI, efficiency of energy use, and energy metabolism due to heterosis and complementarity effects (Mendonça et al., 2014). With our study, we wanted to test the hypothesis that crossbred Holstein x Simmental cows reduce the DMI during the weeks before calving to a lower degree, while showing a higher DMI right after calving combined with a similar milk yield in comparison with purebred Holsteins. Therefore, we aimed at comparing DMI, BCS, body weight, BHB, milk yield, and milk composition during the transition period (3 weeks before until 3 weeks after calving) for both breeding groups.

Material and Methods

All procedures used in this research were approved by the Santa Catarina State University Ethical Committee, protocol n° 6330030517.

Animals and management

A commercial dairy farm located in Santa Catarina, South Brazil served as the experimental unit for the research. The farm used a compost bedded pack barn confinement system (CBP). The herd consists of approximately 280 lactating cows with 60% of them being purebred Holstein cows and 40% being crossbred Holstein x Simmental cows. A total number of 30 multiparous cows with 18 Holstein and 12 crossbred F1 Holstein x Simmental cows entered the study. The research was performed from August to November 2017. All cows with 3 or more parities that calved within the experimental time entered the study. The cows had a dry off period of 60 days before the expected calving day. Each cow entered the study 21 days before the expected calving day (prepartum) and stayed in the research group until day 21 after calving (postpartum) covering a six-week transition period. Cows entered and left the pre- and postpartum groups according to their expected and actual calving dates. The prepartum cows group stayed with all the other prepartum cows of the farm in an area outside the barn with free access to water and shadow. Twice a day, the cows had access to the feed parlor, where they received a total mixed ration based on maize silage and a commercial concentrate specifically composed for this pre lactation transition period. After calving, the research cows were kept in a separate group in the CBP. The diet offered to postpartum cows was the same as offered to the other postpartum and high yielding cows at the farm. It was a total mixed ration (TMR) based on maize silage, ryegrass (fresh and silage), and concentrates. The ingredients and the chemical composition of the pre and postpartum diet are shown in Table 1. The diet offered to the cows was calculated to provide 100% of the nutritional requirement (NRC, 2001).

Cows were mechanically milked 3 times a day (05:00 h, 13:30 h and 20:30 h), and the individual milk yield (MY) was electronically recorded (DeLaval®). Individual milk samples were taken every 7 days in 40-mL bottles containing Bronopol. Each sample consisted of an average mixture of the 3 daily milkings and was sent to the laboratory of milk analysis from the Universidade do Estado de Santa Catarina, UDESC/Lages, SC, Brazil, where the samples were analyzed for milk composition by the infrared method with a DairySpec (Bentley®) equipment.

After milking, the postpartum cows had access to the feed parlor for about 2 hours and 30 minutes. The feed parlor had an individual self-locking feed front, where each cow stayed for the duration of each meal. The offered TMR was weighed and offered individually to allow individual feed intake measurements. The TMR was offered ad libitum for each cow allowing 5-10% residuals and was prepared using a horizontal forage mixer. After each meal, non-consumed feed was weighed back. Samples of the TMR offered and the residual of each cow as well as the individual ingredients of the diet, were collected and then dried in a forced air oven at 55 °C for 72 h. After this procedure, the samples were minced through a 1-mm screen for subsequent chemical analyses. The dry matter content was determined by drying the samples at 105 °C for 24 h. The ash was quantified by combustion in a muffle furnace at 550°C for 4 h, and the organic material was quantified by mass difference. The total nitrogen was assayed using the Kjeldahl method (method 984.13; AOAC International, 1998). The neutral detergent fiber (NDF) concentration was assessed according to Mertens (2002), except that the samples were weighed in filter bags and treated with neutral detergent in Ankom A220 equipment (Ankom Technology, Macedon, NY). The concentrations of acid detergent fiber (ADF) and ADL were analyzed according to AOAC International (1998).

Once a week, body weights and body condition score (BCS) were recorded. We performed the BCS evaluation based on a scale between 1 (extremely thin) and 5 (very fat) (Ferguson et al., 1994). On the same day, we weighed all the cows – after milking the lactating cows - just before the first meal in the morning. On the same day, blood was sampled from the coccygeal vein of each cow for the measurement of β-hydroxybutyrate (BHB). The concentration of BHB was tested immediately using an electronic handheld device (Precision Xtra meter, Abbott Diabetes Care). We performed all these measurements throughout the 6-week observation period. On the calving day, we evaluated each cow for BCS, body weight, and BHB.

To obtain the daily rumination data we used the data collected by the Heatime® (SCR/Allflex) system, an automatic system composed of a neck collar with a tag that records the rumination time (in minutes) of each cow.

Statistical analysis

The energy corrected milk yield (ECM) was obtained by the equation: ECM = (0.327 * MY) + (12.95% * F* MY / 100) + (7.65 % P * MY / 100), where, MY = milk yield in l/day, F = fat percentage, and P = protein percentage (Tyrrell; Reid, 1965)

For the variance analysis, we used the MIXED procedure of the SAS (SAS 2002) statistical package after having the data tested for normality of residuals Kolmogorov–Smirnov test and for homogeneity of the variances using the Levene test. The first variance analysis model included the fixed effects genetic group, period (pre/post partum), and the interaction between them. In a second model, we included the week relative to calving (Table 2) as fixed effect instead of the pre/post partum period. All results are presented graphically.

Results

Holstein and crossbred Holstein x Simmental cows have similar DMI (P=0.5224; Table 3). There is a difference between pre and postpartum DMI (P<0.0001) with a higher value after calving. This difference represents about 6-7 kg/day. Both genetic groups reduce the DMI in the week just before calving (Figure 1, A), with a slight increase at the calving day (week 0). After calving, the DMI increased quickly for both genetic groups. Three weeks after calving, cows have a DMI of around 18 kg/day. We observed, however, an interaction between genetic groups and periods (P = 0.0412). Holstein x Simmental cows show a higher DMI at the third week after calving than purebred Holsteins (Figure 1, A).

The DMI % to body weight, on the other hand, did not differ between Holstein and Holstein x Simmental crossbred cows (P=0.3623; Table 3). Again, during the prepartum period, the DMI % to body weight is lower than during the postpartum period (P<0.0001) - with values around 1.1 and 1.3%. After calving, with a general increase of DMI, the DMI % to body weight also increased, reaching values close to 3% at the third week after calving (Figure 1, B). There was no interaction between genetic groups and periods.

Crossbred Holstein x Simmental cows have a higher BCS than Holstein cows ($P<0.0001$; Table 3). This difference can be observed for both pre and postpartum periods. The difference between the breeds yields about 0.5 points before calving, while this difference increases to about 0.8 points during the postpartum period. It means that crossbred cows lose less BCS after calving than Holstein cows (0.39 vs. 0.63, Figure 2, A). The BCS decreases in both genetic groups after parturition, but the BCS decreases stop earlier for crossbred Holstein x Simmental cows (Figure 2, A). Both genetic groups show similar body weights ($P = 0.5156$; Table 3), and are expectedly heavier pre partum than postpartum ($P<0.0001$). Both genetic groups start to lose weight at week 1 before calving (Figure 2, B). In addition, there was an interaction between the genetic group and transition period ($P=0.0379$). Before calving, the body weight difference between both genetic groups reached about 5 kg, while the difference increased to almost 30 kg postpartum.

For the variable BHB, we just observed a difference for the period ($P=0.0004$), with lower values pre partum for both genetic groups. Holstein and crossbred Holstein x Simmental cows present values below 1 mmol/liter during the three weeks before calving. After calving, the BHB concentration increases to more than 1 mmol/liter (Figure 2, C). Though, there is no significant interaction between genetic group and transition period ($P=0.2610$, Table 3), we do observe a minor interaction between genetic group and week ($P= 0.0736$). As shown in Figure 2 C, crossbred cows reach lower BHB concentrations during weeks 2 and 3 after calving, while there is no difference between the breeds during all other transition weeks.

Because of having similar DMI, there was no difference for rumination time between the genetic groups ($P=0.4961$), as well as no interaction between genetic group and period. After calving, both genetic groups increase their rumination time by about 60-70 minutes/day ($P<0.0001$). It is worth highlighting that before calving cows spend around 51 minutes ruminating for each Kg of DMI. After calving, these values decrease to about 32-34 minutes for each Kg of DMI.

Holstein cows and crossbred Holstein x Simmental cows yielded similar amounts of milk ($P=0.4741$; Table 4). Both genetic groups produced about 21 liters/day at the calving day. During the following days, the milk production increased quickly until reaching amounts around 34 liters/day at the third week after calving (Figure 3, A). Genetic groups did not differ in the protein and lactose content of the milk ($P=0.6316$ and $P=0.8322$, respectively). Yet for fat content, crossbred Holstein x Simmental cows tend to produce milk with higher percentages

(P=0.0593). The time pattern of the milk solids, however, varies during the weeks after calving. The lactose content is increasing, while the fat and protein contents are decreasing between week 1 and week 3 after calving (Figure 3, D). This pattern holds for Holstein as well as for crossbred Holstein x Simmental cows.

Discussion

The dry matter intake did not differ between Holstein cows and crossbred Holstein x Simmental cows between 3 weeks before and 3 weeks after calving (Table 3 and Figure 1, A). The crossbred cows do not have a higher DMI although they produce the same amount of milk (Table 4, Figure 3, A) combined with a higher BCS (Table 3 and Figure 2, A) in comparison with purebred Holsteins. Mendonça et al., (2014), however, observed a tendency towards higher DMI for Holstein cows between 6 weeks before and 6 weeks after calving in comparison with crossbred Holstein x Montbeliarde cows. They recorded values of around 1.4 % for the DMI in relation to the body weight for crossbred Holstein x Montbeliarde cows and 1.6 % for Holstein cows. In another study, also comparing Holstein and crossbred Holstein x Montbeliarde cows, no difference between the genetic groups was found for DMI during the first 150 days of the lactation (Hazel et al., 2013). In the same study, the crossbred cows yield 96 % of the total amount of milk in comparison with the purebred Holstein cows. Our study, however, does not result in any difference for DMI, possibly, because both genetic groups produce similar amounts of milk, ECM combined with an identical milk composition (Table 3, Figure 3, B, C, D). DMI increases as milk yield increases after calving (Weber et al., 2013). Since both genetic groups in our study have similar body weights, it might mean that the cows of both genetic groups have similar maintenance requirements regarding energy and nutrients. Depending on body composition and body weight of the genetic groups, the nutrient requirements may differ what might affect the DMI. By comparing Holstein with Jersey cows or Holstein with crossbred Holstein x Jersey cows, Palladino et al. (2010) and Prendiville et al. (2011) demonstrated that the heavier purebred Holstein cows consume more dry matter than not so heavy purebred Jerseys or crossbred cows, respectively.

With the start of the lactation, the nutrient requirements for milk production increase. Cows have to increase the DMI to meet the energy requirements (Youssef and El-Ashker, 2017). The DMI increases fast after calving. The cows almost double their DMI 21 days after calving starting with about 9 kg/day after calving and reaching almost 18 kg/day at day 21 post partum (Table 3, Figure 3, A). The time pattern looked similar for DMI in relation to body weight (%) in both genetic groups. At the week before calving, cows had a DMI % to body weight of about 1.2 %. This value has more than doubled by reaching values close to 3% of the body weight at the third week of lactation. The increasing relative DMI is not only related to the increased demand of nutrients after calving. It increases more than the absolute DMI, because cows lose weight after calving (Table 3, Figure 2, B) as a consequence of the negative energy balance (NEB) (Carvalho et al., 2014; Esposito et al., 2014). It is interesting to note that at this lactation point, 21 days after calving, cows have almost reached 3% DMI % of body weight. This value is close to the values recommended for high yielding dairy cows (NRC, 2001).

Even with similar DMI, crossbred Holstein x Simmental cows have a better BCS during the pre and postpartum period than the purebred Holsteins (Table 3, Figure 2, A). The crossbred cows do not have a higher DMI to maintain the higher BCS. The more favorable BCS seems to be closely related to the complementarity between the breeds used in the crossbreeding programs, since Simmental is a dual-purpose breed, which has a higher BCS than purebred Holstein cows (Schweizer et al., 2018; Sgorlon et al., 2015). Młynek et al., (2018) have also shown that Simmental cows declined less in BCS after calving than Holstein cows. In our study, Holstein cows lose about 0.6 BCS points within 3 weeks after calving, while the crossbred Holstein x Simmental cows lose about 0.4 BCS points. The BCS values observed for Holstein cows in our study are similar to those found by Kaufman et al., (2016). The lower loss of BCS points in the crossbred cows after calving might also explain their better reproductive performance in comparison with the purebred Holsteins (Janzekovic et al., 2015; Knob et al., 2016, 2019). A higher BCS and body weight losses after calving has a negative effect on reproductive performance (Carvalho et al., 2014).

Although less intense for crossbred cows, we observed BCS and body weight loss for both genetic groups after calving. With the start of the new lactation, the milk production increases quickly demanding a high energy supply for the cow. The DMI increases also after calving (Youssef and El-Ashker, 2017), but not as fast as the milk yield (Barletta et al., 2017; Djoković et al., 2017; Mann et al., 2016), which requires from the cow to use her own body reserves by decreasing BCS and body weight in order to produce milk. This condition is well known as NEB (Esposito et al., 2014). Very similar to the BCS pattern, we did not find a difference for the body weight between breeds (Table2, Figure 2, B). The difference observed for the transition period may be related to NEB after calving. Both genetic groups lose around 100 kg of live weight in the first 3 weeks of lactation. These values represent 12% and 15% of the body weight for crossbred Holstein x Simmental cows and Holstein cows, respectively. The body weight loss started at the week 1 before calving, possibly due to the reduction in DMI, which can amount to 30% (Dewhurst et al., 2000). Part of these differences for BW between pre and post partum can also be related to the calving day, since that, with the birth cows lose at least the calf weight and all the fluids. For these 2 genetic groups, the calf weight represents on average 45 kg (Knob et al., 2016).

Highly related to the NEB and all metabolic changes that happen during the transition period, the BHB values do not differ between the genetic groups (Table 3, Figure 2, C). It seems that the genetic group is not related to BHB, since both of them have similar milk yields and DMI. BHB is highly correlated with NEB, because the compensation of the negative balance between DMI and nutrients requirements for milk production forces the body (energy metabolism pathways) to utilize own body reserves, especially fat tissue. During this process, non esterified fatty acids (NEFA), which are used as an alternative energy source for peripherals tissues, are released into the blood stream (Barletta et al., 2017; Djoković et al., 2017). NEFA can be oxidized as lipoproteins and ketone bodies by the liver metabolism. The main component of the ketone bodies is usually beta-hydroxybutyrate (BHB), which can be used as an energy source by the liver or can be released into the bloodstream to be used by other tissues (Youssef and El-Ashker, 2017). This effect also explains higher BHB values during the weeks after calving, because the energy requirements are higher at this time. By comparing Holstein with crossbred Holstein x Jersey cows between two weeks before and 8 weeks after calving, Pelizza et al., (2019) did not find any difference between breeds for BHB values. They just report a difference between transition periods with higher BHB values after calving. No difference between breeds was also reported by Sgorlon et al., (2015) comparing BHB values for Holstein and Simmental cows in Italy.

Although we could not observe a general difference between genetic groups, we found an interaction between week and genetic groups for the variable BHB. Crossbred Holstein x Simmental cows showed lower values during week 2 and 3 after calving (Figure 2, C) than did Holstein. Holstein cows presented an average value of 1.24 mmol/l after calving, which can be considered as subclinical ketosis (Leblanc, 2010). The higher BHB value in weeks 2 and 3 after calving is a consequence of the higher BCS loss and body weight loss of the Holstein cows in comparison with the crossbred cows (Table 3). It shows that Holstein cows are mobilizing more body reserves to meet the energy demands for milk production (Esposito et al., 2014).

In addition to the parameters described above, the rumination time measured with an electronic device is an important tool to evaluate the health of the cows and the quality of the diet offered to them. In our study, the rumination time, however, did not differ between the genetic groups (Table 3, Figure 2, D). The difference between transition periods with higher rumination times after calving are possibly related to a higher DMI intake after calving (Table 2, Figure 1, A). It might be of interest to note that cows spend around 51 minutes ruminating for each Kg of DMI before calving. After calving, these values decreased to about 32-34 minutes for each Kg of DMI. This difference may be related to the different diets offered to the cows during the prepartum and postpartum period. The amount of concentrate offered to the cows increased from around 27 % to 40 % of the DM postpartum (Table 1). The ingredients of the concentrate ration undergo a rapid degradation in the rumen and do not favor rumination, because they have a lower fiber content (Kargar et al., 2010). Besides a lower DMI especially in the week just before calving, other factors, which contribute to lower rumination time especially at the day before and at the calving day, are the birth mechanisms and changes that the cow's body has to go through around calving. Schirmann et al. (2013) observed a reduction of 60 minutes rumination time and of 3.8 kg DMI 24 hours before calving. Between 24 and 48 hours after calving, the rumination time reaches again the same values as before calving.

Both genetic groups produce similar amounts of milk in the first 3 lactation weeks (Table 3, Figure 3, A). Similar or little lower milk yields for crossbred cows were also reported in other studies that covered the entire lactation (Brähmig, 2011; Knob et al., 2018; Nolte, 2019). Generally, we can see that the milk yield increases fast in the first lactation week starting at about 21 liters at the calving day to close 30 l/d after 7 days of lactation. By lactation day 21, both genetic groups have a milk yield close to 40 liters/day, meaning that they doubled their milk yield during this time. The variables fat and protein content did also not show a difference between the genetic groups. Both variables decreased from the first until the third week after calving, possibly due to dilution effect, because the amount of milk produced doubled during the same period.

Conclusion

We do know that after three weeks of lactation, DMI, milk yield, and all the other parameters keep changing. This study, however, demonstrates how the two different genetic groups, Holstein and crossbred Holstein x Simmental, pass the transition period, which always is a big challenge, especially for high yielding dairy cows. Both genetic groups lose BCS and weight after calving, although the Holstein cows lose more than crossbred cows. Both genetic groups present a similar dry matter intake during the transition period. That means that even with a better BCS before and after calving, crossbred cows do not have higher DMI. The genetic groups also have similar milk yield and composition, so the use of the crossbreds does not negatively affect the milk and solids yield at the beginning of the lactation in Holstein herds.

Acknowledgements

We would like to thank the dairy farmer that kindly collaborated in this research. We also would like to thank to Ordemilk and Allfex® that kindly donate the cervical neckless monitoring system for the realizations of this research. This study was financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001.

Declaration of Interest

The authors declare that they have no conflict of interest.

References

Barletta, R. V., Maturana Filho, M., Carvalho, P. D., Del Valle, T. A., Netto, A. S., Rennó, F. P., Mingoti, R. D., Gandra, J. R., Mourão, G. B., Fricke, P. M., Sartori, R., Madureira, E. H. and Wiltbank, M. C.: Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows, Theriogenology, 104, 30–36, doi:10.1016/j.theriogenology.2017.07.030, 2017.

Brähmig, J.: Einfluss der Wechselkreuzung von Deutschen Holsteins und Deutschem Fleckvieh auf Milchleistung und Milchqualität in einem automatischen Melksystem, Veterinary Medicine Faculty, Ludwig Maximilians University, Munich, Germany, 162 pp., 2011.

Carvalho, P. D., Souza, A. H., Amundson, M. C., Hackbart, K. S., Fuenzalida, M. J., Herlihy, M. M., Ayres, H., Dresch, A. R., Vieira, L. M., Guenther, J. N., Grummer, R. R., Fricke, P. M., Shaver, R. D. and Wiltbank, M. C.: Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows, J. Dairy Sci., 97(6), 3666–3683, doi:10.3168/jds.2013-7809, 2014.

Cassell, B. G. and McAllister, J.: Dairy Crossbreeding Research : Results from Current Projects, Virginia Coop. Extensio, 1–6, 2009.

Dewhurst, R. J., Moorby, J. M., Dhanoa, M. S., Evans, R. T. and Fisher, W. J.: Effects of altering energy and protein supply to dairy cows during the dry period. 1. Intake, body condition, and milk production, J. Dairy Sci., 83(8), 1782–1794, doi:10.3168/jds.S0022-0302(00)75049-1, 2000.

Djoković, R., Kurćubić, V., Ilić, Z., Cincović, M., Lalović, M., Jašović, B. and Bojkovski, J.: Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid lactation, Adv. Diabetes Metab., 5(2), 26–30, doi:10.13189/adm.2017.050202, 2017.

Esposito, G., Irons, P. C., Webb, E. C. and Chapwanya, A.: Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows, Anim. Reprod. Sci., 144(3–4), 60–97, doi:10.1016/j.anireprosci.2013.11.007, 2014.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. Principal descriptors of body condition score in Holstein cows.. J. Dairy Sci. 77:2695–703. doi:10.3168/jds.S0022-0302(94)77212-X, 1994.

Hazel, a R., Heins, B. J., Seykora, a J. and Hansen, L. B.: Montbéliarde-sired crossbreds compared with pure Holsteins for dry matter intake, production, and body traits during the first 150 days of first lactation., J. Dairy Sci., 96(3), 1915–23, doi:10.3168/jds.2012-5667, 2013.

Heins, B. J., Hansen, L. B., Seykora, a J., Johnson, D. G., Linn, J. G., Romano, J. E. and Hazel, a R.: Crossbreds of Jersey x Holstein compared with pure Holsteins for production, fertility, and body and udder measurements during first lactation., J. Dairy Sci., 91(3), 1270–8, doi:10.3168/jds.2007-0564, 2008.

Janzekovic, M., Mocnik, U. and Brus, M.: Ultrasound Measurements for Body Condition Score Assessment of Dairy Cows, DAAAM International Scientific Book 2015, Vienna, Austria., 2015.

Kargar, S., Khorvash, M., Ghorbani, G. R., Alikhani, M. and Yang, W. Z.: Short communication: Effects of dietary fat supplements and forage:concentrate ratio on feed intake, feeding, and chewing behavior of Holstein dairy cows, J. Dairy Sci., 93(9), 4297–4301, doi:10.3168/jds.2010-3168, 2010.

Kaufman, E. I., LeBlanc, S. J., McBride, B. W., Duffield, T. F. and DeVries, T. J.: Association of rumination time with subclinical ketosis in transition dairy cows, J. Dairy Sci., 99(7), 5604–5618, doi:10.3168/jds.2015-10509, 2016.

Knob, D. A., Alessio, D. R. M., Thaler Neto, A. and Mozzaquatro, F. D.: Reproductive performance and survival of Holstein and Holstein x Simmental crossbred cows, Trop. Anim. Health Prod., 48(7), 1409–1413, doi:10.1007/s11250-016-1103-9, 2016.

Knob, D. A., Alessio, D. R. M., Neto, A. T., Mozzaquatro, F. D., Thaler Neto, A. and Mozzaquatro, F. D.: Growth , productive performance , and udder health of crossbred Holstein x Simmental cows and purebred Holstein cows, Semin. Ciências Agrárias, 2597–2606, doi:10.5433/1679-0359.2018v39n6p2597, 2018.

Knob, D. A., Scholz, A. M., Alessio, D. R. M., Mendes, B. P. B., Perazzoli, L., Kappes, R. and Thaler Neto, A.: Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows, Trop. Anim. Health Prod., doi:10.1007/s11250-019-02174-9, 2019.

Leblanc, S.: Monitoring metabolic health of dairy cattle in the transition period introduction—metabolic challenges in peripartum dairy cows and their associations with reproduction, J. Reprod. Dev. Reprod. Dev, 56(56), 29–35, 2010.

Mann, S., Nydam, D. V, Lock, A. L., Overton, T. R. and McArt, J. A. A.: Short communication: Association of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids, J. Dairy Sci., 99(7), 5851–5857, doi:10.3168/jds.2016-10920, 2016.

McCarthy, M. M., Mann, S., Nydam, D. V., Overton, T. R. and McArt, J. A. A.: Short communication: Concentrations of nonesterified fatty acids and β-hydroxybutyrate in dairy cows are not well correlated during the transition period, J. Dairy Sci., 98(9), 6284–6290, doi:10.3168/jds.2015-9446, 2015.

Mendonça, L. G. D., Abade, C. C., da Silva, E. M., Litherland, N. B., Hansen, L. B., Hansen,

W. P. and Chebel, R. C.: Comparison of peripartum metabolic status and postpartum health of Holstein and Montbéliarde-sired crossbred dairy cows., J. Dairy Sci., 97(2), 805–18, doi:10.3168/jds.2013-7159, 2014.

MERTENS, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International, v. 85, n. 6, p. 1217–1240, 2002.

Młynek, K., Głowińska, B., Salomończyk, E., Tkaczuk, J. and Styś, W.: The effect of daily milk production on the milk composition and energy management indicators in Holstein–Friesian and Simmental cows, Turkish J. Vet. Anim. Sci., 42(4), 223–229, doi:10.3906/vet-1711-31, 2018.

Nolte, O.: Effekte der Rückkreuzung auf Fleckvieh aus einer Kreuzungsherde mit Deutschen Holstein-Genanteilen auf Milchleistung und Milchqualität, Veterinary Medicine Faculty, Ludwig Maximilians University, Munich, Germany, 16§ pp., 2019.

Olson, K. M., Cassell, B. G. and Hanigan, M. D.: Energy balance in first-lactation Holstein , Jersey , and reciprocal F 1 crossbred cows in a planned crossbreeding experiment, J. Dairy Sci., 93(9), 4374–4385, doi:10.3168/jds.2010-3195, 2010.

Palladino, R. A., Buckley, F., Prendiville, R., Murphy, J. J., Callan, J. and Kenny, D. A.: A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions, J. Dairy Sci., 93(5), 2176–2184, doi:10.3168/jds.2009-2453, 2010.

Pelizza, A., Hauser, A., Mendes, T. C., Mattiello, C. A., Knob, D. A., Cardozo, L. L., Parizotto Filho, R., Gomes, I. P. O., Alessio, D. R. M., Camera, M. and Thaler Neto, A.: Perfil metabólico de vacas Holandês e mestiças Holandês x Jersey no periparto, Arq. Bras. Med. Veterinária e Zootec., 71(3), 741–751, doi:10.1590/1678-4162-10098, 2019.

Prendiville, R., Pierce, K. M. and Buckley, F.: An evaluation of production efficiencies among lactating Holstein-Friesian , Jersey , and Jersey × Holstein-Friesian cows at pasture, J. Dairy Sci., 92(12), 6176–6185, doi:10.3168/jds.2009-2292, 2009.

Prendiville, R., Pierce, K. M., Delaby, L. and Buckley, F.: Animal performance and production efficiencies of Holstein-Friesian, Jersey and Jersey × Holstein-Friesian cows throughout lactation, Livest. Sci., 138(1–3), 25–33, doi:10.1016/j.livsci.2010.11.023, 2011.

Schirmann, K., Chapinal, N., Weary, D. M., Vickers, L. and von Keyserlingk, M. A. G.: Short communication: Rumination and feeding behavior before and after calving in dairy cows, J. Dairy Sci., 96(11), 7088–7092, doi:10.3168/jds.2013-7023, 2013.

Schweizer, H., Bernau, M. and Scholz, A. M.: Comparison of performance and health parameters of German and New Zealand dairy cows, in Proceedings of the World Congress on Genetics Applied to Livestock Production., 2018. vol. Electronic Poster Session - Biology & Species - Bovine (dairy) 2, p. 669, 2018.

Sgorlon, S., Fanzago, M., Sandri, M., Gaspardo, B. and Stefanon, B.: Association of index of welfare and metabolism with the genetic merit of holstein and simmental cows after the peak of lactation, Ital. J. Anim. Sci., 14(3), 368–373, doi:10.4081/ijas.2015.3841, 2015.

Weber, C., Hametner, C., Tuchscherer, A., Losand, B., Kanitz, E., Otten, W., Singh, S. P., Bruckmaier, R. M., Becker, F., Kanitz, W. and Hammon, H. M.: Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows, J. Dairy Sci., 96(1), 165–180, doi:10.3168/jds.2012-5574, 2013.

Weigel, K. a and Barlass, K. a: Results of a producer survey regarding crossbreeding on US dairy farms., J. Dairy Sci., 86(12), 4148–54, doi:10.3168/jds.S0022-0302(03)74029-6, 2003.

Youssef, M. and El-Ashker, M.: Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period, Trop. Anim. Health Prod., 49(2), 239–244, doi:10.1007/s11250-016-1211-6, 2017.

Table 1. Ingredients and composition of TMR[1] (% of dry matter) offered to the dry (prepartum) and lactating (postpartum) Holstein and crossbred Holstein x Simmental dairy cows.

Ingredients	Dry cows	Lactating cows
Corn silage	72.64	16.23
Ryegrass fresh	-	38.96
Ryegrass silage	-	6.49
Commercial concentrate	27.36	15.58
Brewery waste	-	15.58
Soybean hull	-	1.95
Ground corn	-	4.55
Mineral mix (commercial)	-	0.65
Chemical composition		
Dry Matter %	31.73	34.13
Organic material	93.3	91.81
Ash	6.7	8.19
Crude Protein	12.68	15.44
Ether Extract	3.62	5.04
NDF (neutral detergent fiber)	43.5	43.37
ADF (Acid detergent fiber)	20.92	20.74
NFC (non fiber carbohydrates)	33.91	28.5

[1] Values were obtained from chemical analysis of feed samples. NFC = 100 – (% CP + % NDF + % fat + % ash)

Table 2. Week and period classes according to days relative to calving

	Prepartum (before calving)			Calving day	Postpartum (after calving)		
Days relative to calving	21 - 15	14 - 8	7 - 1	0	1 - 7	8 - 14	15 - 21
Week	-3	-2	-1	0	1	2	3

Table 3. Least Squares Mean ± mean squares error and P-value for Genetic Group (GG), Transition Period, and their interaction for the variables dry matter intake (DMI), milk yield, milk composition, body weight (BW), body condition score (BCS), rumination time (RT), and beta-hydroxybutyrate (BHB) for purebred Holstein (H) and F1 Holstein x Simmental (H x S) crossbred cows.

Variable	GG	Period		P Value		
		Prepartum	Postpartum	GG	Period	GG*Per
DMI (Kg/day)	H	9.23±0.5	15.98±0.5	0.5224	<0.0001	0.0412
	H x S	9.32±0.6	16.44±0.6			
DMI % of Body Weight	H	1.33±0.10	2.40±0.07	0.3623	<0.0001	0.5425
	H x S	1.16±0.14	2.36±0.10			
BCS	H	3.58±0.11	2.95±0.10	<0.0001	<0.0001	0.1287
	H x S	4.13±0.15	3.74±0.13			
Body Weight (Kg)	H	744.7±17.5	632.3±17.1	0.5156	<0.0001	0.0379
	H x S	750.3±21.8	661.9±21.2			
BHB (mmol/l)	H	0.77±0.12	1.26±0.10	0.5226	0.0004	0.2610
	H x S	0.79±0.16	1.04±0.13			
RT (minutes/day)	H	478.7±14.5	552.0±14.1	0.4961	<0.0001	0.3003
	H x S	471.4±18.4	529.7±17.4			

Table 4. Least Squares Mean ± mean squares error and P-value for Genetic Group (GG) Week, and their interaction for the variables related to milk yield and milk composition for purebred Holstein and F1 Holstein x Simmental crossbred cows.

Variable	GG	LSM	P Value		
			GG	Week	GG*Week
Milk yield (Kg)	H	30.51±1.43	0.4741	<0.0001	0.5006
	H x S	28.83±1.81			
ECM*	H	38.19±1.48	0.4123	0.4044	0.7712
	H x S	35.90±2.30			
Fat (%)	H	3.99±0.13	0.0593	0.0040	0.5161
	H x S	4.48±0.20			
Protein (%)	H	3.31±0.07	0.6316	0.0027	0.0807
	H x S	3.37±0.10			
Lactose (%)	H	4.57±0.03	0.8322	0.0009	0.3743
	H x S	4.58±0.06			
Fat + Protein (Kg)	H	2.54±0.09	0.5421	0.7828	0.8578
	H x S	2.42±0.15			

*ECM = Energy corrected milk

Figure 1. Weekly means of absolute dry matter intake and dry matter intake in relation to body weight (%) between three weeks before calving and three weeks after calving for purebred Holstein (H) and crossbred Holstein x Simmental cows (H x S).

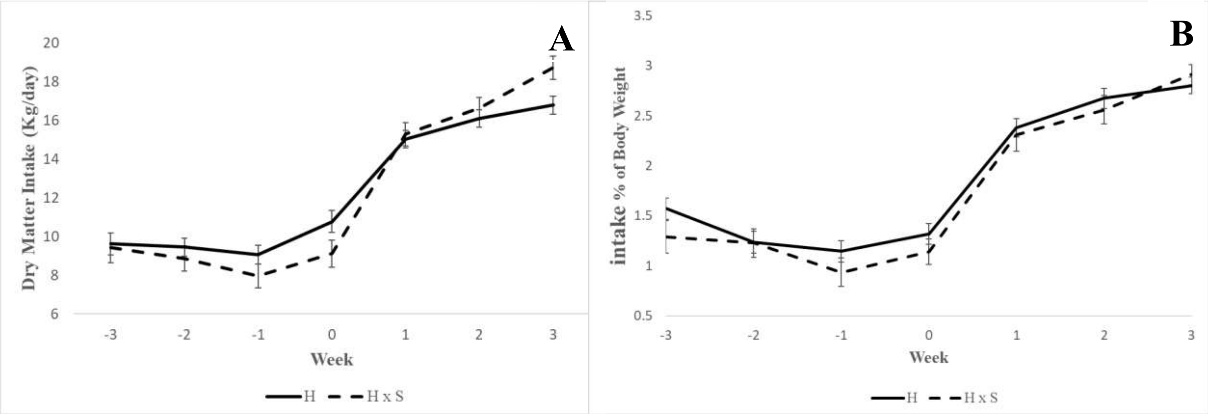


Figure 2. Weekly means of body condition score (BCS), body weight (BW), beta-hydroxybutyrate (BHB) and rumination time between three weeks before calving and three weeks after calving for purebred Holstein (H) and crossbred Holstein x Simmental (H x S) cows.

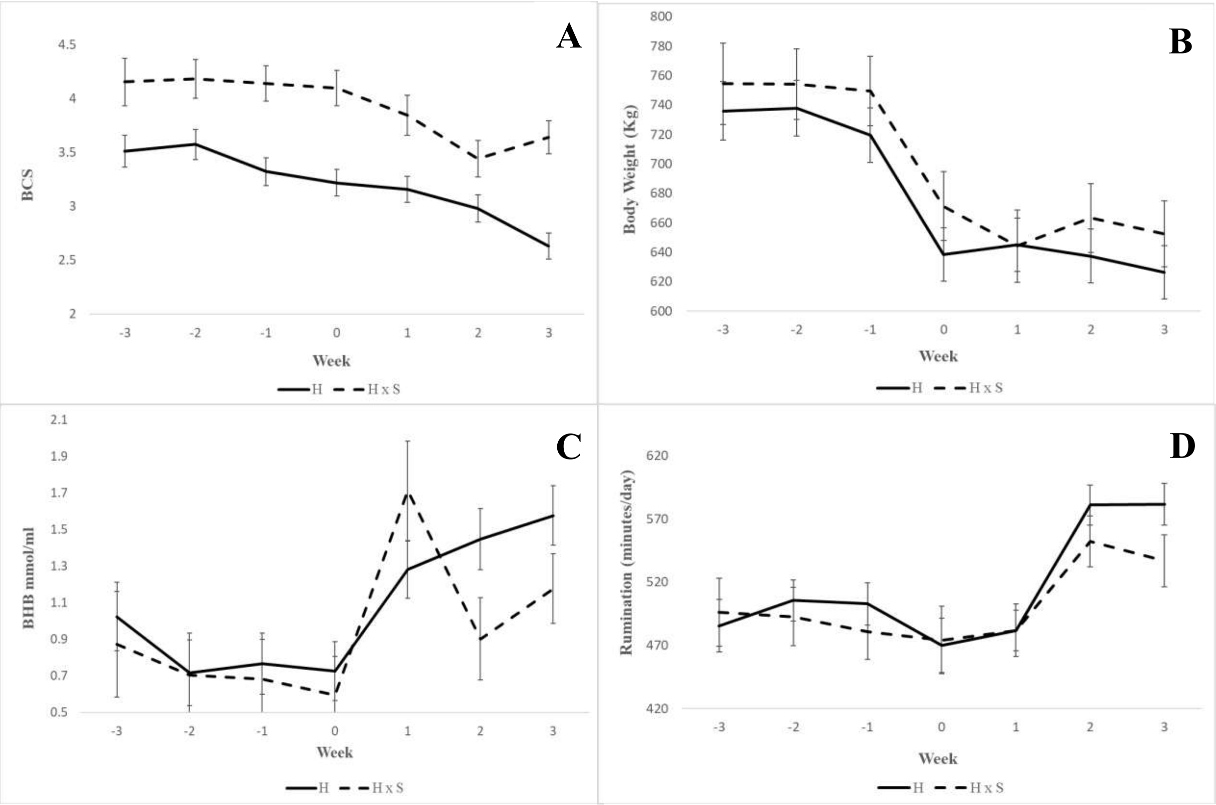
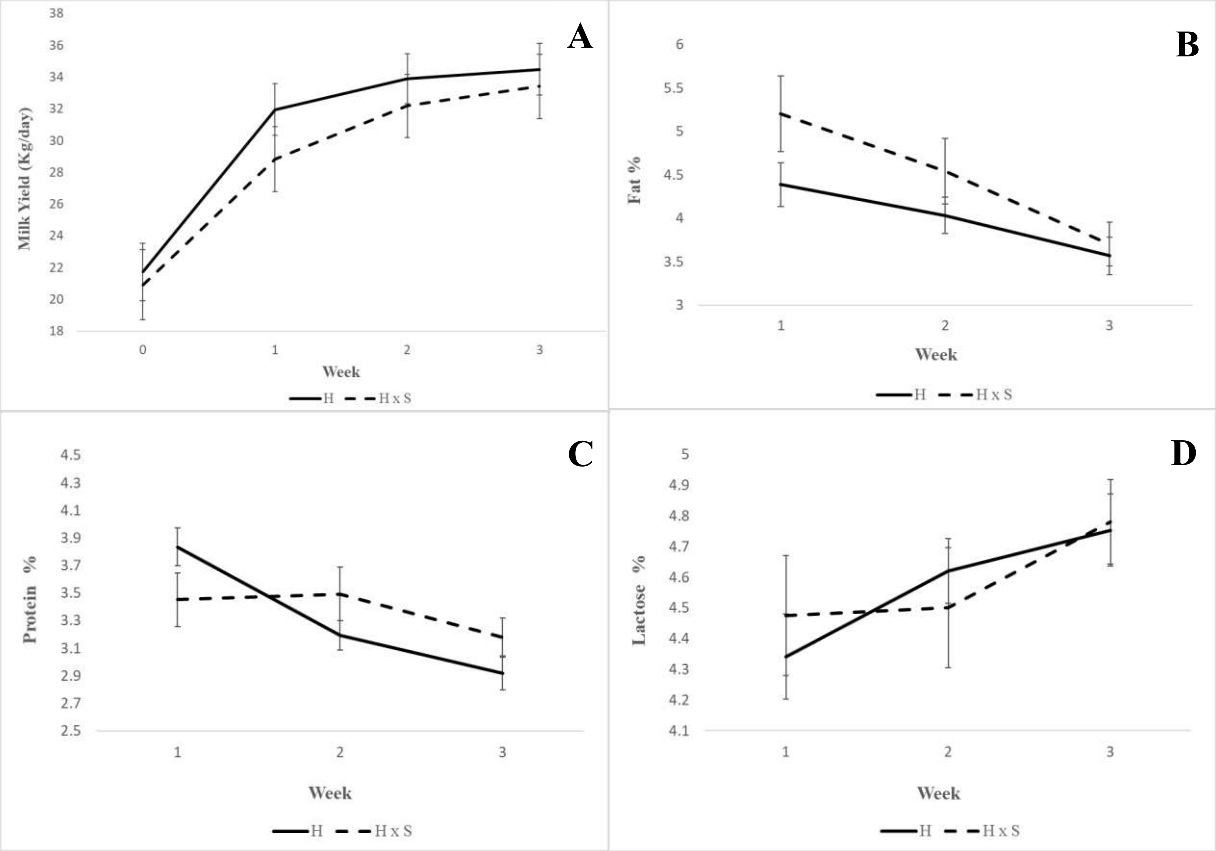


Figure 3. Weekly means of milk yield and milk composition in the three weeks after calving for purebred Holstein (H) and crossbred Holstein x Simmental cows (H x S).



CHAPTER V

Energy balance during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses

Energy balance during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses

Deise Aline Knob[12], André Thaler Neto[2], Helen Schweizer[1], Anna Weigand[1], Roberto Kappes[2], Armin Scholz[1]

[1]Ludwig Maximilians Universität München (LMU), Tierärztlichen Fakultät, Lehr- und Versuchsgut Oberschleißheim, Oberschleißheim, Deutschland.

[2]Programa de Pós-Graduação em Ciência Animal - Centro de Ciências Agroveterinárias - Universidade do Estado de Santa Catarina - CAV/UDESC, Lages – Santa Catarina. Brasil.

*Correspondence: Deise Aline Knob
Avenida Luis de Camões, 1231, ap. 3.
Bairro Coral, Lages, Santa Catarina,
Brasil Cep: 88523-000
Phone +55 49 991046241
Email: deisealinek@gmail.com

Abstract

Crossbreeding in dairy cattle has been used to improve functional traits and milk composition of Holstein herds. The first generation, F1, is the one with the maximum heterosis and so, also presents the best results. Independent of the genetic group, cows usually need to mobilize body fat reserves during early lactation in order to be able meeting the substantial energy demands for milk synthesis. The objective of the study was to compare indicators of the metabolic energy balance, nonesterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), Glucose, Body condition score (BCS) backfat thickness (BFT) as well as of milk yield and milk composition of Holstein and Simmental cows and their crosses from the prepartum period until 100 lactation days. The research was carried out at the Livestock Center of the Ludwig Maximilians University (Munich, Germany). The herd was composed of 120 lactating cows of the Holstein and Simmental breeds as well as their crosses. Cows were divided into 5 genetic groups (GG) according to their theoretic proportion of Holstein and Simmental genes as follows: Holstein (≥ 87.5% Holstein), R1 Holstein (between 62.5 and 87.4% Holstein), "F1" crossbreeds (between 37.5 and 62.4 Holstein), R1 Simmental (between 62.5 and 87.4% Simmental) and Simmental (≥87.5 % Simmental). The research was carried out from April 2018 until February 2019. Cows were evaluated 3 weeks before calving until 14 weeks after calving, 186 cows entered the study. In a weekly routine, BCS (1 = very thin to 5 = very fat) and BHBA (in mmol/l) lameness score (LS) (1 = healthy to 5 = very lame), blood parameters like BHB, glucose, and NEFA were evaluated. A mixed model variance analysis with the fixed effects: genetic group, parity, season, week, interaction between genetic group and week, and the effect of the bedding system was used. The higher the Simmental proportion higher the BCS. All the genetic groups lose BCS, BFT and body weight after calving. Holstein and F1- crossbred cows have the highest milk yield, while the Simmental have the lowest values (P= 0.0013). Simmental cows present the lower NEFA value, F1 – Crossbred cows have the higher value while the other genetic groups have intermediate values (P = 0.0011). For the variables BHB and glucose, we found no differences among genetic groups, only for weeks relative to calving. Simmental and R1 – Simmental cows deal better with a negative energy balance after calving. These genetic groups lose less body weight and backfat thickness than the other genetic groups with greater Holstein proportions. Cows with at least 50% Holstein genes yield greater amounts of milk, which causes higher NEFA and BHB values indicating a stronger body tissue mobilization in order to meet the energy requirements.

Key words: backfat thickness, BHB, body condition score, NEFA, rotational cross

Introduction

Crossbreeding in dairy cattle has been used to improve functional traits and milk composition of Holstein herds (Malchiodi et al., 2011; Hazel et al., 2014; Mendonça et al., 2014; Knob et al., 2016, 2018, 2019). The majority of these studies, however, report only the results of the first crossbreeding generation (F1) in comparison with the purebred line(s), like e.g. Prendiville et al. (2009, 2010) for Holstein-Jersey-F1, or Hazel et al. (2017) for Holstein-Montbeliarde and Holstein–Viking Red-F1, or Laguna et al. (2017) for Holstein-Gir-F1. Only the F1 can reach the maximum heterosis effect often defined as best performance results in terms of productivity, efficiency, reproduction success, and/or vitality of the F1 offspring by surpassing the average of the parental lines (VanRaden and Sanders, 2003). The performance of the subsequent breeding generations in a dairy cattle crossbreeding program stays often unanswered or is not being communicated anymore. In dairy cattle, if at all (Kargo et al., 2012), three different crossbreeding approaches are being used most frequently. 1) Backcrossing, that is actually more related to purebreeding, because after crossing with a "foreign" breed or line, the subsequent matings occur again only within the original parental breed or line leading to a "refined" breed (with some foreign "blood"). German Simmental breeders, for example, use Red Holstein semen to refine the parental breed "Deutsches Fleckvieh". 2) Three-breed rotational crossbreeding works with three dairy breeds (Shonka-Martin et al. 2019). After producing the first F1 generation from two breeds, a third breed is crossed in leading again to a F1 offspring generation that maintains the expected maximum heterosis level also for the second crossbreeding generation. During the following crossbreeding generations, by applying a sire rotation of the three breeds (lines), the heterosis effect will reach a level of 85.7 % of the maximum heterosis effect to be expected in the F1 generation. 3) The most simple approach is a two-breed rotational cross leading to a large variety of the genetic proportions of the two parental breeds in the crossbred population with an average heterosis effect of 66.6 % of the expected maximum The third approach, for example, is a standard procedure in the New Zealand "Kiwi Cross" breeding program by crossing New Zealand Holsteins with New Zealand Jerseys (https://www.lic.co.nz/products-and-services/artificial-breeding/crossbreeding-kiwicross/) (Gama, 2002; Buckley et al., 2014).

A simulation study showed that terminal and rotational crossbreeding strategies using Swedish Red and Swedish Holstein cows can improve profitability in average Swedish organic and conventional dairy herds with purebred Swedish Holstein. The largest economic benefits were shown for rotational crossbreeding, where all animals in the herd were crossbreds and expressed 67% of the full heterosis (Clasen et al., 2019).

Generally, independent of belonging to purebred or crossbred breeding lines, dairy cows usually need to mobilize body fat reserves during early lactation in order to be able meeting the substantial energy demands for milk synthesis (Esposito et al., 2014). The evaluation of body condition scores (BCS) is one technique that can be used to visually and subjectively estimate the intensity of the loss of the subcutaneous fat reserves (Earle, 1982, Edmonson et al. 1989). Ultrasound backfat thickness (BFT) is a (more) objective and direct measure for the evaluation of subcutaneous fat (Schröder and Staufenbiel, 2006). Both BCS and BFT may be therefore used to evaluate the energy status of the cow (Barletta et al., 2017). Differences for these variables between breeds are reported especially by comparing dual purpose breeds or crossbreds with Holstein cows (Knob et al., 2016; Ferris et al., 2018; Shonka-Martin et al., 2019).

During the process of using body tissues as an energy source, the metabolic status and blood parameters of the cow are changing. For example, nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) are being generated (Barletta et al., 2017; Bicalho et al., 2017). NEFA originating from the mobilization of body (energy) reserves, as are e.g. triglycerides (triacylglycerol), are being used directly by peripheral tissues as an alternative source of energy (Kuhla et al., 2016, Li et al., 2020). In the liver, oxidation and generation of metabolites such as acetyl CoA occur, which can be used for the generation of energy by the Krebs cycle or by the tricarboxylic acid cycle. If this oxidation occurs incompletely, ketone bodies like acetoacetate and BHB are generated in order to compensate for the deficit in the glucose precursors intake (Adewuyi et al., 2005 Eisemann, 2010, Figure 1). BHB can also be used as an energy source by the liver itself or is released into the bloodstream to be used as an energy source by other tissues. Another possible route is the re-esterification of NEFA in the form of triacylglycerol, which will again be stored in adipose tissue (Kuhla et al., 2016, Barletta et al., 2017; Youssef and El-Ashker, 2017). In addition, by the stimulation of insulin secretion, NEFA can be used for the gluconeogenesis process in the liver, which will reduce the process of ketogenesis (Adewuyi et al., 2005; Hayirli, 2006). Mann et al. (2016b) conclude, however, that "differences in serum concentrations of NEFA between cows overfed energy prepartum and high blood concentrations of BHB are likely due to greater negative energy balance postpartum reflected in lower circulating concentrations of glucose and insulin and an increase in the total amount of mobilized adipose tissue mass rather than due to changes in adipose tissue insulin signaling".

By comparing Holstein, Brown Swiss, Simmental and crossbred Holstein x Simmental cows, Blum et al. (1983) observed higher NEFA values in Holstein cows. They attribute this observation to the comparably higher milk yield and consequently higher body reserves mobilization. In addition, they observed higher NEFA concentrations at the beginning of the lactation justified by the high milk yield. In the study of Mendonça et al. (2014), no differences were observed between Holstein and crossbred F1 Holstein x Montbeliarde cows for NEFA and BHB values. Sgorlon et al. (2015) also reported no difference for NEFA and BHB concentrations by comparing Holstein and Simmental cows after the lactation peak.

Crossbreds F1 Holstein x Simmental cows in comparison to the parental Holstein breed have a better reproductive performance with similar milk yield (Knob et al., 2016, 2018, 2019; Diepold, 2019; Nolte, 2019). We performed this study, in order to better understand and to compare the crossbred generations following the F1 in a two-breed rotational system with the parental breeds Holstein and Simmental. Especially, we aimed to evaluate the pre partum period and the first 100 lactation days, when the cows are more susceptible to a challenging negative energy balance and all related negative "side" effects. Our hypothesis is that the higher the Simmental proportions the better the cow can pass the transition period combined with a lower negative effect on BCS and BFT (loss) and favorable blood parameters indicating a negative energy balance. The objective of the study was to compare indicators of the metabolic energy balance (BHB, NEFA, Glucose, BCS, BFT) as well as of milk yield and milk composition of Holstein and Simmental cows and their crosses from the prepartum period until 100 lactation days.

Material and Methods

All the procedures carried out with the animals in our research were approved by the animal ethical committee of the Government of Upper Bavaria under the protocol number ROB-55.2-2532.Vet_03-18-60.

Animals and management

The research was carried out at the Livestock Center of the Ludwig Maximilians University (Munich, Germany). The herd consisted of about 120 lactating cows of the Holstein and Simmental breeds as well as their crosses. The crossbred cows are the result of a rotational crossbreeding system that started in the year 1999. The crossbred cows have different gene proportions of the Simmental and Holstein breed. Cows were divided into 5 genetic groups according to their theoretic proportion of Holstein and Simmental genes as follows: Holstein - ≥ 87.5% Holstein (12 cows), R1 Holstein - between 62.5 and 87.4% Holstein (27 cows), "F1" crossbreds - between 37.5 and 62.4 Holstein (32 cows), R1 Simmental - between 62.5 and 87.4% Simmental (75 cows) and Simmental - ≥87.5 % Simmental (40 cows).

The lactating animals were kept in a confinement system throughout the entire year. The freestall barn was divided into two parts. One side of the freestall was equipped with a straw deep box bedding system and the other side used soft rubber lying mats as bedding system. The cows were randomly housed at one of both sides, so that the proportion of each genetic group and parity was similar on both sides. In each part of the barn, cows had free access to an automatic milking system (AMS) from Lely Industries N.V Maasland/Netherlands (Astronaut A3 and Astronaut A3 next). Cows entered the AMS through an electronic identification system positioned in the neck collars. Information regarding milking frequency, milk yield, somatic cell count, and body weight were recorded daily and per milking. The diet offered to the cows was a partial mixed ration composed of corn silage, grass and clover silage, hay, wheat straw and concentrates. The cows received the concentrates during milking at the AMS according to their milk yield and days in milk (DIM).

Data collection

The research was carried out between April 2018 and August 2019. Multiparous (n = 120) and primiparous (n = 66) cows were evaluated 21 days before the expected day of calving until day 100 of lactation (the first 14 weeks after calving). We recorded data and took samples of each cow participating in the study weekly. We evaluated the following traits: body condition score (BCS), backfat thickness (BFT), lameness score (LS), blood parameters like beta-hydroxybutyrate (BHB), glucose, and non-esterified fatty acids (NEFA). BCS was evaluated as a visual score from 1 (very thin cow) to 5 (very fat cow) according to Ferguson et al. (1994). On the day of body condition scoring, we also measured the BFT with an ultrasonic device (KX5200, Kaixin Electronic Instrument CO, Xuzhou, Jiangsu, China) using a linear probe (6.5 MHz). The measurement point was chosen on an imaginary line between the tuber ischia and the tuber coxae about 10 cm cranial of the tuber ischia (Schröder and Staufenbiel, 2006). The image was frozen on the screen of the ultrasound device and the layer of subcutaneous fat was measured to the nearest millimeter. To minimize potential errors, the evaluations were conducted always by the same researcher. The BFT was always assessed on the right body side of the cow. Similarly, with a weekly routine, blood was sampled from the coccygeal vein of each cow using plastic syringes equipped with a vacuum system and a clot activator. After 3 hours, the blood samples were centrifuged for the serum separation with 3000 rpm for 10 minutes. At the time of blood sampling, we also performed the BHB and glucose tests. A portable measurement device from Pharmadoc (Lüdersdorf, Germany) served as a tool for the determination of the BHB concentration (mmol/l). The glucose (mg/dL) concentration was measured by means of the portable ACCU-CHEK Guide device (Roche Diabetes Care Deutschland GmbH). The serum samples provided the basis for the NEFA (mmol/l) evaluations using commercial test kits from Diaglobal® (Berlin, Germany). After the analysis, the serum samples were frozen at -20 °C.

Lameness scoring (LS) was carried out with the help of a 5-point scoring system by looking at the cow while standing and walking. Cows received marks between 1 (no lameness, back straight in standing and walking, normal treading) and 5 (severe lameness, back is bent in walking and standing, with one or more legs only partially or no treading). The LS evaluations were performed by the same researcher during the entire research period.

Statistical analysis

Data were classified according to the week relative to the calving date. Parity was grouped as first, second and third or more parturitions. To obtain normality of data, SCC was transformed to SCS by the logarithmic scale applying the following equation: $SCS = \log2 (SCC/100) + 3$. The energy corrected milk yield (ECM) was obtained by the equation: $ECM = (0.327*MY) + (12.95\% * F*MY/100) + (7.65\% P*MY/100)$, where, MY = milk yield in l/day, F = fat percentage, and P = protein percentage. A REML (restricted maximum likelihood) analysis of variance (ANOVA) using the MIXED model procedure of SAS 9.4 with the random effect of "cow" was performed. The model contained the following fixed effects: genetic group, parity, season, week, interaction between genetic group and week, and the effect of the bedding system. Before variance analysis, data were tested for normality of the residuals using the Kolmogorov–Smirnov test. A Tukey-Kramer adjustment was used for the t-test of least squares means (LSM). The significance level was set to $P \leq 0.05$.

Results

The results of our study show a difference among genetic groups for most of the variables related to the energy balance before calving until day 100 after calving. For BCS, for example, the higher the Simmental proportion, higher the BCS ($P < 0.0001$). The average difference between the purebred Simmental and Holstein cows reached 1 point (Table 1). The genetic groups R1-Hol and F1-Crossbred have similar BCS as the Holstein cows. For BFT, we also found a slight difference between the genetic groups, with Simmental showing the higher values (P= 0.0727), although, for this variable the difference between the Holstein and the Simmental breed is lower (0.3 cm). When we look at the difference across weeks for each genetic group, we can see that all of them lose BCS and BFT after calving (Figure 2, A and B). The lowest BCS is observed around weeks 6 to8, while for BFT, cows reach the lowest value around week 10. Holstein cows lose 1 BCS point until week 8 after calving representing 27% of initial BCS. With an increasing Simmental proportion, the BCS loss decreases 20%, 23%, 17%, and 13% for R1-Hol, F1- Crossbred, R1 – Sim and Simmental cows, respectively. BFT loss in comparison to the values before calving is higher than the BCS % for all the genetic groups. Holstein cows, for example, lose 47% of body fat, while the 3 groups of crossbred cows lose around 40%, and Simmental 27%. Holstein, R1-Hol and F1-Crossbred cows have a similar BW. These values are lower than the BW of Simmental and R1-Sim cows (P= 0.0050; Table 1). The difference represents around 45 kg (Figure 2, D).

Holstein and F1-crossbred cows have the highest milk yield, while the Simmental have the lowest values (P= 0.0013; Table 2). The difference represents about 84 % of the yield reached by the Holstein cows. The R1 – Hol and R1-Sim cows have intermediate values. The 5 genetic groups reach the lactation peak between week 6 and 8 (Figure 2, C). By comparing the results for ECM, we found lower values for R1-Simmental and Simmental cows in comparison to the other genetic groups (P=0.0006). All the genetic groups have similar fat and protein contents in milk (P=0.6820; P=0.4697; respectively; Table 2). Although, by comparing fat + protein yields, Holstein, R1-Hol and F1-Crossbred cows yielded higher amounts than the other genetic groups (P=0.0009; Table 2), we found no difference for the variable SCS among the genetic groups during the first 100 days of lactation (P=0.4673; Table 2).

The blood parameters evaluated as indicators of the cow's energy balance show also differences among genetic groups, and also the week relative to calving has an effect on all the variables. Simmental cows present the lower NEFA value, F1 – Crossbred cows have the higher value while the other genetic groups have intermediate values (P = 0.0011; Table 1). When we look at how NEFA concentration changes according to lactation week, we observed a slight increase in the last week before calving and higher values in the first week after calving (Figure 3, C). For this variable (NEFA), we observe an interaction between genetic group and week (P=0.0317). During the first week after calving, Holstein and F1 – Crossbred cows present higher NEFA values than do the other genetic groups. Holstein cows present higher values also during the third week after calving. From week 4 until the end of the experimental period, there are no differences among genetic groups anymore (Figure 3). For the variables BHB and glucose, we found no differences among genetic groups (P=0.2418 and P= 0.3688, respectively, Table 1). Glucose values increase in the week just before calving, and decrease during the first week after calving. Then, Glucose concentration increases slightly until week 4 after calving and remains stable until week 14 (Figure 3, B). This pattern was similar for all genetic groups (P=0.2309). For BHB, we observed an interaction between genetic group and week (P=0.0074). For example, F1 – crossbred cows reach 1.2 mmol/l during the second week of lactation, while Holstein cows reach the highest value during week 4 (1.27 mmol/l). For the other genetic groups, no difference was found (Figure 3, A). The highest value for R1-Hol was found also during week 4 (1.13 mmol/l), while R1 – Sim reached higher glucose concentrations during week 1 after calving (1.05 mmol/l) and Simmental cows during week 7 after calving (1.01 mmol/l).

Discussion

This study investigates the potential differences among Holstein, Simmental and their crosses regarding the energy balance before calving until 100 DIM. The main limitation of our study is the fact that the cows were not equally distributed within each genetic group. We had just 12 cows for the Holstein group, for example. However, we found consistent results about performance parameters and indicators of energy balance for all genetic groups.

By comparing the BCS of purebred and crossbred Holstein x Simmental cows, it is noticed that the higher the Simmental proportion the higher the BCS, possibly because of the fact that Simmental, as a dual purpose breed, has a higher BCS than the Holstein breed (Schweizer et al., 2018; Sgorlon et al., 2015). In addition, due to complementarity of the crossbreeding system, this characteristic is also highlighted in crossbred cows. Higher BCS for crossbred Holstein x Simmental cows in comparison with the Holstein cows was also reported in other studies (Knob et al., 2016; Ledinek et al., 2018). Similar to our finding, Ledinek, et al. (2018) reported an increasing BCS with an increasing Simmental proportion by comparing Holstein, Simmental and crossbred cows with different proportions of Holstein and Simmental genes. The favorable BCS and BFT values, and, especially the lower BCS loss of cows with higher Simmental proportion is possibly related to the different nutrient partitioning between milk production and body reserves (Yan et al., 2006; Ledinek et al., 2018). Holstein cows allocate more energy to milk production (Veerkamp et al., 2003). The lowest BCS and BFT of Holstein cows may reflect the genetic selection for production and conformation, whereas for dual purpose breeds like Simmental, the selection has been focused on body condition and milk production (Mendonça et al., 2014).

Cows in our research, especially the Holstein cows during the prepartum period, have a higher BCS than in other studies (Kaufman et al., 2016; Knob et al., 2016). The higher the Holstein proportion the higher is the milk yield (Figure 2, C). These genetic groups demand a higher energy supply in order to be able to meet the energy requirements for milk production. As a consequence, they mobilize more body fat for the conversion into energy leading to higher values of NEFA and BHB (see also Figure 1). Šamanc et al. (2015) reported that cows that lose more than 0.75 BCS points have higher NEFA values. In our study, the genetic groups Holstein and F1 – crossbred cows are the groups that lose more than 0.75 BCS points (Figure 2, A).

With the high milk yield, especially during the first weeks after calving, a decrease in BCS and BFT is observed for all genetic groups (Figure 2, A and B). This is possibly related to the negative energy balance. Right after calving, the dry matter intake increases but not as fast as the milk yield, and so, to support the energy requirement for milk production, the cows start to use body reserve as energy source (Mann et al., 2016a; Barletta et al., 2017; Djoković et al., 2017). The body tissue mobilization, especially adipose tissue, is converted into energy sources to be used by the cows through hepatic gluconeogenesis (Youssef and El-Ashker, 2017). An indicator of this lipomobilization can be observed by increasing serum concentrations NEFA and BHBA (Figure 3) in the periods before and immediately after calving (Ospina et al., 2010).

In a review regarding NEFA in dairy cattle, Adewuyi et al. (2005) cited that for healthy cows the NEFA values are below 0.20 mmol/L. In the week before calving, these values increased slowly and can reach values between 0.5 and 2 mmol/L during the first week after calving. After calving, NEFA levels decrease, while values greater than 0.7 mmol/L indicate a severe negative energy balance after calving. Six weeks after calving, NEFA values reach again a level below 0.3 mmol/L. In our study, we observed this behavior for the NEFA concentration for all genetic groups (Figure 3). We observed that for the genetic groups Holstein and F1-crossbred cows the NEFA peak during the first week after calving is higher than for the other genetic groups, which is possibly related to the higher milk yield of these genetic groups (Table 1). To support the higher milk yield, these genetic groups have a higher body tissue mobilization (Figure 1). These periods are those of highest energy challenge, in the first week after calving due to low DMI and the beginning of lactation (Mendonça et al., 2014; Djoković et al., 2017). Glucose consumption of the mammary gland in dairy cows is responsible for 50% to 85% of whole-body glucose consumption, and amplifies the glucose demand by 2.5-fold at the third week of lactation in comparison with the demand during the end of the dry period (De Koster and Opsomer, 2013). These higher NEFA values for Holstein and F1-crossbred cows are combined with a longer lasting BHBA peak during the following weeks. A similar behavior was observed by Mendonça et al. (2014) comparing Holstein and crossbred Holstein x Montbeliarde cows.

Another reason for higher NEFA values with an increasing Holstein proportion in cows is that the body tissue mobilization of these genetic groups is not always just subcutaneous fat. They can also mobilize visceral fat (Figure 1). Therefore, BCS and BFT loss do not completely reflect the body tissue mobilization of the cows. Even with higher BCS and BFT loss, since Holstein and F1 – crossbred cows have the higher milk yield (Table 2, Figure 2, C), it is also possible that they are mobilizing other fat depots to fully meet the energy requirements. Weber et al. (2013), by comparing high yielding cows with medium and low yielding cows, reported that visceral fat was more readily mobilized in cows in the high yielding group and thus contributed relatively stronger to elevated NEFA concentrations. Akter et al. (2011), by comparing different fat depots in primiparous cows, found that visceral fat depots were more readily mobilized than subcutaneous fat during the first 15 weeks of lactation.

The glucose values for all the genetic groups are in accordance with the reference values for dairy cows ranging between 45 and 75mg/dL (González e Silva, 2006). We could not find any difference among the genetic groups for glucose levels. That is in accordance with other studies that also compared glucose levels of Holstein and crossbred cows. Pelizza et al. (2019) reported no difference for glucose levels by comparing Holstein and crossbreed Holstein x Jersey cows. The values ranged between 59 and 63 (mg/dL) for both genetic groups. Blum et al. (1983) comparing Holstein, Swiss Brown, Simmental and crossbred Holstein x Simmental cows, Mendonça et al. (2014) comparing Holstein and crossbred Holstein x Montbeliarde and Sgorlon et al. (2015) comparing Holstein and Simmental cows reported no difference in serum glucose concentrations among the genetic groups evaluated. The variation is mainly related to lactation stage, like pre and post partum and DIM (Djoković et al., 2017).

In our study, we also observed a glucose variation according to the lactation stage (Figure 3, B). At the beginning of lactation, due to the high milk yield, glucose levels are lower due to its uptake to the mammary gland for lactose synthesis. Another possible reason is due to fatty infiltration due to negative energy balance, which will reduce the capacity of hepatic gluconeogenesis and reduced glycogen stores (Djoković et al., 2017; González e Silva, 2017). González et al. (2011) observed values of 3.37 ± 0.74 (mmol /L) at the beginning of the lactation, and 3.82 ± 0.41 (mmol /L) at the middle third of lactation. Djoković et al. (2017) observed values of 2.29 ± 0.48 mmol /L at the beginning of lactation, while at mid-lactation the values ranged from 2.5 to 4.2 (mmol/L). In another study by Djokovic et al. (2011), mean glucose levels in cows ranged from 2.2 to 4.0 mmol/L, however for cows in the puerperal period, blood glucose was significantly lower than in pregnant cows.

Cows with more than 50% of Holstein genes showed a higher milk yield compared to R1- Sim and Simmental cows. These results have already been reported in other studies (Schwaiger, 2008; Brähmig, 2011; Knob et al., 2018; Ledinek et al., 2018; Nolte, 2019), where crossbred F1 Holstein x Simmental cows have similar milk yield as the Holstein cows. The genetic selection has been more intensified for the Holstein breed, and this may reflect the better performance for cows with higher Holstein proportions in comparison to the Simmental cows (Sgorlon et al., 2015). A lower milk yield of Simmental cows in comparison to the Holstein cows has been reported by (Urdl et al., 2015; Kara and Koyuncu, 2018; Nolte, 2019). Simmental cows reach about 70 to 80% of the milk yield of the Holstein cows.

In contrast to other studies, where Simmental cows or crossbred cows have a higher fat content than the Holstein cows (Kara and Koyuncu, 2018; Ledinek et al., 2018; Knob et al., 2019; Nolte, 2019), there was no difference between the genetic groups in our study. Besides the genetic group, other factors can affect the milk fat content, like e.g. the diet offered to the cows and the lactation stage. In our study, the similar fat content for all genetic groups can be a consequence of the equal diet management for all groups. Another reason why cows with 50% or more Holstein genes have the same milk fat content as the Simmental cows can be related to the body tissue mobilization. These genetic groups are those that have a high initial BCS and show also a higher BCS and BFT loss. They reach higher NEFA values. This alternative energy source, besides changing the fatty acids profile in milk, can also induce a higher milk fat content. Pires et al. (2013) compared the metabolic status with the milk yield and milk composition according to different initial BCS. They reported that cows with higher initial BCS have higher NEFA and BHB levels. The same group of cows has a higher milk fat content reflecting the availability of body fat.

The ECM yield and especially fat + protein yield of the crossbred cows is higher than in other studies that compare Holstein and crossbred cows (Table 2). Auldist et al. (2007) by comparing Holstein and crossbred Holstein x Jersey cows reported a total fat + protein amount of around 1.9 kg/day with no difference between the genetic groups. Fellipe et al. (2017), by comparing the same genetic groups, reported also no difference between them with about 1.7 Kg fat + protein /day. By comparing the first 150 DIM of Holstein and crossbred Holstein x Montbeliarde cows, Hazel et al. (2013) report no difference between the genetic groups with a fat + protein yield of about 2.06 Kg/day. With the results of our study, we can show that crossbred cows with 50% or more Holstein genes can be as competitive as the Holstein cows for fat + protein yield. This is an important economic factor for the different milk payment programs.

Conclusion

Simmental and R1 – Simmental cows deal better with a negative energy balance after calving. These genetic groups lose less body weight and backfat thickness than the other genetic groups with greater Holstein proportions. Cows with at least 50% Holstein genes yield greater amounts of milk, which causes higher NEFA and BHB values indicating a stronger body tissue mobilization in order to meet the energy requirements.

References

Adewuyi, A.A., E. Gruysi, and F.J.C.M.V. Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet. Q. 27:117–126. doi:10.1080/01652176.2005.9695192.

Auldist, M.J., M.F.S. Pyman, C. Grainger, and K.L. Macmillan. 2007. Comparative Reproductive Performance and Early Lactation Productivity of Jersey × Holstein Cows in Predominantly Holstein Herds in a Pasture-Based Dairying System. J. Dairy Sci. 90:4856–4862. doi:10.3168/jds.2006-869.

Barletta, R. V., M. Maturana Filho, P.D. Carvalho, T.A. Del Valle, A.S. Netto, F.P. Rennó, R.D. Mingoti, J.R. Gandra, G.B. Mourão, P.M. Fricke, R. Sartori, E.H. Madureira, and M.C. Wiltbank. 2017. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. Theriogenology 104:30–36. doi:10.1016/j.theriogenology.2017.07.030.

Bicalho, M.L.S., E.C. Marques, R.O. Gilbert, R.C. Bicalho. 2017. The association of plasma glucose, BHBA, and NEFA with postpartum uterine diseases, fertility, and milk production of Holstein dairy cows. Theriogenology 88: 270–282. http://dx.doi.org/10.1016/j.theriogenology.2016.09.036

Blum, J.W., P. Kunz, H. Leuenberger, K. Gautschi, and M. Keller. 1983. Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. Anim. Prod. 36:93–104. doi:10.1017/S0003356100039982.

Brähmig, J. 2011. Einfluss der Wechselkreuzung von Deutschen Holsteins und Deutschem Fleckvieh auf Milchleistung und Milchqualität in einem automatischen Melksystem 162.

Buckley, F., N. Lopez-Villalobos, and B.J. Heins. 2014. Crossbreeding : implications for dairy cow fertility and survival. Animal 8:122–133. doi:10.1017/S1751731114000901.

Clasen, J.B., W.F. Fikse, M. Kargo, L. Rydhmer, E. Strandberg, and S. Østergaard. 2019. Economic consequences of dairy crossbreeding in conventional and organic herds in Sweden. J. Dairy Sci. 103: 514–528. doi:10.3168/jds.2019-16958.

Diepold, D.A. 2019. Effekte der Fleckvieh - Rückkreuzung auf Fruchtbarkeit und Gesundheit. Dissertation Thesis, Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München.

Djokovic, R., Z. Ilic, V. Kurcubic, M. Petrovic, and V. Doskovic. 2011. Functional and morphological state of the liver in Simmental dairy cows during transitional period. Rev. Med. Vet. (Toulouse). 162:574–579.

Djoković, R., V. Kurćubić, Z. Ilić, M. Cincović, M. Lalović, B. Jašović, and J. Bojkovski. 2017. Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid lactation. Adv. Diabetes Metab. 5:26–30. doi:10.13189/adm.2017.050202.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. 1989. A Body Condition Scoring Chart for Holstein Dairy Cows. Journal of Dairy Science, 72 (1): 68–78. DOI 10.3168/jds.S0022-0302(89)79081-0.

Eisemann, Joan H. 2010. Diseases: Metabolic Disorders of Ruminants. Encyclopedia of Animal Science, Second Edition, 1: 1, 290 — 293

Esposito, G., P.C. Irons, E.C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60–97. doi:10.1016/j.anireprosci.2013.11.007.

Fellipe, E.W., I.P. de O. Gomes, and A. Thaler Neto. 2017. Comparação de vacas mestiças Holandês x Jersey com vacas puras quanto à eficiência produtiva e reprodutiva. Arch. Vet. Sci. 22:48–54. doi:10.5380/avs.v15i3.16979.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows.. J. Dairy Sci. 77:2695–703. doi:10.3168/jds.S0022-0302(94)77212-X.

Ferris, C.P., P.J. Purcell, A.W. Gordon, T. Larsen, and M. Vestergaard. 2018. Performance of Holstein and Swedish-Red × Jersey/Holstein crossbred dairy cows within low- and medium-concentrate grassland-based systems. J. Dairy Sci. 101:7258–7273. doi:10.3168/jds.2017-14107.

GAMA, L. T.. Melhoramento Genético Animal. Escolar Editora. 2002.

González, F.H.D.; Silva, S.C. Introdução à bioquimica clínica veterinária. 2.ed. Porto Alegre: UFGRS, 2006.

González, F.D., R. Muiño, V. Pereira, R. Campos, and J.L. Benedito. 2011. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. J. Vet. Sci. 12:251–255. doi:10.4142/jvs.2011.12.3.251.

Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Commun. 30:749–774. doi:10.1007/s11259-006-3320-6.

Hazel, a R., B.J. Heins, a J. Seykora, and L.B. Hansen. 2013. Montbéliarde-sired crossbreds compared with pure Holsteins for dry matter intake, production, and body traits during the first 150 days of first lactation.. J. Dairy Sci. 96:1915–23. doi:10.3168/jds.2012-5667.

Hazel, A.R., B.J. Heins, A.J. Seykora, and L.B. Hansen. 2014. Production, fertility, survival, and body measurements of Montbéliarde-sired crossbreds compared with pure Holsteins during their first 5 lactations. J. Dairy Sci. 1–14. doi:10.3168/jds.2013-7063.

Hazel, A.R., B.J. Heins, and L.B. Hansen. 2017. Fertility, survival, and conformation of Montbéliarde × Holstein and Viking Red × Holstein crossbred cows compared with pure Holstein cows during first lactation in 8 commercial dairy herds. J. Dairy Sci. 100:9447–9458. https://doi.org/10.3168/jds.2017-12824.

Kara, N., and M. KOYUNCU. 2018. A research on longevity, culling reasons and milk yield traits in between Holstein and Simmental cows. Mediterr. Agric. Sci. 31:325–328. doi:10.29136/mediterranean.443409.

Kargo, M., P. Madsen, and E. Norberg. 2012. Short communication: Is crossbreeding only beneficial in herds with low management level? J. Dairy Sci. 95 :925–928. doi:10.3168/jds.2011-4707.

Kaufman, E.I., S.J. LeBlanc, B.W. McBride, T.F. Duffield, and T.J. DeVries. 2016. Association of rumination time with subclinical ketosis in transition dairy cows. J. Dairy Sci. 99:5604–5618. doi:10.3168/jds.2015-10509.

Knob, D.A., D.R.M. Alessio, A.T. Neto, F.D. Mozzaquatro, A. Thaler Neto, and F.D. Mozzaquatro. 2018. Growth , productive performance , and udder health of crossbred Holstein x Simmental cows and purebred Holstein cows. Semin. Ciências Agrárias 2597–2606. doi:10.5433/1679-0359.2018v39n6p2597.

Knob, D.A., D.R.M. Alessio, A. Thaler Neto, and F.D. Mozzaquatro. 2016. Reproductive performance and survival of Holstein and Holstein x— Simmental crossbred cows. Trop. Anim. Health Prod. 48:1409–1413. doi:10.1007/s11250-016-1103-9.

Knob, D.A., A.M. Scholz, D.R.M. Alessio, B.P.B. Mendes, L. Perazzoli, R. Kappes, and A. Thaler Neto. 2019. Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows. Trop. Anim. Health Prod.. doi:10.1007/s11250-019-02174-9.

De Koster, J.D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. - Food Anim. Pract. 29:299–322. doi:10.1016/j.cvfa.2013.04.002.

Laguna, J.G., M.S. Cardoso, J.A. Lima, R.B. Reis, A.U. Carvalho, H.M. Saturnino, and S.M.R. Teixeira. 2017. Expression of hepatic genes related to energy metabolism during the transition period of Holstein and F1 Holstein-Gir cows. J. Dairy Sci. 100:9861–9870. https://doi.org/10.3168/jds.2016-12459

Kuhla, B., C.C. Metges, H.M. Hammon. 2016. Endogenous and dietary lipids influencing feed intake and energy metabolism of periparturient dairy cows. Domestic Animal Endocrinology 56: S2–S10. http://dx.doi.org/10.1016/j.domaniend.2015.12.002

Ledinek, M., L. Gruber, F. Steininger, K. Zottl, M. Royer, K. Krimberger, M. Mayerhofer, C. Egger-Danner, and B. Fuerst-Waltl. 2018. Analysis of lactating cows in commercial Austrian dairy farms: diet composition, and influence of genotype, parity and stage of lactation on nutrient intake, body weight and body condition score. Ital. J. Anim. Sci. 18:202–214. doi:10.1080/1828051X.2018.1504632.

Li, J., Liang Li, DongMing Guo, SuYun Li, YuXin Zeng, ChuHao Liu, Ru Fu, MengQian Huang, Wei Xie. 2020. Triglyceride metabolism and angiopoietin-like proteins in lipoprotein lipase Regulation. (Review). Clinica Chimica Acta 503: 19–34. https://doi.org/10.1016/j.cca.2019.12.029

Malchiodi, F., M. Penasa, F. Tiezzi, and G. Bittante. 2011. Milk yield traits, Somatic cell score, milking time and age at calving of pure Holstein versus crossbred cows. Agric. Conspec. Sci. 76:259–261.

Mann, S., D. V Nydam, A.L. Lock, T.R. Overton, and J.A.A. McArt. 2016a. Short communication: Association of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids. J. Dairy Sci. 99:5851–5857. doi:10.3168/jds.2016-10920.

Mann, S., D.V. Nydam, A. Abuelo, F.A. Leal Yepes, T.R. Overton, and J.J. Wakshlag (2016b): Insulin signaling, inflammation, and lipolysis in subcutaneous adipose tissue of transition dairy cows either overfed energy during the prepartum period or fed a controlled-energy diet. J. Dairy Sci. 99:6737–6752. http://dx.doi.org/10.3168/jds.2016-10969

Mendonça, L.G.D., C.C. Abade, E.M. da Silva, N.B. Litherland, L.B. Hansen, W.P. Hansen, and R.C. Chebel. 2014. Comparison of peripartum metabolic status and postpartum health of Holstein and Montbéliarde-sired crossbred dairy cows.. J. Dairy Sci. 97:805–18. doi:10.3168/jds.2013-7159.

Nolte, O. 2019. Effekte der Rückkreuzung auf Fleckvieh aus einer Kreuzungsherde mit Deutschen Holstein-Genanteilen auf Milchleistung und Milchqualität. Dissertation Thesis, Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München.

Ospina, P.A., D.V. Nydam, T. Stokol, and T.R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546–554. doi:10.3168/jds.2009-2277.

Pelizza, A., A. Hauser, T.C. Mendes, C.A. Mattiello, D.A. Knob, L.L. Cardozo, R. Parizotto Filho, I.P.O. Gomes, D.R.M. Alessio, M. Camera, and A. Thaler Neto. 2019. Perfil metabólico de vacas Holandês e mestiças Holandês x Jersey no periparto. Arq. Bras. Med. Veterinária e Zootec. 71:741–751. doi:10.1590/1678-4162-10098.

Pires, J.A.A., C. Delavaud, Y. Faulconnier, D. Pomiès, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. J. Dairy Sci. 96:6423–6439. doi:10.3168/jds.2013-6801.

Prendiville, R., K.M. Pierce, and F. Buckley. 2009. An evaluation of production efficiencies among lactating Holstein-Friesian, Jersey, and Jersey × Holstein-Friesian cows at pasture. J. Dairy Sci. 92 :6176–6185. doi:10.3168/jds.2009-2292.

Prendiville, R., K.M. Pierce, and F. Buckley. 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 cross with regard to milk yield, somatic cell score, mastitis, and milking characteristics under grazing conditions. J. Dairy Sci. 93 :2741–2750. doi: 10.3168/jds.2009-2791

Šamanc, H., D. Gvozdić, N. Fratrić, D. Kirovski, R. Djoković, Ž. Sladojević, and M. Cincović. 2015. Body condition score loss, hepatic lipidosis and selected blood metabolites in holstein cows during transition period. Anim. Sci. Pap. Reports 33:35–47.

Schröder, U.J., and R. Staufenbiel. 2006. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J. Dairy Sci. 89:1–14. doi:10.3168/jds.S0022-0302(06)72064-1.

Schwaiger, V. 2008. Kreuzungszucht Beim Milchvieh - Ein Ausblick Vor Und Nachteile Der Kreuzungszucht Zwishen Deutchem Fleckvieh Und Deutschen Holstein.

Schweizer, H., M. Bernau, and A.M. Scholz. 2018. Comparison of performance and health parameters of German and New Zealand dairy cows. Page in Proceedings of the World Congress on Genetics Applied to Livestock Production. vol. Electronic Poster Session - Biology & Species - Bovine (dairy) 2, p. 669, 2018.

Sgorlon, S., M. Fanzago, M. Sandri, B. Gaspardo, and B. Stefanon. 2015. Association of index of welfare and metabolism with the genetic merit of holstein and simmental cows after the peak of lactation. Ital. J. Anim. Sci. 14:368–373. doi:10.4081/ijas.2015.3841.

Shonka-Martin, B.N.N., A.R.R. Hazel, B.J.J. Heins, and L.B.B. Hansen. 2019. Three-breed rotational crossbreds of Montbéliarde , Viking Red , and Holstein compared with Holstein cows for dry matter intake, body traits, and production. J. Dairy Sci. 102:1–12. doi:10.3168/jds.2018-15318.

Urdl, M., L. Gruber, W. Obritzhauser, and A. Schauer. 2015. Metabolic parameters and their relationship to energy balance in multiparous Simmental, Brown Swiss and Holstein cows in the periparturient period as influenced by energy supply pre- and post-calving. J. Anim. Physiol. Anim. Nutr. (Berl). 99:174–189. doi:10.1111/jpn.12178.

VanRaden, P.M. and A.H. Sanders. 2003. Economic Merit of Crossbred and Purebred US Dairy Cattle. J. Dairy Sci. 86:1036–1044.

Veerkamp, R.F., B. Beerda, and T. Van Der Lende. 2003. Effects of genetic selection for milk yield on energy balance, levels of hormones, and metabolites in lactating cattle, and possible links to reduced fertility. Livest. Prod. Sci. 83:257–275. doi:10.1016/S0301-6226(03)00108-8.

Weber, C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S.P. Singh, R.M. Bruckmaier, F. Becker, W. Kanitz, and H.M. Hammon. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96:165–180. doi:10.3168/jds.2012-5574.

Yan, T., C.S. Mayne, T.W.J. Keady, and R.E. Agnew. 2006. Effects of dairy cow genotype with two planes of nutrition on energy partitioning between milk and body tissue. J. Dairy Sci. 89:1031–1042. doi:10.3168/jds.S0022-0302(06)72170-1.

Youssef, M., and M. El-Ashker. 2017. Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period. Trop. Anim. Health Prod. 49:239–244. doi:10.1007/s11250-016-1211-6.

Table 1. Least square means ± standard errors (SEM) for body condition score (BCS), backfat thickness (BFT), beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, body weight, and lameness score (LS) of Holstein, R1-Holstein, F1-Crossbred, R1-Simmental and Simmental cows.

Variable	Genetic Group*				
	Holstein	R1-Hol	F1 Hol-Sim	R1-Sim	Simmental
BCS	3.29±0.11c	3.41±0.08c	3.54±0.07c	3.87±0.05b	4.22±0.06a
BFT (cm)	1.54±0.14ab	1.47±0.09b	1.59±0.08ab	1.78±0.06a	1.71±0.08ab
BHB (mmol/l)	0.95±0.05	0.94±0.03	0.94±0.03	0.93±0.02	0.85±0.03
NEFA (mmol/l)	0.24±0.02ab	0.22±0.02ab	0.25±0.01a	0.21±0.008b	0.18±0.01c
Glucose (mg/dL)	59.6±1.0	59.1±0.8	58.8±0.6	60.3±0.4	60.1±0.6
Body Weight (Kg)	667±18bc	675±13bc	672±11c	703±7ab	715±10a
LS	1.67±0.15	1.47±0.1	1.45±0.09	1.38±0.6	1.31±0.8

*Genetic groups according to their theoretic proportion of Holstein and Simmental genes: Holstein - ≥ 87.5% Holstein, R1-Holstein - between 62.5 and 87.4% Holstein, F1-Crossbreds - between 37.5 and 62.4 Holstein, R1-Simmental - between 62.5 and 87.4% Simmental and Simmental - ≥87.5 % Simmental.

Table 2. Means adjusted to the model ± standard error (SEM) for variables related to milk yield and composition and somatic cell score (SCS) of Holstein, R1-Holstein, F1-Crossbred, R1-Simmental and Simmental cows.

Variable	Genetic Group*				
	Holstein	R1-Hol	F1-Crossbred	R1-Sim	Simmental
Milk yield (liters)	39.6±1.7a	37.2±1.2ab	38.4±1.05a	35.4±0.7bc	33.5±0.9c
ECM (Kg)**	43.06±1.92a	40.74±1.52a	41.03±1.22a	36.93±0.76b	36.03±1.11b
Fat (%)	3.81±0.18	4.08±0.14	3.94±0.11	3.86±0.07	3.90±0.10
Protein (%)	3.28±0.07	3.39±0.05	3.28±0.04	3.31±0.03	3.34±0.04
Fat + Protein (Kg)	2.84±0.13a	2.73±0.10a	2.71±0.08a	2.44±0.05b	2.40±0.07b
Concentrate intake (kg/day)	5.47±0.21	5.55±0.15	5.61±0.13	5.55±0.08	5.47±0.12
SCS	2.18±0.27	2.39±0.19	2.33±0.17	2.53±0.11	2.22±0.15

*Genetic groups according to their theoretic proportion of Holstein and Simmental genes: Holstein - ≥ 87.5% Holstein, R1-Holstein - between 62.5 and 87.4% Holstein, F1-Crossbreds - between 37.5 and 62.4 Holstein, R1-Simmental - between 62.5 and 87.4% Simmental and Simmental - ≥87.5 % Simmental.

**ECM: Energy corrected milk yield

Figure 1. Metabolic pathways of NEFA in the liver

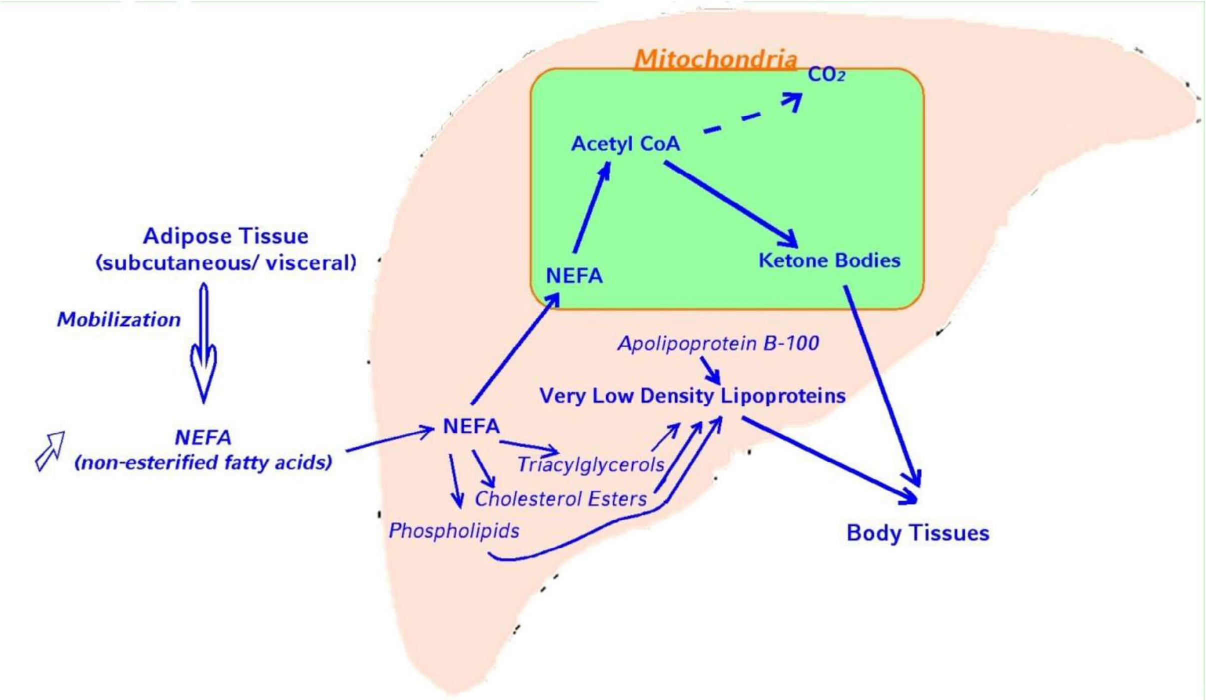


Figure 2. Weekly means of body condition score (BCS) (A), backfat thickness (BFT) (B), milk yield (C) and body weight (D) between three weeks before calving and fourteen weeks after calving for purebred Holstein, Simmental cows and their crosses.

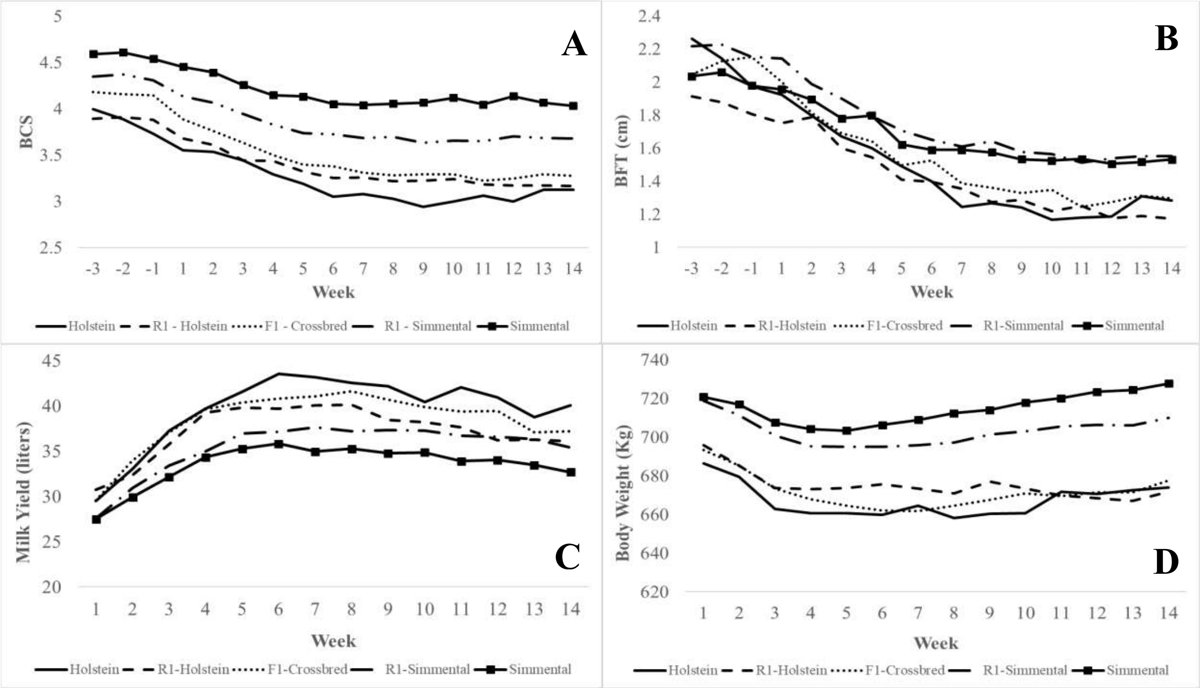
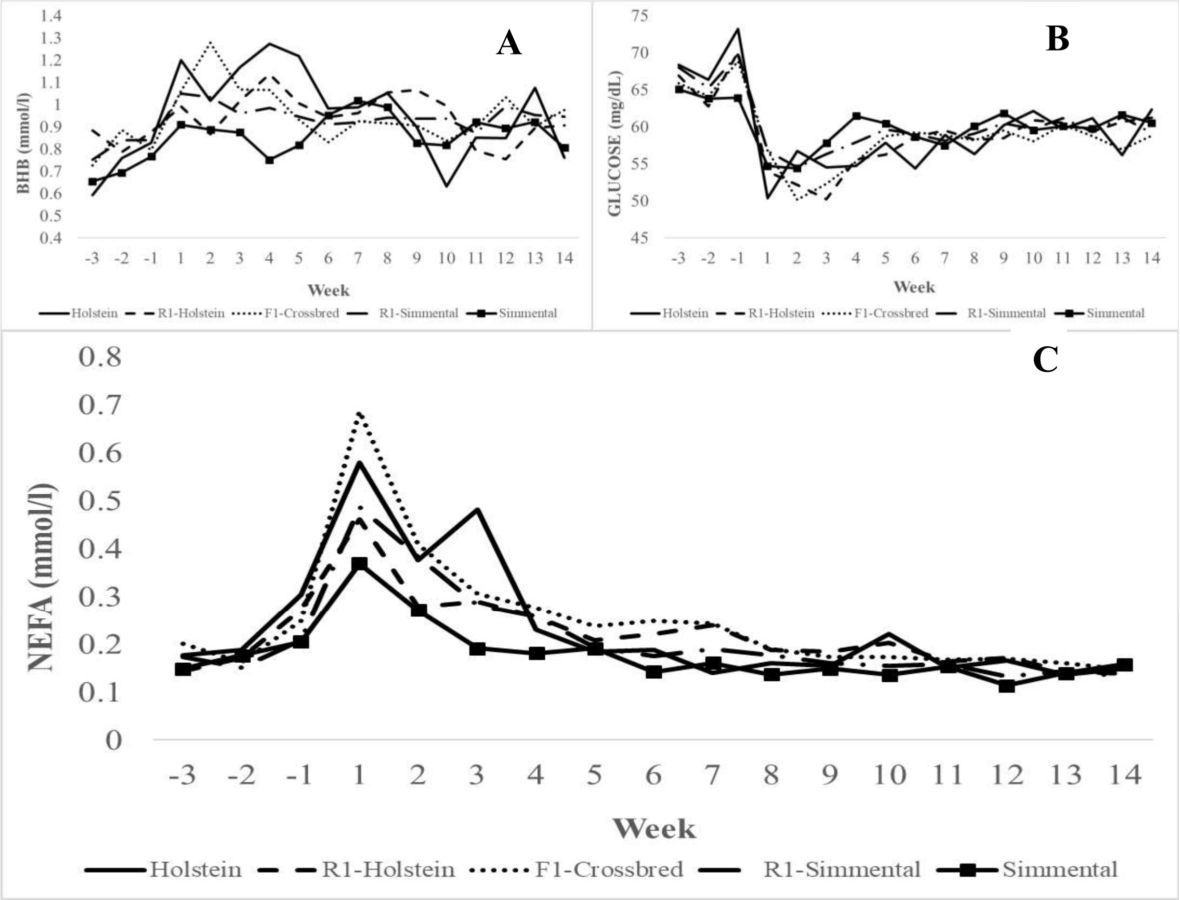


Figure 3. Weekly means of beta-hydroxybutyrate (BHB) (A), glucose (B) and nonesterified fatty acids (NEFA) (C) between three weeks before calving until fourteen weeks after calving for purebred Holstein, Simmental cows and their crosses.



FINAL CONSIDERATIONS

The results of our study show that crossbred Holstein x Simmental cows are an alternative to use in high productive systems. In comparison to Holstein cows the crossbred ones shown to reach the same feed efficiency for milk yield and composition. They produce similar amounts of milk with better reproductive performance. As higher the Simmental genes proportion the better the cows deal with the negative energy balance after calving. These genetic groups lose less body condition score and backfat thickness. Cows with at least 50% Holstein genes have the higher milk yield which impact on higher NEFA and BHB values, as indicators of higher body tissue mobilization to attempt the energy requirements.

All the parameters evaluated in our research have an economic impact in a dairy productive system. Better reproductive performance impact on higher productive life of the cow. By reaching the same milk yield and feed efficiency, which is one of the highest costs for a dairy farm, shown that the crossbred cows are as competitive as the Holstein cows. One of the biggest questions on a crossbreeding system is how to manage the generation after the F1, which always is the one with the best performance because of the maximum heterosis. With our research we shown that also the generations after the F1 can be competitive, despite the slight lower milk yield for example, this one’s presented higher milk solids content, better reproductive performance and some evidences that they can handle better the transition period, which is always a critical period for the high yielding cows.

Besides the economic impact on the dairy production system, we have to take into consideration the fact that as a result of the crossbreeding program with a dual purpose breed, like the Simmental, the male calves and the culling cows have a higer economic value as the Holstein ones. Even though we do not evaluate that, by talking to the farmers they mentioned the additional income as a positive effect of the crosbreeding program. On the other hand, the negative impact of the use of a crossbreeding programs is the herd heterogeneity due to the different blood levels of each cow. It can be a challenge by planning the diet offered to the cows, for example.