Comparison of Holstein and Montbéliarde-sired Crossbred Dairy Cows during the Transition Period

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Luís Gustavo Delamanha Mendonça

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Advisor: Ricardo C. Chebel
Co-advisor: Sandra M. Godden

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Dedication

This thesis is dedicated to my family that always supported me.

Table of Contents

List of Tables

List of Figures

CHAPTER 1: Literature Review

Crossbreeding and Inbreeding

Crossbreeding is used with the intent of improving phenotypic characteristics (e.g. health, fertility, longevity, milk components) through the benefits of heterosis. Heterosis resulting from crossbreeding eliminates chances of inbreeding depression within breed. Inbreeding is the probability of 2 alleles at any locus to be identical by descent (Falconer and Mackay, 1996) and is more likely to occur in purebred individuals because purebred individuals have greater relationship among them. Inbreeding depression is mostly characterized by reduced productivity (Charlesworth and Willis, 2009). Young and Seykora (1996) demonstrated an increased relationship among individuals of Holstein (**HO**) breed, the most predominant breed in the United States dairy herd (NAHMS, 2007), which could result in increased inbreeding and impact negatively the United States' dairy industry. Therefore, crossbreeding of dairy breeds could be a solution to increase the genetic diversity in the United States' dairy herd, reduce relationships among dairy animals, and avoid inbreeding depression.

Since the first decades of the 1900s scientists have been comparing productive parameters of crossbred and purebred dairy cows in an attempt to characterize the advantages of heterosis (Cole and Johansson, 1948; Wriedt, 1930). Anecdotal evidence suggests that crossbred cows are less likely to develop diseases, but scientifically-based research evaluating the association between crossbreeding and incidence of periparturient diseases is lacking. Furthermore, in many research trials the comparison of reproductive, health, and longevity parameters between purebred and crossbred cows is confounded by

differences in milk yield and body weight between breeds. Thus, it is a challenge for researchers to determine if the improvements in performance observed in crossbreeding trials are indeed a result of heterosis.

Transition Period of Dairy Cows

The transition period of lactating dairy cows, three weeks prepartum to three weeks postpartum, is marked by physiological and metabolic changes that affect immune function and increase susceptibility for postpartum diseases (Goff and Horst; 1997). Dairy cows that developed metritis postpartum had reduced dry matter intake (**DMI**) prepartum (Hammon et al., 2006; Huzzey et al., 2007) and compromised neutrophil function on the day of calving (Hammon et al., 2006) compared with healthy cows. Insufficient DMI to meet energy demands peripartum results in negative energy balance and increased plasma non-esterified fatty acids (**NEFA**) concentrations, an indicator of fat mobilization (Grummer et al., 2004). On the other hand, the capacity of complete fatty acid oxidation in the liver can be indirectly measured by plasma beta-hydroxybutyrate (**BHBA**), which is a product of beta-oxidation of fatty acids in the liver (Palmquist, 1972). Increasing concentrations of these metabolites (NEFA and BHBA) during the peripartum period are associated with increased incidence of diseases peripartum and compromised productive and reproductive performances of dairy cows (LeBlanc, 2010).

Periparturient Immunity and Uncoupling of the Somatotropic Axis

As already discussed, highly inbred populations have increased homozygosity and inbreeding depression, characterized by reduced performance. O'Brien and Evermann (1988) suggested that immune responses may be impaired in populations with elevated inbreeding as a result of decreased genetic variability. Inbred wild bird populations have compromised cell mediated (Reid et al., 2003) and innate (Townsend et al., 2010) immune responses.

The comparison of components of the immune system between purebred and crossbred populations, which have a greater genetic variation, is a valid approach to examine the association between heterosis and immune responses in dairy cattle. Cartwright et al. (2011) demonstrated that purebred HO calves (2 to 6 mo of age) had reduced antibody-mediated immunity compared with Norwegian Red-HO crossbred calves. Research evaluating the association between heterosis and immune responses of periparturient dairy cows, however, is lacking.

Innate immunity plays a critical role on the health of dairy cows during the transition period, three weeks pre- to three weeks post-partum (Cai et al., 1994). During the transition period the functionality and activity of polymorphonuclear neutrophilic leukocyte (**PMNL**) are of particular importance because they are involved with the expulsion of placenta following parturition (Kimura et al., 2002) and because PMNL are the first line of defense of the uterus against invading pathogens (Singh et al., 2008). The functionality and activity of PMNL of dairy cows is influenced by the negative energy balance during the periparturient period (Hammon et al., 2006) and high NEFA

concentrations are associated with impaired innate (Hammon et al., 2006) and adaptive (Lacetera et al., 2005) immune function.

The continued increase in milk yield of periparturient dairy cows even during negative energy balance is a consequence of the uncoupling of the somatotropic axis, characterized by decreased growth hormone receptor (**GHR**) expression in the liver, reduced insulin-like growth facor (**IGF**)-1 synthesis by the liver, reduced negative feedback of IGF-1 on growth hormone (**GH**) secretion, and, consequently, increased GH and reduced IGF-1 concentrations (Lucy et al., 2009). Increasing concentration of GH during periods of severe negative energy balance exacerbates lipolysis and production of NEFA destined to *de novo* fat synthesis in the mammary gland (Etherton and Bauman, 1998). The degree of uncoupling of the somatotropic axis during the periparturient period seems to be dependent on genetic strains and appears to be exacerbated in HO cows of North American genetics (Lucy et al., 2009; Grala et al., 2011).

Objectives

The objectives of this thesis were to determine whether crossbreeding improves innate immune responses, health and ovarian function during the transition period of dairy cows. Therefore, the specific objectives of the studies described herein are:

Chapter 2: Compare postpartum health, metabolic parameters peripartum, and uterine involution and ovarian function postpartum of purebred HO and Montbéliarde (**MO**)-sired crossbred cows.

Chapter 3: Compare innate immune responses and the degree of uncoupling of the somatotropic axis of purebred HO and MO-sired crossbred cows during the transition period.

Hypotheses

The hypotheses of the study described in chapter 2 were that purebred HO cows would have greater incidence of health disorders and delayed uterine involution compared with MO-sired crossbred cows.

The hypotheses of the study described in chapter 3 were that MO-sired crossbred cows would have improved innate immune responses during the transition period compared with HO cows; and, that the degree of uncoupling of the somatotropic axis of MO-sired crossbred cows would be reduced compared with HO cows.

CHAPTER 2: Comparison of Peripartum Metabolic Status and Postpartum Health of Holstein and Montbéliarde-Sired Crossbred Dairy Cows

Chapter Summary

Objectives of the current study were to compare the metabolic status peripartum and health and ovarian function postpartum of Holstein (**HO**) and Montbéliarde (**MO**) sired crossbred cows. Cows (52 HO and 52 crossbred) were enrolled in the study 45 d before expected calving date and were followed until 90 DIM (study d 0 = calving). Cows had body weight and body condition score recorded on study d -45, - 14, 0-1, 28, and 56. Dry matter intake was calculated for a sub-group of cows (25 HO and 38 crossbred) from 6 weeks before to 6 weeks after calving. Blood was sampled weekly from study d -14 to 56 for determination of glucose, NEFA, and BHBA concentrations; from study d -7 to 21 for determination of haptoglobin concentration; and, from study d 14 to 56 for determination of progesterone concentration. Cows were examined at calving and on study d 4, 7, 10, and 14 for diagnosis of postparturient diseases, on study d 24 for diagnosis of endometritis, and on study d 42 for diagnosis of subclinical endometritis. Uterus and ovaries were examined by ultrasonography every 3 d from study d 14 to 41. Milk yield and composition were measured monthly and yield of milk, fat, protein, and energy corrected milk were recorded for the first 90 DIM. Holstein and crossbred cows had similar BW throughout the study, but HO cows had reduced BCS compared with crossbred cows. Even though DMI from 6 weeks before to 6 weeks after calving tended to be greater for HO cows (16.8 ± 0.7 vs 15.3 ± 0.5 kg/d), HO cows had more pronounced decline in DMI expressed in percentage of BW from study d -15 to 0. Yield

of ECM and NEFA and BHBA concentrations were not different between breeds. No differences were observed in incidence of retained fetal membranes, metritis, and subclinical endometritis, but HO cows tended to be more likely to have pyrexia from study d 0 to 15 (50.0 vs 31.4%) and greater incidence of clinical endometritis (44.2 vs 26.5%) than crossbred cows. Holstein cows were more likely to have at least one uterine disorder postpartum than crossbred cows (63.5 vs 36.7%). No differences between breeds were observed in involution of previously pregnant and non-pregnant uterine horns. Holstein cows had larger subordinate follicles (10.1 ± 0.4 vs 8.9 ± 0.5) and greater number of class III follicles (1.6 ± 0.1 vs 1.2 ± 0.1) than crossbred cows. Furthermore, the first corpus luteum postpartum of HO cows was diagnosed at slower rate compared with crossbred cows. Crossbred cows had improved uterine health compared with HO cows and this may have been a consequence of heterosis and less pronounced decrease in DMI during the last days of gestation.

Materials and Methods

Cows, Breeds, Enrollment Period, Dry Period

The study was conducted at the University of Minnesota's St. Paul dairy. In this study 52 purebred HO (19 nulliparous and 33 lactation $\geq$ 1) and 52 MO-sired crossbred (6 nulliparous and 46 lactation $\geq$ 1) cows were used. Cows that had their first calving during the study will be referred to as primiparous and cows that had their second or more calving during the study will be referred to as multiparous.

Cows were enrolled in the study 45 d before expected calving date and were followed until 90 days in milk (**DIM**) (study d 0 = calving). The enrollment period was from October 2009 – January 2010 (first season of parturition) and during the month of September 2010 (second season of parturition). The mean (± SEM) and median length of the dry period were 51.8 (± 1.9) and 49 d, respectively.

Body Weight and Body Condition Score

Data regarding body weight (**BW**) and body condition score (**BCS**) were collected on study d -45, -14, 0-1, 28, and 56. Body condition score was assessed by one person using the visual technique on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) with 0.25 increment as described by Ferguson et al. (1994).

Diets and Calculation of Dry Matter Intake

Cows were fed a total mixed ration (**TMR**) once daily and water was available *ad-libitum*. The TMR offered during the dry period of the first season of parturition consisted of wheat straw, corn silage, alfalfa hay, ground dry corn, soybean meal, and a protein mix and contained 54.3% dry matter, 13.2% crude protein, 26.0% ADF, 39.8% NDF and 1.51 Mcal/kg of NE_L. The dry cow ration for the second season of parturition consisted of wheat straw, corn silage, alfalfa hay, ground dry corn, and a protein mix, and contained 61.5% dry matter, 17.0% crude protein, 28.2% ADF, 41.9% NDF and 1.47 Mcal/kg of NE_L. The lactating cow TMR for the first season of parturition consisted corn silage, alfalfa hay, ground dry corn, soybean meal and a protein mix, and contained

55.6% dry matter, 17.8% crude protein, 18.3% ADF, 29.6% NDF and 1.75 Mcal/kg of NE_L. The lactating cow ration for the second season of parturition consisted of corn silage, alfalfa hay, ground dry corn, whole cottonseed and a protein mix, and contained 64.0% dry matter, 16.6% crude protein, 22.2% ADF, 31.6% NDF and 1.66 Mcal/kg of NE_L.

The separation between the feed bunks in the tie-stall barn allowed for calculation of daily DMI from study d - 45 to 42 , approximately from 6 weeks before to 6 weeks after calving, for a subgroup of multiparous cows (HO = 25 and crossbred = 38). Amount of feed offered and orts were measured and daily DMI was calculated based on dry matter (**DM**) content of the ration. For purposes of statistical analysis daily DMI and weekly averages of DMI were used.

Productive Parameters

Cows were milked twice daily. The dairy was enrolled in a regular milk recording Dairy Herd Improvement association program. Data regarding production, milk composition, and somatic cell count were collected monthly in the first 90 DIM. First test was limited to tests occurring 5-34 DIM, second test was limited to tests occurring 35-64 DIM, and third test was limited to tests occurring 65-94 DIM. Energy corrected milk (**ECM**) was calculated according to the formula (Tyrrell and Reid, 1965):

ECM (kg) = (0.327 x milk yield) + (12.95 x fat yield) + (7.2 x protein yield).

Physical Exams and Uterine Health

At enrollment, cows underwent a physical examination to determine eligibility for the study and cows diagnosed with health disorders or extremely lame were deemed not eligible to be enrolled in the study. Throughout the study, cows were monitored daily and cows that presented decreased appetite, depression, or dehydration were examined by a veterinarian.

Physical exams were performed in all cows on study d 0, 4, 7, 10, and 14. The parameters evaluated were presence of retained fetal membranes (**RFM**), uterine discharge and attitude score. Rectal temperature was measured on a daily basis from study d 0 to 15. Retained fetal membrane was characterized as the failure of detachment of the fetal membranes within 12 h postpartum. Cows with rectal temperature > 39.4 ^{0}C were considered to have pyrexia. Puerperal metritis was characterized by the presence of fever and a red/brown watery fetid uterine discharge.

Cows were examined by vaginoscopy on study d 24 for determination of endometritis. The visual inspection of the external os of the cervix was performed with the aid of a flashlight and cows in which pus composed > 50% of the exudate were considered to have clinical endometritis. Subclinical endometritis was diagnosed on study d 42 ± 3 by cytological examination of endometrial samples collected by the cytobrush technique. The cytobrush technique consisted of using a stainless steel instrument to introduce the cytobrush device (Cytobrush Plus®, Cooper Surgical, Inc) in the uterus through the vagina and cervix. While palpating the uterus per rectum, the instrument was guided to the previously pregnant uterine horn where the cytobrush device was exposed and slowly rotated for the collection of an endometrial sample. The cytobrush was rolled

onto a clean glass slide, which was stained with modified Wright-Giemsa stain (Protocol-Hema3, Biochemical Sciences, Swedesboro, New Jersey, USA). Cytological evaluation of the slides at 400X magnification was done three times by one examiner who was blinded to the breed of the cow from which the sample was collected. The examiner counted a minimum of 100 cells at each reading and subclinical endometritis was determined by > 10% of total cells classified as PMNL (Kasimanickam et al., 2004).

Blood Sample Collection and Analyses

Blood was sampled weekly from study d -14 to 56. Blood samples were collected from the coccygeal vein/artery into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed on ice until centrifugation for plasma separation (3,000 rpm for 15 min at 4 ^{0}C). Plasma was aliquoted into microcentrifuge tubes and stored at -32 ^{0}C until analysis.

Plasma NEFA was quantified using a colorimetric assay using a commercial kit (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009). Beta-hydroxybutyrate concentration was determined enzymatically using a commercially available kit (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009). Glucose concentration was determined by enzymatic reaction using a commercially available kit (Stanbio Laboratory, Boerne, TX). Haptoglobin concentrations were measured by a colorimetric procedure as described by Hulbert et al. (2011).

A plate reader (Spectramax 340; Molecular Devices, Sunnyvale, CA) was used to measure the absorbance for the colorimetric and enzymatic assays. Control serum

(Randox Control Sera, Antrim, UK) was used for the NEFA, BHBA and glucose assays. The intra-assay coefficients of variation for the assays were: 5.5% for NEFA, 8.8% for BHBA, 4.0% for glucose, and 3.9% for haptoglobin. The inter-assay coefficients of variation for the assays were: 10.1% for NEFA, 10.8% for BHBA, 5.1% for glucose, and 9.1% for haptoglobin, respectively.

Progesterone concentration was determined by a solid-phase radioimmunoassay kit (Coat-a-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) and the intra- and inter-assay coefficients of variation were 9.3% and 12.3%, respectively. Cows were considered to have had a short luteal phase when progesterone concentration < 1 ng/mL in the sample immediately following the sample in which progesterone concentration $\geq$ 1 ng/mL.

Ultrasound Evaluation of Uterus and Ovaries

In a subgroup of cows [HO = 45 (19 primiparous and 26 multiparous) and crossbred = 41 (6 primiparous and 35 multiparous)], ultrasound examinations of the genital tract was performed at 3 d intervals from study d 14 to 42 using an ALOKA SSD 500 ultrasound (Aloka, Tokyo, Japan) equipped with a 5-MHz rectal probe to determine uterine involution and evaluate ovarian structures. Uterine involution was determined by monitoring changes in diameter of previously pregnant and non-pregnant uterine horns. The previously pregnant uterine horn was identified at one of the clinical examinations performed from study d 0 to 14 by rectal palpation as being the horn with bigger diameter. The uterine horns were scanned immediately anterior to the external uterine

bifurcation. Cross-sectional diameters of the uterine horns were recorded, and standardized measure of estimated volume was calculated by the formula:

$$V = \{4/3 \times \pi \times [(Dv/2 \times Dh/2)/2]^3\};$$

where Dv and Dh are the vertical and horizontal diameters, respectively, of the uterine horn.

Ovaries were scanned to determine number and size of follicles greater than 5 mm in diameter and volume of corpus luteum (**CL**). Follicles were classified as class II (5 to 9 mm) and class III (≥ 10 mm) and CL volume was calculated by the following formula:

$$\sum(\{4/3 \times \pi \times [(d_a/2 + d_b/2)/2]^3\} - \{4/3 \times \pi \times [(c_a/2 + c_b/2)/2]^3\});$$

where $d_{a,b}$ and $c_{a,b}$ are orthogonal luteal and cavity dimensions, respectively, for the n^{th} CL.

Statistical Analysis

The study was prospective observational. A sample size of 50 experimental units per treatment (HO vs MO-sired crossbred) was expected to provide enough replicates to detect statistical significance with a 15% unit difference in incidence of metritis when incidence of metritis ranges from 10 to 30% ($\alpha = 0.05$; $\beta = 0.20$; one-tailed test). In addition, a sample size of 50 experimental units per treatment (HO vs MO-sired crossbred) was expected to provide enough replicates to detect statistical significance with a 0.115 mmol/L difference in concentration of NEFA when NEFA concentration ranges from 0.020 to 1.500 mmol/L ($\alpha = 0.05$; $\beta = 0.20$; one-tailed test). Furthermore, a sample size of 50 experimental units per treatment (HO vs MO-sired crossbred) was

expected to provide enough replicates to detect statistical significance with a 0.210 mmol/L difference in concentration of BHBA when BHBA concentration ranges from 0.030 to 1.500 mmol/L ($\alpha = 0.05$; $\beta = 0.20$; one-tailed test).

Data collected during the study was entered and organized in an Excel spreadsheet (Microsoft Corporation, Redmond, WA) and analyzed statistically by using SAS, version 9.2 (SAS/STAT®, SAS Inst. Inc., Cary, NC).

Body weight, BCS, DMI, milk data, blood metabolites data, uterine horn involution, and number and size of follicles were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. Unstructured, compound symmetry, and autoregressive(1) covariance structures were tested and the covariance structure used was chosen based on Akaike's Information Criterion (AIC). Dichotomous outcomes were analyzed by logistic regression using the LOGISTIC procedure of SAS. Models used for the statistical analysis included breed (HO vs MO-sired crossbred), parity (primiparous vs multiparous), season of parturition (first vs second), and the interactions between breed and parity. For analysis of repeated measurements study day and the interaction between breed and study day were included in the model.

The Cox regression proportional hazard model was used to evaluate the association between breed and the interval from calving to detection of first CL or detection of progesterone concentration $\geq$ 1 ng/mL using the PHREG procedure of SAS. The model included breed (HO vs MO-sired crossbred), parity (primiparous vs multiparous), season of parturition (first vs second) and the interaction between breed and parity.

Statistical significance was defined as $P \leq 0.05$ and statistical tendencies as $0.05 < P \leq 0.10$.

Results

Three cows were excluded from the study for the following reasons: uterine prolapsed (n = 1 crossbred cow), cow unresponsive to treatment for milk fever (n = 1 crossbred cow), and teat injury (n = 1 HO cow).

Breed was not associated with occurrence of stillbirth ($P = 0.68$), incidence of dystocia ($P = 0.68$), and twin births ($P = 0.54$; Table 1). Multiparous cows were ($P = 0.03$) less likely to have a stillborn calf compared with primiparous cows [adjusted odds ratio (**AOR**) = 0.21; 95% confidence interval (95% **CI**) = 0.05, 0.87].

Body Weight and Body Condition Score

There was no ($P = 0.41$) association between breed and BW from study d -45 to 56 but the interaction between breed and study day was ($P = 0.04$) associated with BW (Table 1; Figure 1A). Body weight differed ($P < 0.01$) between multiparous (668.0 ± 8.9 kg) and primiparous (596.8 ± 15.4 kg) cows, but the interaction between breed and parity was not ($P = 0.17$) associated with BW. Body condition score differed ($P < 0.01$) between breeds as HO cows had lesser BCS than crossbred cows (Table 1, but the interaction between breed and study day was not ($P = 0.26$) associated with BCS (Figure 1B). Primiparous cows tended ($P = 0.07$) to have greater BCS throughout the study than

multiparous cows (3.4 ± 0.07 vs 3.2 ± 0.04), but the interaction between breed and parity was not (P = 0.51) associated with BCS.

Milk Production

There was no (P = 0.72) association between breed and average ECM yield (Table 1), but, as expected, average ECM yield differed (P < 0.01) between primiparous (36.9 ± 1.6 kg/d) and multiparous (41.7 ± 0.7 kg/d) cows. Average daily milk yield during the first 90 DIM, however, was (P = 0.05) greater for HO cows than crossbred cows (Table 1). There was a tendency (P = 0.10) for the interaction between breed and parity to be associated with daily milk yield because the average daily milk yield of primiparous HO (41.4 ± 2.8 kg/d) and crossbred (35.0 ± 3.6 kg/d) cows was (P = 0.05) different, but no differences (P = 0.71) were observed in average daily milk yield of multiparous HO (43.2 ± 1.5 kg/d) and crossbred cows (42.6 ± 1.4 kg/d).

Average daily fat yield (P = 0.81) and protein yield (P = 0.74) were not associated with breed (Table 1). The interaction between breed and study day was (P < 0.01) associated with average daily protein yield because on the first test HO cows had (P = 0.04) reduced protein yield than crossbred cows (1.15 ± 0.05 vs 1.26 ± 0.04 kg/d), but on the second (P = 0.85) and third (P = 0.15) tests no differences in protein yield were observed between breeds. Primiparous cows had (P = 0.02) greater average daily fat yield than multiparous cows (1.5 ± 0.03 vs 1.4 ± 0.07 kg/d) but parity was not (P = 0.74) associated with average daily protein yield. Season (P < 0.01) was associated with

average daily protein yield in the first 90 DIM (first season = 1.19 ± 0.02 vs second season = 1.37 ± 0.06 kg/d).

Dry Matter Intake, Glucose, NEFA and BHBA

Holstein (16.5 ± 0.7 kg/d) cows tended (P = 0.08) to have greater DMI than crossbred (14.9 ± 0.6 kg/d) cows from 6 weeks before to 6 weeks after calving, but the interaction between breed and study day was not (P = 0.29) associated with DMI (Figure 2A). Interestingly, breed (HO = 1.7 ± 0.1 vs crossbred = 1.5 ± 0.1 % of BW; P = 0.10) and the interaction between breed and study day (P = 0.10; Figure 2B) tended to be associated with daily DMI from study d -15 to 0 expressed as percentage of BW on d -15.

Plasma glucose concentration from study d -14 to 56 was not (P = 0.54) different between breeds (77.0 ± 0.8 mg/dL), but the interaction between breed and study day was (P < 0.01) associated with glucose concentration (Figure 3A). Primiparous cows had greater (P < 0.01) average glucose concentration than multiparous cows from study d -14 to 56 (79.2 ± 1.2 vs 74.8 ± 0.6 mg/dL).

Breed (0.40 ± 0.01 mmol/L; P = 0.56) and the interaction between breed and study day (P = 0.93) were not associated with plasma NEFA concentration from study d -14 to 56 (Figure 3B). Parity was (P < 0.01) associated with plasma NEFA concentration from study d -14 to 56 (primiparous = 0.43 ± 0.02 vs multiparous = 0.36 ± 0.01 mmol/L).

There was no (P = 0.95) association between breed and plasma BHBA concentration from study d -14 to 56 (Figure 3C). The interaction between breed and study day, however, was associated (P < 0.01) with plasma BHBA concentration (Figure

3C). Primiparous cows had ($P < 0.01$) reduced BHBA concentration from study d -14 to 56 compared with multiparous cows (0.66 ± 0.03 vs 0.56 ± 0.04 mmol/L).

Postpartum Health Parameters and Haptoglobin

Breed was not associated with incidence of retained fetal membranes ($P = 0.82$) or metritis ($P = 0.11$). Nonetheless, HO cows tended ($P = 0.07$) to be more likely to have pyrexia (AOR = 2.15; 95% CI = 0.95, 4.88; Table 2) and had ($P = 0.02$) greater daily rectal temperature from study d 0 to d 15 than crossbred cows (39.1 ± 0.1 vs 38.8 ± 0.1 ºF). The associations between breed and incidence of pyrexia and between breed and rectal temperature was a consequence of metritis, because when metritis was included in the models as a covariate breed was not associated with incidence of pyrexia ($P = 0.28$) or rectal temperature ($P = 0.16$). On the other hand, cows diagnosed with metritis were more likely to have pyrexia (100.0 vs 35.5%; $P = 0.02$) and had greater rectal temperature (39.3 ± 0.1 vs 38.9 ± 0.1 ºC; $P < 0.01$) than cows that were not diagnosed with metritis.

On study d 24, HO cows tended ($P = 0.07$) to be more likely to have clinical endometritis than crossbred cows (Table 2). There was no ($P = 0.12$) association between breed and incidence of subclinical endometritis on study d 42 (Table 2). Interestingly, crossbred cows diagnosed with subclinical endometritis had ($P < 0.01$) greater percentage of PMNL in the uterine cytology than HO cows (18.4 ± 2.9 vs 11.8 ± 2.5% of total cells classified as PMNL), whereas, among cows without subclinical endometritis, there was no ($P = 0.33$) difference between breeds in percentage of PMNL in the uterine cytology (3.3 ± 0.1 % of total cells classified as PMNL).

Combining the results of all uterine diseases (RFM, metritis, and clinical and subclinical endometritis), HO cows were ($P < 0.01$) more likely to have at least one uterine disease compared with crossbred cows (Table 2). On the other hand, breed ($P = 0.40$) and the interaction between breed and study day ($P = 0.85$) were not associated with haptoglobin concentration from study d -7 to 21 (Figure 4).

Uterine Involution

Volume estimates of the previously pregnant uterine horn from study d 14 to 41 were not ($P = 0.74$) different between breeds and the interaction between breed and study day was not ($P = 0.48$) associated with volume estimates of the previous pregnant horn (Figure 5). Interestingly, the interaction between breed and parity was ($P = 0.01$) associated with the mean volume estimates of the previously pregnant uterine horn because, among HO cows, parity was not associated with volume estimates of the previously pregnant uterine horn ($P = 0.55$) but, among crossbred cows, multiparous cows had ($P < 0.01$) greater uterine volume estimates than primiparous cows (13,306 ± 918.3 vs 10,178 ± 1,220.2 mm^3). The volume estimates of the previously non-pregnant uterine horn from study d 14 to 41 was not ($P = 0.15$) different between HO and crossbred cows. Similarly, the interaction between breed and study day also was not ($P = 0.45$) associated with the volume estimates of the previously non-pregnant uterine horn from study d 14 to 41 (Figure 5). The volume estimates of the previously non-pregnant uterine horn was not ($P = 0.18$) different between multiparous and primiparous cows.

Postpartum Ovarian Activity and Progesterone Concentration

The diameter of the largest follicle from study d 14 to 41 did not (P = 0.85) differ between breeds (16.8 ± 0.5 mm) and the interaction between breed and study day was not (P = 0.44) associated with diameter of the largest follicle from study d 14 to 41. On the other hand, the diameter of the subordinate follicle from study d 14 to 41 was (P = 0.03) greater for HO cows compared with crossbred cows, but the interaction between breed and study day was not (P = 0.90) associated with diameter of the subordinate follicle from study d 14 to 41 (Figure 6). Parity tended (P = 0.07) to be associated with the diameter of the subordinate follicle from study d 14 to 41 (multiparous = 10.2 ± 0.3 vs primiparous = 8.8 ± 0.7 mm).

The number of class II follicles from study d 14 to 41 was not (P = 0.17) different between breeds (Figure 7A). On the other hand, HO cows had (P < 0.01) a greater number of follicles class III than crossbred cows, but the interaction between breed and study day was not (P = 0.80) associated with number of class III follicles from study d 14 to 41 (Figure 7B). Parity, tended (P = 0.10) to be associated with the number of class III follicles from study d 14 to 41 (primiparous = 1.3 ± 0.1 vs multiparous = 1.6 ± 0.1).

From study d 14 to 56, the plasma progesterone concentration did not (P = 0.24) differ between HO and crossbred cows (Figure 8). Similarly, the interaction between breed and study day was not (P = 0.88) associated with plasma progesterone concentration from study d 14 to 56 (Figure 8). Breed was not (P = 0.36) associated with the speed at which cows were detected to have progesterone concentration $\geq$ 1 ng/mL [adjusted hazard ratio (**AHR**) = 1.24; 95% CI = 0.79, 1.93). The mean and median

intervals from calving to detection of progesterone concentration ≥ 1 ng/mL was 34.6 ± 1.3 d and 28 d, respectively. Cows with short luteal phase had (P < 0.01) reduced progesterone concentration compared with cows that did not have short luteal phase (2.43 ± 0.16 vs 1.54 ± 0.24 ng/mL). Although HO cows were (P = 0.03) more likely to have short luteal phase compared to crossbred cows (AOR = 3.00; 95% CI = 1.01, 8.00), breed was not (P = 0.81) associated with plasma progesterone when occurrence of short luteal phase was included in the model.

Among cows that ovulated from study d 14 to 41, CL volume was not (P = 0.97) different between breeds (4,861 ± 188 mm^3). According to the Cox proportional hazard ratio analysis, the speed at which the first CL was diagnosed by ultrasonography after calving was (P = 0.03) faster for crossbred cows compared with HO cows (AHR = 1.81; 95% CI = 1.07, 3.08). According to the survival analysis, however, breed was no (P = 0.47) associated with interval from calving to detection of the first CL by ultrasonography. The mean and median intervals from calving to detection of the first CL for crossbred cows were 28.4 ± 1.4 d and 27 d, respectively, and for HO cows were 30.2 ± 1.6 d and 27 d, respectively.

Discussion

In the current study, live BW was similar between HO and MO-sired crossbred cows throughout the transition period. Walsh et al. (2008) reported similar BW of HO and MO-HO crossbred cows from the week 2 to 44 of lactation. This is a relevant observation of this study because frame size is generally believed to be associated with

physical capacity for feed intake; thus, similar live BW is more likely to result in equitable comparison of DMI between breeds. On the other hand, BCS was different between breeds throughout the study supporting the idea that energy partitioning is determined, at least in part, by genetics (Veerkamp et al., 2003). The reduced BCS of HO cows compared to MO-sired crossbred cows observed in the current study and by Walsh et al. (2008) likely reflects genetic selection of the former for milk yield and angularity and genetic selection of the latter for milk production and body condition.

Even though HO cows had greater milk yield than crossbred cows during the first 90 DIM, the yield of ECM within the first 90 DIM was not different between breeds. This was mainly a consequence of the greater protein content in the milk of MO-sired crossbred cows (data not shown). The scarce literature comparing productive parameters between MO-sired crossbred and purebred HO cows is as scarce as the literature on the comparison of productive parameters among HO and other types of crossbred dairy cows under United States production systems. One study conducted by researchers from the University of Minnesota demonstrated that HO cows had greater yield of milk and greater yield of fat plus protein in comparison with MO-HO crossbred cows during the first lactation (Heins et al., 2006). On the other hand, Walsh et al. (2008) demonstrated no difference in total yield of solids-corrected milk between HO and MO-sired crossbred cows during an entire lactation. The aim of the current study was not to compare productive parameters of crossbred and HO cows, but the observation that ECM yield in the first 90 DIM was similar between breeds is an important indication that energy requirements for ECM production was similar among cows of different breeds.

Furthermore, crossbred and HO cows had similar concentrations of NEFA and BHBA and, as discussed above, similar BW throughout the study. Therefore, it is possible to speculate that, in the current study, energy balance of crossbred and HO cows may have been similar. This is an important aspect of the current study because correlations between indicators of energy balance during the transition period and health parameters (Hammon et al., 2006) and between indicators of energy balance during the transition period and resumption of ovarian cycles postpartum (Butler and Smith, 1989) have been demonstrated.

Despite the data described above that suggests that HO and crossbred cows had similar energy balance during the transition period, HO cows tended to have greater DMI than crossbred cows from 6 weeks before to 6 weeks after calving. It is important to note that the data regarding DMI comprises a sub-sample of mature cows (lactation $\geq$ 1 at the start of the study) that were randomly selected. Nonetheless, this is an intriguing finding that may suggest that, in the current study, HO cows were less feed efficient than crossbred cows during the transition period because no differences in BW and concentrations of NEFA and BHBA were observed. Even though in the current study calves were not weighed at birth, MO-HO calves have been described as being heavier at birth than HO calves (Heins et al., 2010) reducing the likelihood that more nutrients were diverted by HO cows for growth of calves compared with MO-sired crossbred cows. Another possible explanation for the tendency of HO cows to have had greater DMI is their greater milk yield compared with MO-sired crossbred cows during the first 90 DIM.

Therefore, HO cows may have diverted more ingested nutrients to the production of lactose and milk compared with MO-sired crossbred cows.

Holstein cows tended to have greater DMI expressed in percentage of BW in the last 15 d of gestation than crossbred cows, but the interaction between breed and study day tended to be associated with DMI expressed in percentage of BW. Such an interaction was observed because DMI of HO cows decreased from 1.9 to 1.4% of BW from 15 to 1 d before calving, whereas DMI of crossbred cows was 1.5 and 1.4% of BW 15 and 1 d before calving, respectively. The importance of DMI during the transition period for productive, health, and reproductive parameters has been the focus of much research (Drackley, 1999; LeBlanc, 2010). The magnitude of the decrease in DMI in the last few weeks of gestation, however, has been suggested to be more important than total DMI prepartum. Zamet et al. (1979) described that DMI of cows that had at least one peripartum disorder decreased from 1.8% to 0.9% of BW during the last 27 d of gestation, whereas normal cows had smaller decrease in DMI, from 1.8% to 1.2% of BW, during the last 27 d of gestation. Huzzey et al. (2007) demonstrated that cows that developed severe metritis had reduced feed intake as early as 2 weeks before calving compared with cows that did not develop metritis. On the other hand, cows that developed mild metritis had similar feed intake up to approximately 4 d before calving compared with healthy cows, but thereafter cows that developed mild metritis had reduced DMI compared with healthy cows (Huzzey et al., 2007). It is not clear why HO cows tended to have more pronounced decline in DMI in the last days of gestation than crossbred cows. Several endocrine and metabolic factors are likely to be involved with

control of DMI during the transition period (Allen et al., 2009). In chapter 3 of this thesis we demonstrated that HO cows had greater concentrations of cortisol from 14 d before to 42 d after calving and greater concentrations of GH from 7 d before to 56 d after calving than crossbred cows, but peripartum concentrations of IGF-1, insulin, and leptin were not different between breeds.

Despite the fact that the incidences of metritis and sub-clinical endometritis were not different between breeds, it is important to note that the number of experimental units may have been too small to detect differences. On the other hand, HO cows tended to be more likely to be diagnosed with clinical endometritis than crossbred cows. This could suggest that HO cows were less likely to resolve postpartum uterine bacterial contamination as the persistence of pathogenic bacteria for up to 3 weeks postpartum is associated with clinical endometritis in dairy cows (Dohmen et al., 1995). Finally, HO cows were more likely to be diagnosed with at least one uterine disease. As mentioned above, reduced prepartum feed intake is a predisposing factor for metritis (Huzzey et al., 2007) likely due to compromised neutrophil function during the peripartum (Hammon et al., 2006). In the current study, HO cows tended to have more pronounced decrease in DMI in the last 15 d of gestation. In chapter 3 we demonstrated that peripheral PMNL from HO cows had reduced phagocytic activity on the day of calving compared with 7 d before calving, whereas phagocytic activity of PMNL from MO-sired crossbred cows did not change in the last 7 d of gestation. Furthermore, PMNL from HO cows had reduced expression of CD18 (an adhesion molecule) from 7 d before calving to 21 d after calving compared with crossbred cows as demonstrated in chapter 3. In light of the feed intake

differences between breeds described above but lack of differences in metabolic parameters (NEFA and BHBA), the importance of heterosis on improved health of postpartum crossbred cows should not be underestimated. Heterosis is observed as improved performance or health of crossbred animals compared with their parental average because of heterosis, which is resultant from increased heterozygosity, alleviating inbreeding depression (VanRaden and Sanders, 2003). In a large retrospective study, Snowder et al. (2005) demonstrated that crossbred beef calves, animals with more stable concentrations of hormones and metabolic parameters than transition cows, had reduced incidence of bovine respiratory disease compared with purebred beef calves of different breeds.

Interestingly, even though the incidence of subclinical endometritis did not differ between HO and MO-sired crossbred cows, crossbred cows positive for subclinical endometritis had a greater percentage of PMNL in the uterine cytology than HO cows positive for subclinical endometritis. In chapter 3 of this thesis we demonstrated that there were no differences in peripheral PMNL expression of adhesion molecules or function between crossbred and purebred HO cows at 42 DIM, when cows were examined for sub-clinical endometritis. Therefore, the difference between breeds in number of neutrophils present in the uterus of cows positive for subclinical endometritis could be due to differences between breeds in total number of circulating PMNL or secretion of chemokines and cytokines by the endometrial cells that influence influx of PMNL to the uterus (Sheldon et al., 2009).

In spite of the fact that HO cows had increased incidence of uterine disorders and greater rectal temperature from calving to 15 DIM, there were no differences in haptoglobin concentration from 7 d before calving to 21 DIM between breeds. Haptoglobin is an acute phase protein produced mainly in the liver that is generally elevated in metritic cows (Huzzey et al., 2009). Therefore, the lack of difference in haptoglobin concentration between breeds is difficult to explain, but it could reflect differences in systemic inflammatory response between breeds or differences in liver functionality between breeds.

Even though HO cows had greater incidence of uterine disorders compared to crossbred cows, there was no difference in uterine involution postpartum. On the other hand, ovarian structure dynamics differed between breeds. The diameter of the subordinate follicle present in the ovaries of HO cows was greater than the diameter of the subordinate follicle of crossbred cows and the number of class III follicles was greater for HO than crossbred cows. These ovarian characteristics are typical of cows with follicle codominance. Postpartum uterine infections have been suggested to affect ovarian function indirectly through the hypothalamic-pituitary axis (Sheldon et al., 2009). As suggested by Sheldon et al (2009), lipopolysaccharide (**LPS**) appears to affect the release of gonadotropin releasing hormone and luteinizing hormone (**LH**), which affects the rate of growth of ovarian follicles and ovulation (Diskin et al., 2003). Because HO cows had greater incidence of uterine disorders than crossbred cows, we speculate that HO cows may have had greater bacteria load in the uterus during the postpartum period, which may have affected LH release resulting in greater incidence of follicle codominance. Another

mechanism that may be associated with the fact that HO had larger subordinate follicles and greater number of class III follicles may be the greater concentration of cortisol in HO cows compared with crossbred cows as demonstrated in chapter 3. Increasing concentrations of ACTH and cortisol have been suggested to cause a decrease in LH secretion by the pituitary in what is considered a hypothalamus-pituitary-adrenal axis (Li and Wagner, 1983).

In the current study, the speed at which the first CL was diagnosed by ultrasonography in HO cows was slower compared with crossbred cows. Interestingly, breed was not associated with the speed at which cows were detected to have progesterone concentration $\geq$ 1 ng/mL. Such difference is likely to be a consequence of sampling schedule because blood samples were collected once weekly, whereas ultrasound exams were performed at 3 d intervals. The slower rate of diagnosis of the first CL in HO cows may be associated with a possible impairment of LH secretion as a consequence of different incidence of uterine disorders between breeds or a consequence of different concentrations of cortisol between breeds. The slower rate of diagnosis of the first CL in HO cows cannot be explained by breed differences in plasma concentration of IGF-I, a key hormone involved in resumption of cyclicity postpartum (Butler, 2000), because there were no differences in IGF-I concentrations from 7 d before calving to 56 DIM between HO and MO-sired crossbred cows as demonstrated in chapter 3. It is not clear why HO cows that resumed estrus cycles were more likely to have short luteal phase following the first ovulation compared with MO-sired crossbred cows, but this is an important observation because it may indicate that HO cows necessitate a more

prolonged recovery period postpartum until the start of breeding and may be associated with improved fertility in crossbred cows.

Conclusions

In the current study, crossbred cows had reduced incidence of uterine disorders postpartum. Two main factors may be involved with improved health of postpartum crossbred cows, namely: heterosis and feed intake peripartum. Holstein cows demonstrated ovarian function similar to that of cows that have codominance and we speculate that this may be cause by increased uterine disorders and cortisol concentrations, which have been associated with compromised LH secretion. The lack of difference in metabolic parameters, BW, and ECM yield, however, indicate that negative energy balance was unlikely involved with differences in health and ovarian function between HO and MO-sired crossbred cows.

Table 1. Body weight, body condition score, and milk production and composition in HO and MO-sired crossbred dairy cows

	HO	Mo-sired crossbred	*P* – value		
			Breed	Parity	Breed x Parity
Body weight, kg	625.7 ± 11.4	639.2 ± 13.0	0.41	< 0.01	0.17
Body condition score	3.0 ± 0.05	3.6 ± 0.06	< 0.01	0.07	0.51
Milk yield, kg/d	42.3 ± 1.9	38.8 ± 2.2	0.05	0.05	0.10
Fat yield, kg/d	1.5 ± 0.05	1.4 ± 0.05	0.81	0.02	0.38
Protein yield, kg/d	1.3 ± 0.04	1.3 ± 0.03	0.74	0.13	0.14
ECM yield, kg/d	39.6 ± 1.1	39.1 ± 1.2	0.72	< 0.01	0.22
Log somatic cell count	2.7 ± 0.2	2.5 ± 0..2	0.61	0.71	0.17

Table 2. Calving and postpartum health parameters in HO and MO-sired crossbred dairy cows

Parameter	HO		MO-sired crossbred	*P*–value
	AOR (95% CI)*	Incidence	Incidence	
Stillbirth [1], %	1.38 (0.29 – 6.52)	11.5	5.8	0.68
Calving difficulty [2] $\geq$ 4, %	1.28 (0.40 – 4.14)	15.4	11.5	0.68
Twins, %	0.68 (0.20 – 2.31)	9.6	13.5	0.54
Retained fetal membranes[3], %	1.18 (0.27 – 5.30)	7.7	7.7	0.82
Metritis[4], %	3.73 (0.74 – 18.91)	13.5	4.0	0.11
Clinical endometritis[5], %	2.20 (0.95 – 5.07)	44.2	26.5	0.07
Subclinical endometritis[6], %	2.09 (0.82 – 5.33)	32.0	18.4	0.12
At least one uterine disorder, %	2.99 (1.33 – 6.72)	63.5	36.7	< 0.01
Pyrexia[7], %	2.15 (0.95 – 4.88)	50.0	31.4	0.07

*Adjusted odds ratio and 95% confidence interval

[1] Stillbirth: dead calf within 48 hours of parturition.

[2] Calving difficulty: calving difficulty score of 4 (use of obstetrics chains) or 5 (use of a calf jack) were coded as difficult births.

[3] Retained fetal membranes: retained fetal membranes for more than 12 hours.

[4]Metritis: presence of fever and red brown watery fetid vaginal discharge.

[5]Clinical endometritis: presence of mucopurulent or purulent discharge (> 50% pus) on study d 24.

[6]Subclinical endometritis: presence of > 10% neutrophils in the uterine cytology examination on study d 42.

[7]Pyrexia: rectal temperature > 103.0 °F.

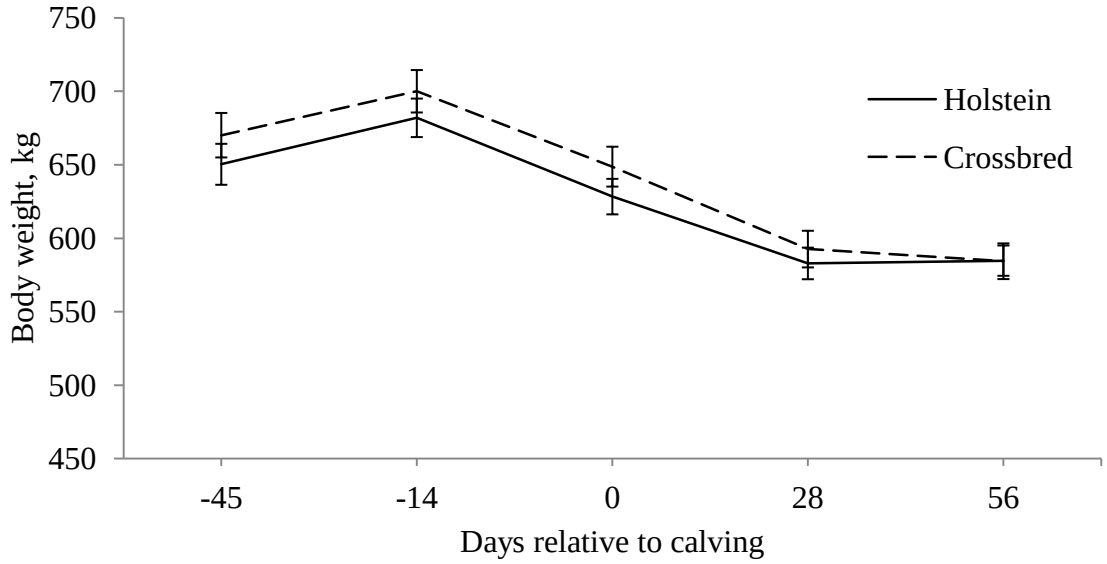

Figure 1A. Body weight of HO and MO-sired crossbred cows throughout the study. Breed – $P = 0.41$; day – $P < 0.01$; and, breed by day– $P = 0.04$.

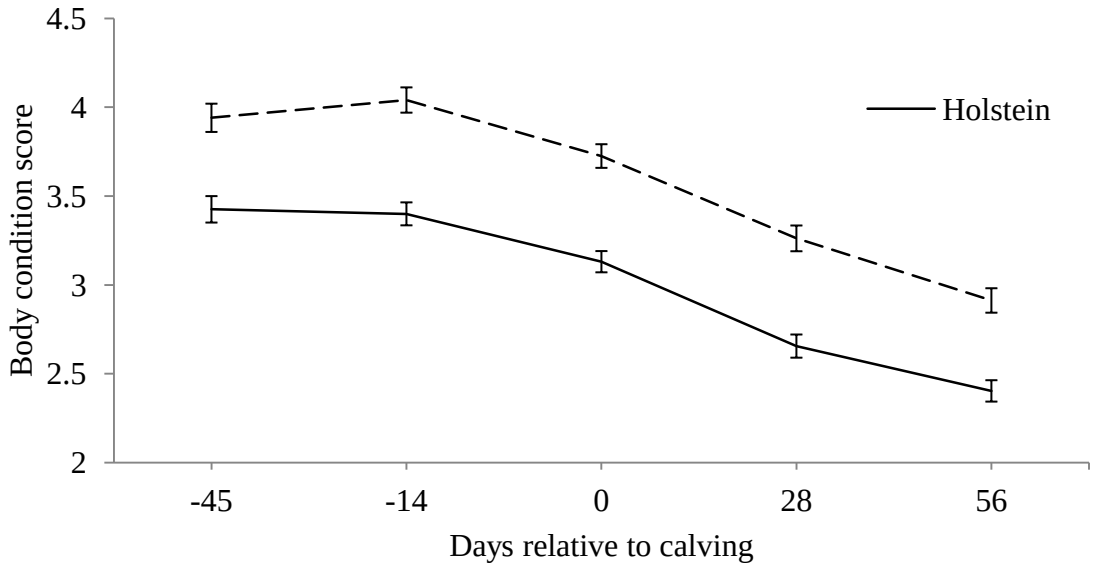

Figure 1B. Body condition score of HO and MO-sired crossbred cows throughout the study. Breed – $P < 0.01$; day – $P < 0.01$; and, breed by day– $P = 0.26$.

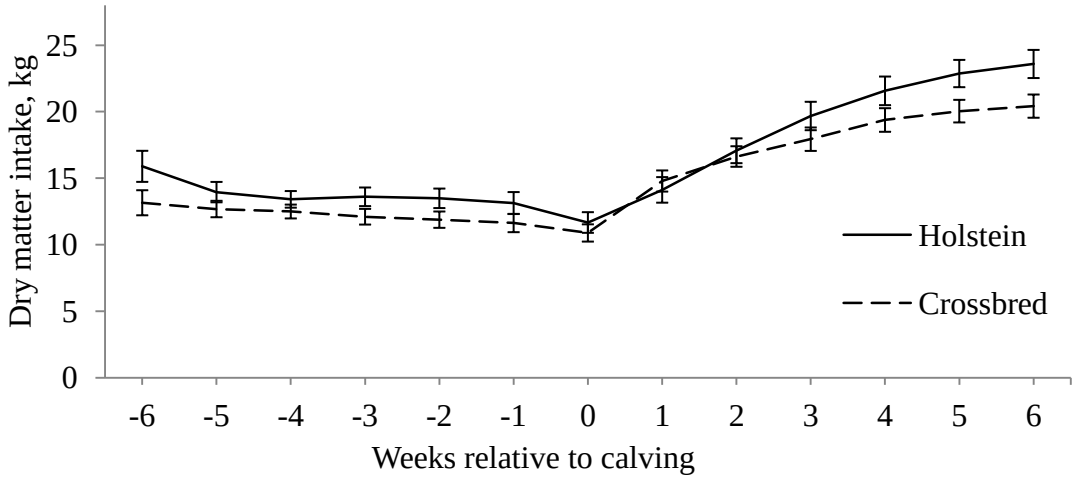

Figure 2A. Dry matter intake of HO and MO-sired crossbred cows from 6 before calving to week 6 after calving. Breed – $P = 0.08$; week – $P < 0.01$; and, breed by week– $P = 0.29$.

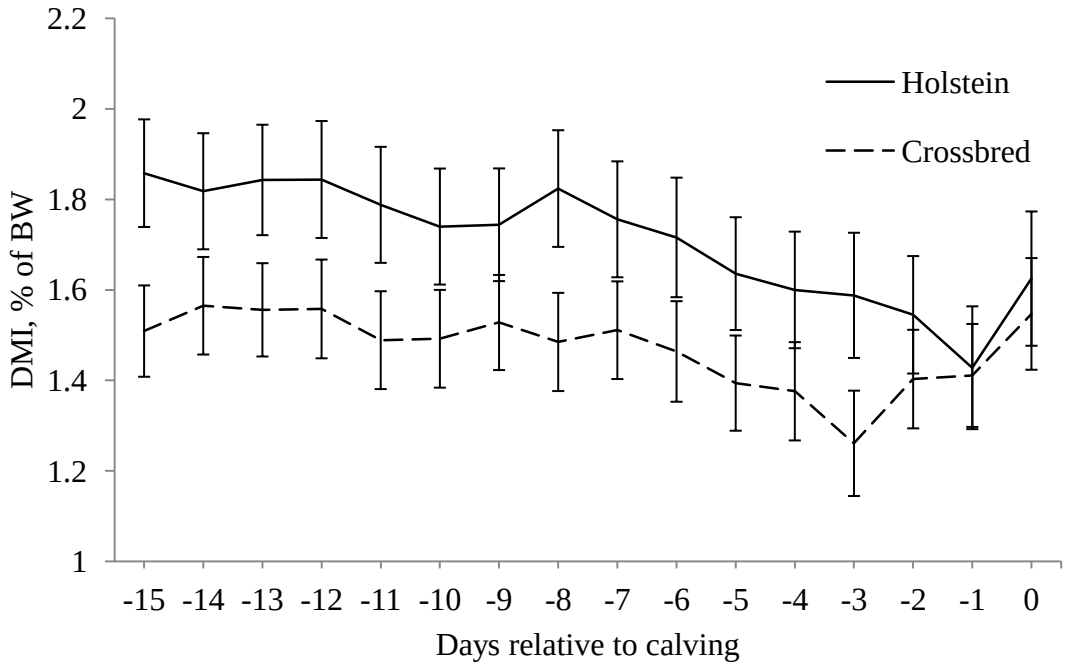

Figure 2B. Dry matter intake expressed in percentage of BW of HO and MO-sired crossbred cows from 15 d before calving to calving. Breed – $P = 0.10$; day – $P < 0.01$; and, breed by day– $P = 0.10$.

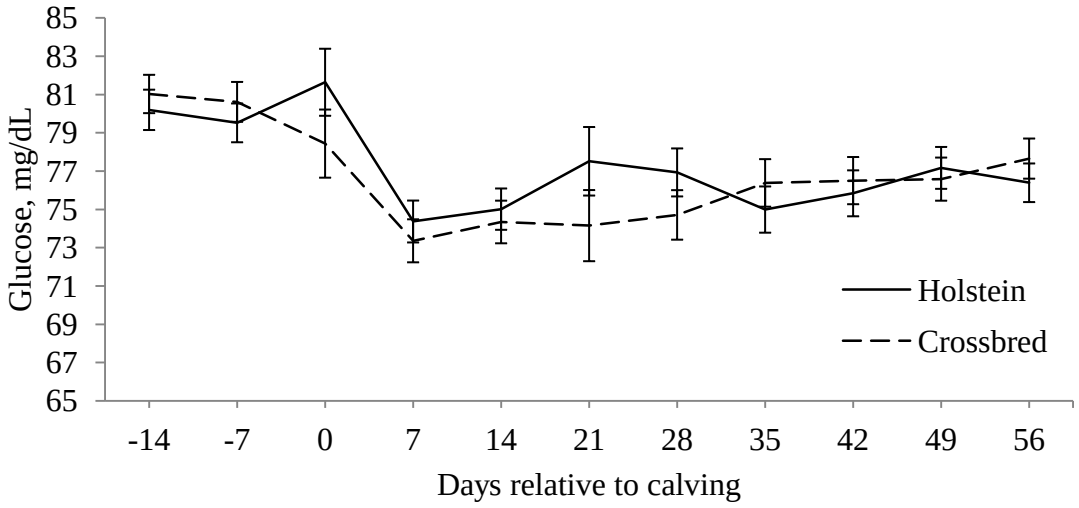

Figure 3A. Association among breed and metabolic parameters. Association between breed and glucose concentration: breed – $P = 0.54$; day – $P < 0.01$; and, breed by day– $P < 0.01$

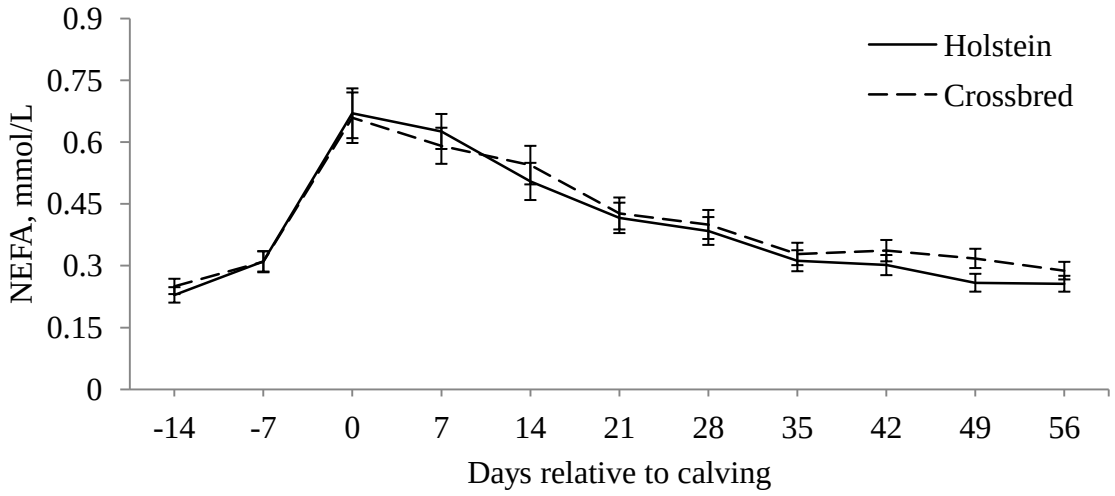

Figure 3B. Association among breed and metabolic parameters. Association between breed and NEFA concentration: breed – $P = 0.56$; day – $P < 0.01$; and, breed by day– $P = 0.93$

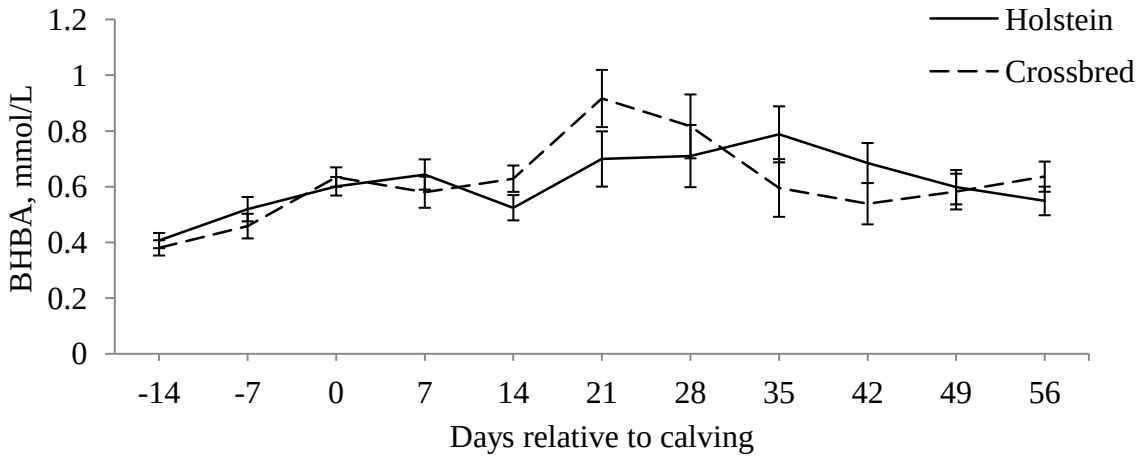

Figure 3C. Association among breed and metabolic parameters. Association between breed and BHBA concentration: breed – $P = 0.95$; day – $P < 0.01$; and, breed by day– $P < 0.01$

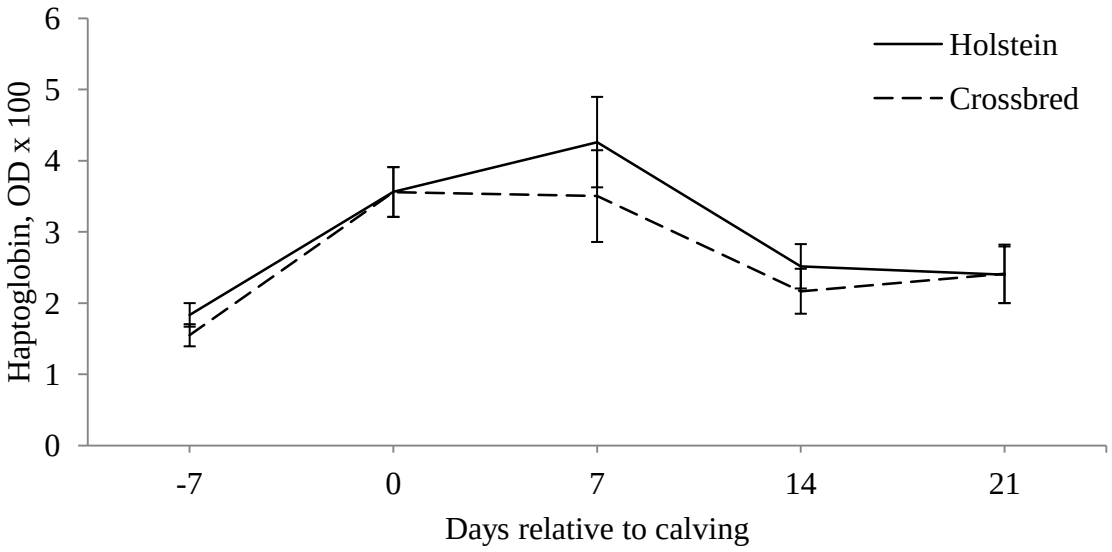

Figure 4. Association between breed and haptoglobin concentration from 7 d before to 21 d after calving. Breed – $P = 0.40$; day – $P < 0.01$; and, breed by day– $P = 0.85$

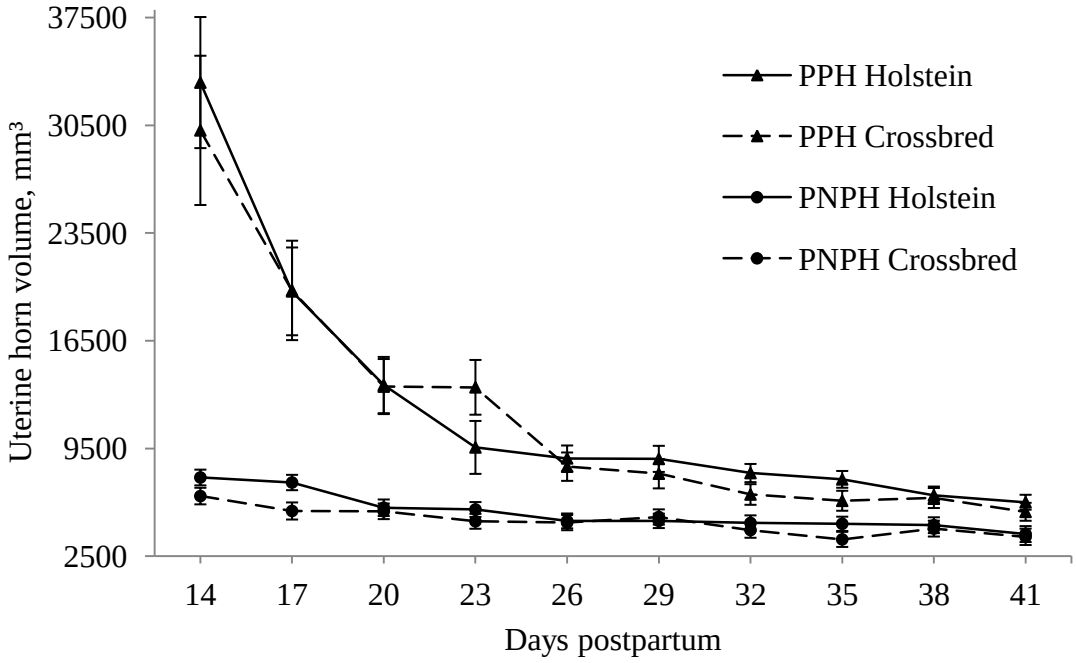

Figure 5. Association between breed and volume of previously pregnant and previously non-pregnant uterine horns from study d 14 to 41. Previous pregnant horn (PPH): breed – $P = 0.73$; day – $P < 0.01$; and, breed by day– $P = 0.48$. Previous non pregnant horn (PNPH): breed – $P = 0.15$; day – $P < 0.01$; and, breed by day– $P = 0.45$

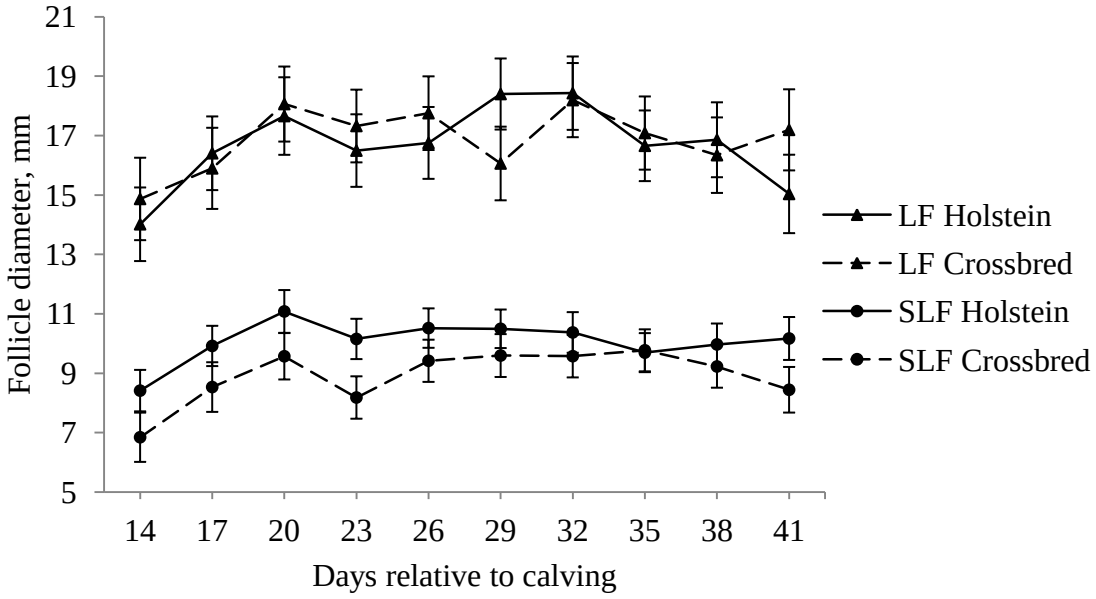

Figure 6. Association between breed and follicle diameter from study d 14 to 41. Largest follicle (LF): breed – $P = 0.85$; day – $P = 0.05$; and, breed by day– $P = 0.44$. Subordinate follicle (SLF): breed – $P = 0.03$; day – $P < 0.01$; and, breed by day– $P = 0.90$.

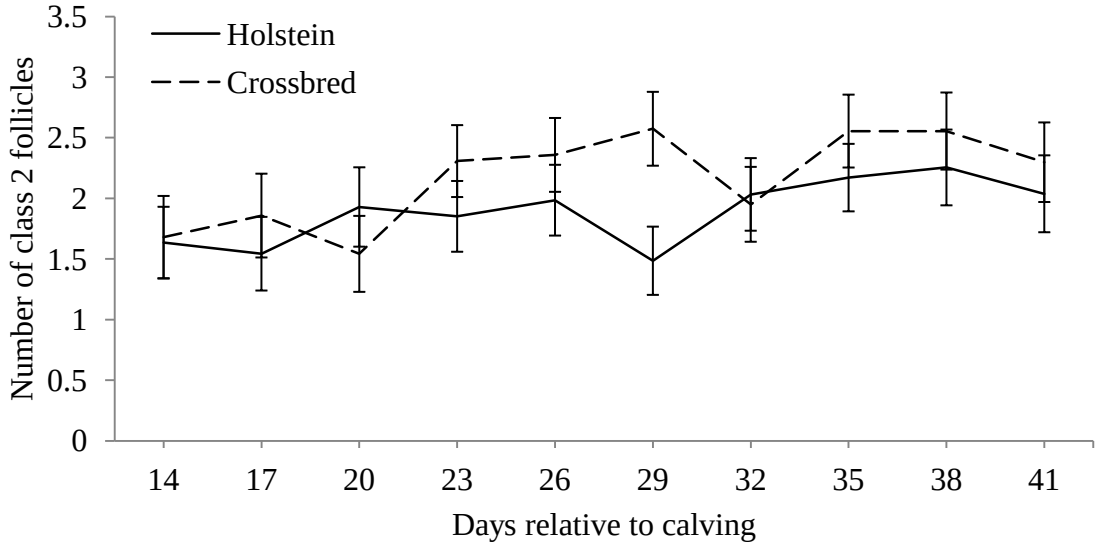

Figure 7 – Panel A.

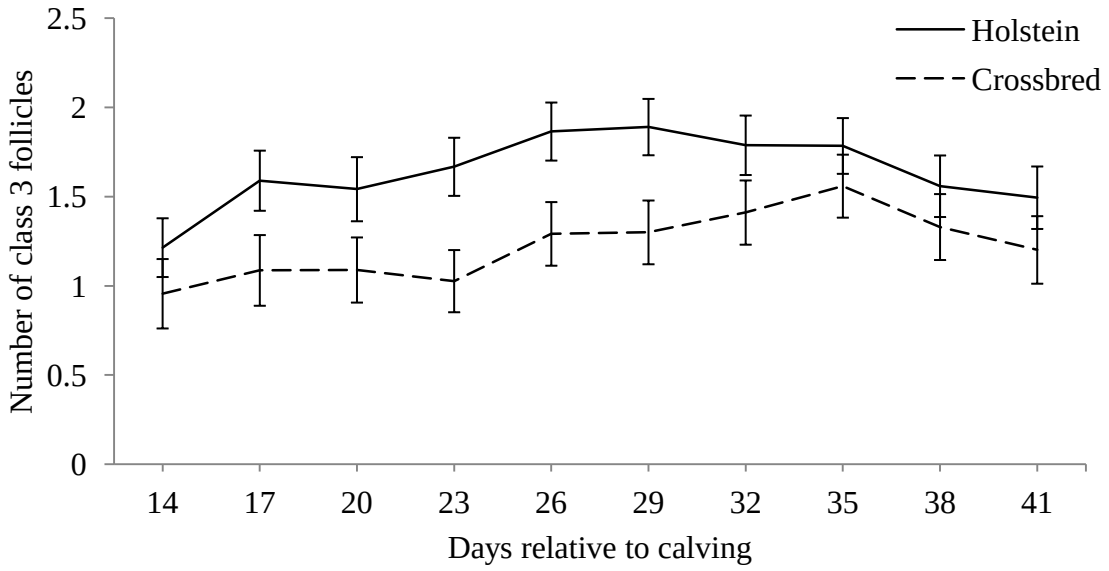

Figure 7 – Panel B.

Figure 7. Association between breed and number of follicles. (A) Number of class II follicles from study d 14 to 41. Breed – $P = 0.17$; day – $P = 0.11$; and, breed by day– $P = 0.51$. (B) Number of class III follicles from study d 14 to 41. Breed – $P < 0.01$; day – $P < 0.01$; and, breed by day– $P = 0.79$

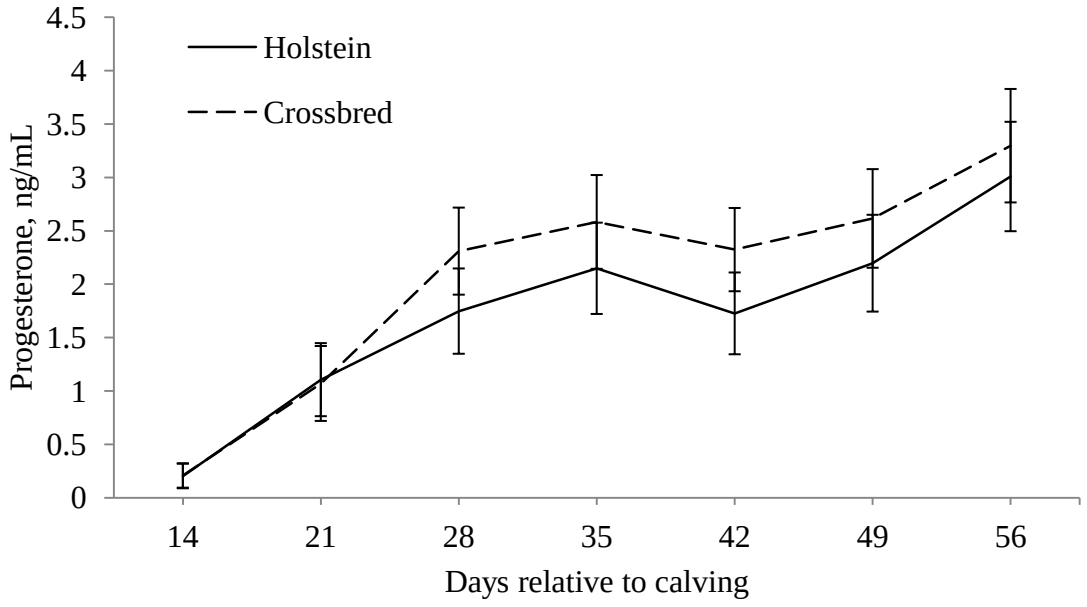

Figure 8. Progesterone concentration of HO and MO-sired crossbred cows from study d 14 to 56. Breed – $P = 0.24$; day – $P < 0.01$; and, breed by day– $P = 0.88$

CHAPTER 3: Comparison of innate immune responses and somatotropic axis components of Holstein and Montbéliarde-sired crossbred dairy cows

Chapter Summary

Objectives were to compare parameters related to innate immune responses and somatotropic axis of peripartum Holstein (**HO**) and Montbéliarde(**MO**)-sired crossbred cows. Cows (40 HO and 47 MO-sired crossbred) were enrolled in the study 45 d before expected calving date (study d 0 = calving). Polymorphonuclear leukocytes (PMNL) isolated from blood samples collected weekly from study d -7 to 21 and on study d 42 were used for determination of percentage of PMNL positive for phagocytosis (PA+) and oxidative burst (OB+), intensity of PA and OB, percentage of PMNL expressing CD18 (CD18+) and L-selectin (LS+), and intensity of CD18 and LS expression. Blood was sampled weekly from study d -7 to 14 and on study d 28, 42, and 56 for determination of insulin, growth hormone (**GH**), leptin, and insulin like growth factor (**IGF**)-1 concentrations. Blood sampled weekly from study d -14 to 21 and on study d 42 was used to determine cortisol concentration. Liver biopsies were performed on study d -14, 7, 14, and 28 for determination of mRNA expression for insulin receptor B (**IRB**), total GH receptor (**GHRtot**), GHR variant 1A (**GHR1A**), and for IGF-1 (**IGF-1**). Intensity of CD18 expression was greater in PMNL from crossbred cows compared with PMNL from HO cows (1,482.1 ± 82.3 vs 1,286.6 ± 69.8 GMFI). Furthermore, among HO cows, percentage of PA+ PMNL on study d -7 (64.4 ± 5.2%) tended to be greater than on study d 0 (57.1 ± 5.1%), but no differences in percentage of PA+ PMNL between study d -7 and 0 was observed in crossbred cows. Similarly, intensity of PA in PA+ PMNL from

HO cows decreased from study d -7 to 0 (4,750.6 ± 1,217.0 vs 1,964.7 ± 1,227.9 GMFI), but no changes in intensity of PA in PA+ PMNL from crossbred cows was observed. On study d 0, intensity of PA tended to be reduced in PA+ PMNL from HO cows compared with PA+ PMNL from crossbred cows (4,688.1 ± 1,271.8 vs 1,964.7 ± 1,227.9 GMFI). Concentrations of GH (7.4 ± 0.4 vs 5.1 ± 0.4 ng/mL) and cortisol (9.5 ± 0.8 vs 7.1 ± 0.8 ng/mL) were greater for HO than crossbred cows. Expression of liver IRB mRNA increased on study d 7 and 14 in HO cows, but not in crossbred cows. Transition crossbred cows had improved innate immune responses. Furthermore, HO cows appeared to be less sensitive to the negative feedback of IGF-1 on GH secretion indicating a possible uncoupling of the somatotropic axis at the hypothalamus.

Materials and Methods

Cows, Breeds, Enrollment Period

The study was conducted at the University of Minnesota's St. Paul dairy. Cows used in the current study are a subset of cows (n = 87) enrolled in the study described in chapter 2. In the current study 40 HO (31 lactation $\geq$ 1 and 9 nulliparous) and 47 MO-sired crossbred (42 lactation $\geq$ 1 and 5 nulliparous) cows were used. Cows that had their first calving during the study will be referred to as primiparous and cows that had their second or more calving during the study will be referred to as multiparous.

Cows were enrolled in the study 45 days before expected calving date and were followed until 90 DIM (study d 0 = calving date). The enrollment period was from October 2009 – January 2010 (first season of parturition) and during the month of

September 2010 (second season of parturition). Data regarding diets fed during the dry and lactating periods, body weight, body condition score, dry matter intake, health and metabolic parameters were already reported in chapter 2.

Blood Sample Collection

Blood samples were collected via coccygeal vein/artery. Blood samples collected weekly from study d -7 to 21 and on study 42 into heparinized evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) were used for evaluation of *ex vivo* innate immune function. Blood sampled weekly from study d -7 to 14 and on study d 28, 42 and 56 into evacuated tubes without anticoagulant and into evacuated tubes with EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) was used for determination of serum concentrations of insulin, GH, and leptin and plasma concentration of IGF-1. Blood samples collected weekly from study d -14 to 21 and on study d 42 into tubes without anticoagulant were used for determination of cortisol concentration. Tubes were placed on ice until centrifugation for serum/plasma separation (1,200 x g for 15 min at 4^{0}C) and serum/plasma harvested was stored at -32 ^{0}C until analysis.

Innate Immune Response Assays

In a subgroup of cows [HO = 29 (20 multiparous and 9 primiparous) and crossbred = 33 (29 multiparous and 4 primiparous)], *ex vivo* innate immune parameters were evaluated as described by Hulbert et al. (2011). Briefly, expression of L-selectin

(LS) and CD18 by peripheral PMNL was determined by indirect immunofluorescence staining. Briefly, the assay consisted of incubating 200 µL of whole blood at 4° C for 30 minutes with 5 µg/mL of anti-bovine CD62L (VMRD Inc., Pullman, WA) monoclonal antibody produced in mouse or 2.5 µg/mL of anti-bovine CD11a/CD18, CD11b/CD18 and CD11c/CD18 (VMRD Inc., Pullman, WA, USA) monoclonal antibody produced in mouse. Prior to the incubation of cells with an anti-mouse IgG-FITC secondary polyclonal antibody (AbD Serotec, Raleigh, NC, USA) diluted 1:400 in PBS solution (Sigma-Aldrich, St. Louis, MO, USA), erythrocytes were lysed with hyperconcentrated PBS solution. After washing the cells with PBS solution, samples were analyzed by flow cytometry.

Phagocytic (**PA**) and oxidative burst (**OB**) activity of peripheral PMNL were determined upon challenge with enteropathogenic bacteria (*Escherichia coli* 0118:H8). Briefly, the assay to determine PA and OB consisted of incubating 200 µL of whole blood with 100 µM of dihydrorhodamine 123 (Molecular Probes, Invitrogen, USA) and 40 µL of fluorescently labeled bacteria (10^9 cfu/mL) at 38.5 °C for 15 minutes, with external bacteria quenched using the Trypan Blue Solution (0.4%) (Sigma-Aldrich, St. Louis, MO, USA). After washing with milliQ water to remove excess dye, erythrocytes were lysed by the addition of hyperconcentrated PBS solution (Sigma-Aldrich, St. Louis, MO, USA). Lastly, the cells were resuspended in PBS solution for immediate flow cytometry analyses.

All flow cytometry data were collected on a BD FACSCANTO II (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using FlowJo 7.6.4 software (Tree

Star Inc., San Carlos, CA, USA). Data are reported as PMNL intensity of phagocytosis, oxidative burst, and expression of CD18 and L-selectin molecules expressed in geometric mean fluorescence intensity (**GMFI**). Furthermore, percentages of PMNL positive for phagocytosis (**PA+**), oxidative burst activity (**OB+**), and expression of CD18 (**CD18+**) and L-selectin (**LS+**) molecules were calculated.

Hormone Assays

Serum cortisol concentrations were determined by a solid-phase radioimmunoassay kit (Coat-a-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation were 5.8% and 11.7%, respectively.

Insulin, GH, IGF-1 and leptin concentrations were determined in a subgroup of multiparous cows (HO = 29 and crossbred = 31). Serum insulin, GH and leptin concentrations were determined in triplicates. Insulin concentration was determined using a specific, double-antibody, equilibrium radioimmunoassay as described by Kolath et al. (2006). Growth hormone concentration was quantified using a modified radioimmunoassay from an ovine GH assay (Lalman et al., 2000). Leptin concentration was determined using a competitive, liquid-liquid phase, double-antibody radioimmunoassay described by Geary et al. (2003). Plasma IGF-1 concentrations were quantified using a commercially available kit (DSL-10-2800 Nonextraction IGF-1 ELISA; Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The intra- and inter-assay coefficients of variation for the assays were lower than 5%.

Liver Tissue Collection

In a subgroup of multiparous cows (HO = 10 and crossbred = 10), liver biopsies were performed on study d -14, 7, 14 and 28. The actual days (mean ± SEM) for each sampling point were -14.6 ± 1.2, 7.1 ± 0.2, 14.3 ± 0.3 and 29.5 ± 0.6. Hair in the biopsy area, 11^{th} intercostal space on the right rib cage, was clipped and cleaned aseptically with betadine scrub followed by 70% ethanol rinse. The area was locally anesthetized (5 mL of 2% lidocaine hydrochloride solution) and a curved surgical blade (no. 12) was used to make a 2 cm incision in length. A stainless steel trocar was used to insert a cannula through muscle, peritoneum and liver. Liver tissue samples were obtained as vacuum was created by drawing back the trocar into the cannula. Liver samples were stored in microcentrifuge tubes and immediately placed in liquid nitrogen. Samples were stored at -80 °C until analysis.

Real-time RT-PCR

Polymerase chain reaction assays were done according to the protocol previously described (Okamura et al, 2009). Briefly, RNA was extracted from liver tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and it was transcribed into cDNA by using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The cDNA samples were analyzed in triplicate using 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Quantitative real-time reverse transcription-PCR was performed using an ABI Prism 7700 machine (Applied

Biosystems, Foster City, CA, USA) and specific primers for insulin receptor B (**IRB**), total GHR (**GHRtot**), GHR variant 1A (**GHR1A**), IGF-1 (**IGF-1**), ubiquitin and cyclophilin A. The specific primers sequences were as follows: IRB forward, 5'-TGCACAACGTGGTTTTCATC-3'; IRB reverse, 5'-GTTTCCTCGAAGGCCTAGC-3'; GHRtot forward, 5'-GGTATGGATCTCTGGCAGCTG-3'; GHRtot reverse, 5'-CTCTGACAAGGAAAGCTGGTGTG-3'; GHR1A forward, 5'-CCAGCCTCTGTTTCAGGAGTGT-3'; GHR1A reverse, 5'-TGCCACTGCCAAGGTCAAC-3'; IGF-1 forward, 5'-TTGGTGGATGCTCTCCAGTTC-3'; IGF-1 reverse, 5'-GCACTCATCCACGATTCCTGT-3'; ubiquitin forward, 5'-ATGCAGATCTTTGTGAAGAC-3'; ubiquitin reverse, 5'-CTTCTGGATGTTGTAGTC-3'; cyclophilin A forward, 5'-CACCGTGTTCTTCGACATCG-3' and cyclophilin A reverse, 5'-ACAGCTCAAAAGAGACGCGG-3'. The fluorescence threshold cycle (**Ct**) values were used to calculate the relative abundance of mRNA concentrations by using the formula $2^{\Delta Ct}$, where ΔCt, difference in threshold cycle value, was calculated by subtracting medium control, cyclophilin A, Ct value from sample Ct value.

Statistical Analysis

Data collected during the study was entered and organized in an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA) and analyzed using SAS (SAS/STAT version 9.2; SAS Inst. Inc., Cary, NC, USA).

Data were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS or by ANOVA using the GLM procedure of SAS. Models included breed (HO vs MO-sired crossbred), parity (multiparous vs primiparous), season of parturition (first vs second), and the interaction between breed and parity and between breed and study day. Ante-dependence covariance structure was used for analysis of concentrations of insulin, GH, IGF-1, and leptin and mRNA expressions of IRB, GHRtot, GHR1A, and IGF-1. On the other hand, unstructured, compound symmetry, and autoregressive(1) covariance structures were tested for analysis of innate immune responses parameters (samples collected weekly from study d -7 to 21) and analysis of cortisol concentration (samples collected weekly from study d -14 to 21) and the covariance structure used was chosen based on Akaike's Information Criterion (AIC). Innate immune responses parameters and cortisol concentration on study d 42 were analyzed by ANOVA using the GLM procedure of SAS. Models included breed (HO vs crossbred), parity (multiparous vs primiparous), season of parturition (first vs second), and the interaction between breed and parity.

Statistical significance was defined as $P \leq 0.05$ and statistical tendencies as $0.05 < P \leq 0.10$.

Results

Innate Immune Responses

Breed was not associated with percentage of LS+ PMNL (52.5 ± 2.0%; $P = 0.41$) or intensity of LS expression (942.2 ± 24.1 GMFI; $P = 0.68$) from study d -7 to 21.

Similarly, breed was not associated with percentage of LS+ PMNL (49.7 ± 3.6%; P = 0.39) or intensity of LS expression (1,118.9 ± 41.1 GMFI; P = 0.88) on study d 42.

Breed was not (P = 0.30) associated with percentage of CD18+ PMNL (75.5 ± 2.0%) from study d -7 to 21. On the other hand, breed was (P = 0.04) associated with intensity of CD18 expression from study d -7 to 21 (Figure 9), because CD18+ PMNL from crossbred cows had greater intensity of CD18 expression than CD18+ PMNL from HO cows (1,482.1 ± 82.3 vs 1,286.6 ± 69.8 GMFI). On study d 42, however, breed was not associated with percentage of CD18+ PMNL (65.8 ± 4.1%; P = 0.47) and intensity of CD18 expression (1,835.0 ± 150.0 GMFI; P = 0.30). Parity tended (P = 0.07) to be associated with intensity of CD18 expression from study d -7 to 21 (multiparous = 1,495.0 ± 47.3 vs primiparous = 1,273.0 ± 113.1 GMFI). Similarly, parity tended (P = 0.08) to be associated with intensity of CD18 expression on study d 42 (multiparous = 1,795.2 ± 170.7 vs primiparous = 1,056.9 ± 397.3 GMFI).

There was no (P = 0.15) association between breed and percentage of PA+ PMNL from study d -7 to 21, but the interaction between breed and study day was (P = 0.03) associated with percentage of PA+ PMNL (Figure 10). Such an interaction was observed because, among HO cows, percentage of PA+ PMNL on study d -7 tended (P = 0.09) to be greater compared with percentage of PA+ PMNL on study d 0, whereas, among crossbred cows, percentage of PA+ PMNL between study d -7 and 0 was not (P = 0.89) different (Figure 10). Furthermore, HO cows had ($P \leq 0.05$) greater percentage of PA+ PMNL on study d 7, 21, and 42 than crossbred cows and HO cows tended to (P = 0.10) have greater percentage of PA+ PMNL on study d 14 than crossbred cows. There was (P

< 0.01) an association between parity and percentage of PA+ PMNL from study d -7 to 21 (multiparous = 75.5 ± 2.5 vs primiparous = 52.3 ± 5.8%). Parity was not (P = 0.25) associated with percentage of PA+ PMNL on study d 42 (74.6 ± 3.4%). Intensity of PA from study d -7 to 21 did not (P = 0.54) differ between PA+ PMNL from HO and crossbred cows, but the interaction between breed and study day tended to be (P = 0.09) associated with PA intensity of PA+ PMNL (Figure 11). This interaction occurred because, on study d 0, PA+ PMNL from HO cows tended to have (P = 0.09) reduced PA intensity compared with PA+ PMNL from crossbred cows. Furthermore, PA intensity in PA+ PMNL from HO cows decreased (P = 0.03) between study d -7 and 0, but there was no (P = 0.20) change in PA intensity in PA+ PMNL from crossbred cows during the same period. On study d 42, breed was not (P = 0.27) associated with intensity of PA of PA+ PMNL. Parity was (P < 0.01) associated with intensity of PA from study d -7 to 21 (multiparous = 7,890.2 ± 504.9 vs primiparous = 2,814.3 ± 1,203.0 GMFI). Parity, however, was not (P = 0.19) associated with intensity of PA on study d 42 (10,028.0 ± 770.0 GMFI).

Breed was not (P = 0.61) associated with percentage of OB+ PMNL from study d -7 to 21 (93.8 ± 0.5%). Similarly, breed was not (P = 0.81) associated with percentage of OB+ PMNL on study d 42 (91.4 ± 1.0 %). Breed was not (P = 0.18) associated with intensity of OB in OB+ PMNL from study d -7 to 21 (22,178.0 ± 1,612.0 GMFI) and on study d 42 (29,781.0 ± 3,980.0 GMFI; P = 0.89). Oxidative burst positive PMNL from multiparous cows had greater (P < 0.01) OB intensity from study d -7 to 21 compared with OB+ PMNL from primiparous cows (26,085.0 ± 1,891.0 vs 7,883.0 ± 4,608.4

GMFI). On study d 42, parity was not (P = 0.20) associated with OB intensity (29,781.0 ± 3,980.0 GMFI).

Insulin, Growth Hormone, Insulin Like Growth Factor-1, Cortisol and Leptin

Breed was not (P = 0.69) associated with insulin concentration from study d -7 to 56 (Figure 12A). Holstein cows, however, had (P < 0.01) greater GH concentration from study d -7 to 56 than crossbred cows (7.4 ± 0.4 vs 5.1 ± 0.4 ng/mL; Figure 12B). Breed was not associated with IGF-1 (P = 0.82; Figure 12C) or leptin (P = 0.30; Figure 13) concentrations from study d -7 to 56.

Holstein cows had greater (P < 0.01) cortisol concentration from study d -14 to 21 than crossbred cows (9.5 ± 0.8 vs 7.1 ± 0.8 ng/mL; Figure 14). On study d 42, cortisol concentration was (P < 0.01) greater for HO cows compared with crossbred cows (11.7 ± 1.6 vs 4.5 ± 1.5 ng/mL). Primiparous cows had (P < 0.01) greater cortisol concentration from study d -14 to 21 than multiparous cows (10.9 ± 1.2 vs 5.6 ± 0.5 ng/mL). On study d 42, primiparous cows had (P < 0.01) greater cortisol concentration than multiparous cows (11.3 ± 2.0 vs 4.9 ± 1.0 ng/mL). The interaction between breed and parity was (P < 0.01) associated with cortisol concentration on study d 42 because, among crossbred cows, parity was not (P = 0.79) associated with cortisol concentration but, among HO cows, primiparous cows had (P < 0.01) greater cortisol concentration than multiparous cows (18.5 ± 2.8 vs 4.8 ± 1.4 ng/mL).

Liver mRNA Expression

There was no (P = 0.69) association between breed and expression of IRB mRNA, but the interaction between breed and study day was (P = 0.02) associated with liver expression of IRB mRNA (Figure 15A). Such an interaction was observed because no changes in IRB mRNA expression were observed in crossbred cows, but, among HO cows, IRB mRNA expression on study d 7 tended to be (P = 0.10) and was (P < 0.01) greater compared with IRB mRNA expression on study d -14 and 28, respectively, and IRB mRNA expression on study d 14 was ($P \leq 0.04$) greater compared with IRB mRNA expression on study d -14 and 28. There was no association between breed and expression of GHRtot mRNA (P = 0.81; Figure 15B), expression of GHR1A (P = 0.83; Figure 15C), and expression of IGF-1 mRNA (P = 0.70; Figure 15D).

Discussion

As demonstrated in chapter 2, HO cows had increased incidence of uterine diseases in the postparturient period compared with crossbred cows. Because PMNL function is fundamental for host defense (Cai et al., 1994) and is associated with occurrence of retained fetal membranes and metritis (Kimura et al., 2002; Hammon et al., 2006), parameters associated with PMNL function were evaluated in the peripartum period of HO and MO-sired crossbred cows. Holstein cows had reduced intensity of expression of CD18, a molecule responsible for the firm adherence of PMNL to the endothelium near areas of inflammation (Burton et al., 1995), compared with crossbred cows but the intensity of expression of L-selectin was similar between breeds. Furthermore, the interaction between breed and study day was associated with intensity

of PA because, among HO cows, the intensity of PA on study d 0 was reduced compared with intensity of PA on study d -7 and 7, whereas, among crossbred cows, the intensity of PA on study d 0 was not different than intensity of PA on study d -7 or 7. These are important findings because cows that develop metritis seem to have a significant reduction in activity of PMNL around the time of calving (Hammon et al., 2006) similar to what was observed in HO cows but not in MO-sired crossbred cows. On the other hand, the increased PA activity of PMNL from HO cows from study d 7 to 21 compared with crossbred cows was likely associated with the tendency for greater incidence of pyrexia in HO cows compared with crossbred cows, as demonstrated in chapter 2, because pyrexia is triggered by circulating endogenous pyrogens that also stimulate PMNL activity (Dinarello, 2004).

Several endocrine and nutritional factors have been associated with PMNL activity around the time of calving. In the last week of gestation, concentrations of progesterone decrease as concentrations of cortisol, estradiol, prostaglandin $F_{2\alpha}$, and prolactin increase (Stevenson, 2007). Cortisol is known to suppress immune response because it down regulates the neutrophil expression of L-selectin and CD18, adhesion molecules involved in the trafficking of neutrophils from the endothelium to the site of inflammation (Burton et al., 1995). Even though we expected that the elevated concentrations of cortisol observed in HO cows during the periparturient period would be involved with compromised PMNL expression of CD18, when cortisol concentration was included in the model it was not associated with CD18 expression and it did not alter the association between breed and expression of CD18. Therefore, it is unlikely that

increased cortisol concentrations in HO cows was involved with suppressed PMNL activity and reduced intensity of CD18 expression.

During the periparturient period dairy cows have reduced feed intake and increased energy expenditure, which result in negative energy balance, elevated adipose tissue mobilization, and increased circulating NEFA concentrations. Holstein cows tended to have reduced DMI in the last 15 d of gestation compared with crossbred cows, but concentration of NEFA was not different between breeds as demonstrated in chapter 2. Reduced feed intake and increased concentrations of NEFA have been associated with reduced PMNL activity and increased incidence of metritis postpartum (Hammon et al., 2006). Polymorphonuclear leukocytes are primarily dependent on glucose and glycogen as energy sources (Galvão et al., 2010) but the uptake of glucose by PMNL appears to be independent of insulin. Because HO and crossbred cows had similar NEFA and glucose concentrations throughout the study as reported in chapter 2, it is not possible to explain the differences in PMNL morphology and function between breeds based on differences in DMI, negative energy balance, or glucose availability.

Holstein and MO-sired crossbred cows demonstrated a decoupling of the somatotropic axis characterized by increased GH concentration and reduced IGF-1 concentrations (discussed below). Several studies have demonstrated an association between IGF-1 and PMNL function and apoptosis *in vitro* (Inoue et al., 1998; Kooijman et al., 2002). Despite the importance of IGF-1 for the immune system, IGF-1 concentrations were not different between breeds; therefore, it is not likely that IGF-1

concentration was involved with the differences in intensity of expression of CD18 and intensity of PA between breeds.

As hypothesized, it is likely that heterosis was associated with improved PMNL functionality on the day of calving in the MO-sired crossbred cows compared with HO cows. Reduced genetic variability has been suggested to impair immune responses in populations with elevated inbreeding (O'Brien and Evermann, 1988) and, as mentioned previously, populations of inbred wild birds have compromised cell mediated and innate immune responses (Reid et al., 2003; Townsend et al., 2010). Increased genetic variation resulting from crossbreeding has been associated with greater primary antibody response in Norwegian Red-HO crossbred calves and cows compared with purebred HO calves and cows, respectively (Cartwright et al., 2011; Begley et al., 2009). On the other hand, bovine leukocyte adhesion deficiency, a recessive autosomal genetic disease characterized by mutation in the CD18 gene and impairment of PMNL adhesion (Nagahata, 2004), is an example of how reduced genetic variability and increased inbreeding may compromise innate immune responses.

Uncoupling of the somatotropic axis during the periparturient period is an adaptive characteristic of high genetic merit dairy cows in order to maintain elevated milk yield even during periods of negative energy balance, a characteristic not observed in beef cows (Jiang et al., 2005). As such, during the uncoupling of the somatotropic axis, cows have reduced GHR1A expression in the liver, reduced IGF-1 secretion from the liver, reduced negative feed-back of IGF-1 on GH secretion, and, consequently increased GH and reduced IGF-1 concentrations (Lucy et al., 2009). In the current study, purebred

HO and MO-sired crossbred cows demonstrated uncoupling of the somatotropic axis during the periparturient period. Holstein cows, however, had greater GH concentration than MO-sired crossbred cows and similar concentration of IGF-1 and liver expression of GHR1A mRNA compared with MO-sired crossbred cows. Lucy et al. (2009) and Grala et al. (2011) demonstrated that strains of the HO breed with elevated genetic merit for milk yield had a more pronounced uncoupling of the somatotropic axis after calving compared with genetic strains with lesser genetic merit for milk yield. On the other hand, Okamura et al (2009) demonstrated that Guernsey cows had similar changes in the somatotropic axis in the periparturient period compared with HO cows. We are unaware of studies evaluating the somatotropic axis of Montbéliarde cows during the periparturient period. The increased concentration of GH in HO cows compared with MO-sired crossbred but similar IGF-1 concentrations and mRNA expression of GHR1A and IGF-1 in the liver between breeds may suggest that the hypothalamus of purebred HO cows was less sensitive to the negative feed of IGF-1 compared with MO-sired crossbred, explaining the greater GH concentration in the former. This may be further evidence that the selection of HO cows over the years may have favored cows that are more prone to lipolysis during the transition period.

Holstein and crossbred cows had similar insulin concentrations throughout the study, but the interaction between breed and study day was associated with glucose concentration as reported in chapter 2. This may be an indication that the degree of insulin resistance of HO cows was greater compared with crossbred cows. Interestingly, HO cows had increased expression of liver IRB mRNA on study d 7 and 14 compared

with study d -14 and 28; this pattern of expression of liver IRB mRNA, however, was not observed in crossbred cows. Upregulation of expression of liver IRB mRNA is expected during periods of negative energy balance, particularly during early lactation (Gross et al., 2011). The differences between breeds on liver expression of IRB mRNA may be a consequence of the greater decrease in dry matter intake in the last 15 d of gestation observed in HO cows as described in chapter 2. Midlactation feed restricted cows and 21 d old fetal growth restricted lambs had upregulation of insulin receptor mRNA in the liver and muscle, respectively (Muhlhausler et al., 2009; Gross et al., 2011). Even though HO cows had greater expression of IRB mRNA on study d 7 and 14, concentrations of insulin and glucose were similar between breeds, corroborating the hypothesis that the level of insulin resistance in HO cows may have been greater.

In the current study, HO cows had greater GH and cortisol concentrations from 7 d before calving to 56 d postpartum compared with MO-sired crossbred cows. Studies that compared hormonal profiles of HO cows of different genetic strains demonstrated that cows with increased genetic merit for milk yield have elevated plasma GH (Lucy et al., 2009; Grala et al., 2011). One possible cause of increased GH in HO cows of elevated genetic merit is the observed increased ghrelin concentrations (Roche et al., 2006) compared with HO cows of lesser genetic merit for milk yield. Ghrelin is an endogenous ligand of the GH secretagogue receptor and stimulates GH secretion and increases appetite (Korbonits and Grossman, 2004), which are believed to be associated with greater milk yield. In the current study and in the study described in chapter 2 HO cows had greater GH concentration and greater milk yield than crossbred cows, respectively.

Furthermore, administration of ghrelin to cattle stimulates GH (Itoh et al., 2005) and cortisol (Itoh et al., 2006) secretion. Unfortunately, in the current study, ghrelin concentration was not determined.

Even though it was expected that HO cows would have reduced leptin concentration compared with MO-sired cows because the former had reduced BCS throughout the study as reported in chapter 2, breed was not associated with leptin concentration. Leptin, a hormone produced mainly in adipose tissue, is involved in the regulation of feed intake, energy homeostasis, and reproductive and immune functions (Ingvartsen and Boisclair, 2001). Therefore, it is not possible to explain the differences in innate immune responses in the current study based on leptin concentrations. The lack of difference between breeds in leptin concentration despite the difference in BCS may be explained by the differences between breeds on GH and cortisol concentrations. Treatment of steers with GH for 3 days and *in vitro* treatment of adipose tissue with dexamethasone resulted in upregulation of leptin mRNA expression in adipose tissue (Houseknecht et al., 2000). Therefore, HO cows, despite having reduced BCS compared with crossbred cows, had greater concentrations of GH and cortisol that may have caused upregulation of leptin mRNA expression and may have resulted in similar leptin concentrations between HO and crossbred cows. This is, however, a simplistic hypothesis because the effects of glucocorticoid and GH on expression of leptin mRNA in adipose tissue in *in vivo* systems may be interdependent.

Conclusions

In the current study HO cows tended to have reduced intensity of PA on the day of calving compared with crossbred cows and had reduced expression of CD18 during the periparturient period compared with crossbred cows. Differences in innate immune responses between HO and crossbred cows could not be explained by differences in concentrations of metabolites or hormones, indicating that heterosis was likely the main reason for differences in innate immune responses. Holstein and MO-sired crossbred cows presented uncoupling of the somatotropic axis during the transition period. The greater GH concentration in HO cows than MO-sired crossbred cows and similar IGF-1 concentration and liver mRNA expression of GHR1A and IGF-1 between breeds, however, indicate that the hypothalamus of HO cows may have been less sensitive to the negative feedback of IGF-1, predisposing HO cows to more lipolysis during the transition period. These differences between breeds are likely a consequence of the different genetic composition and selection of HO and MO breeds.

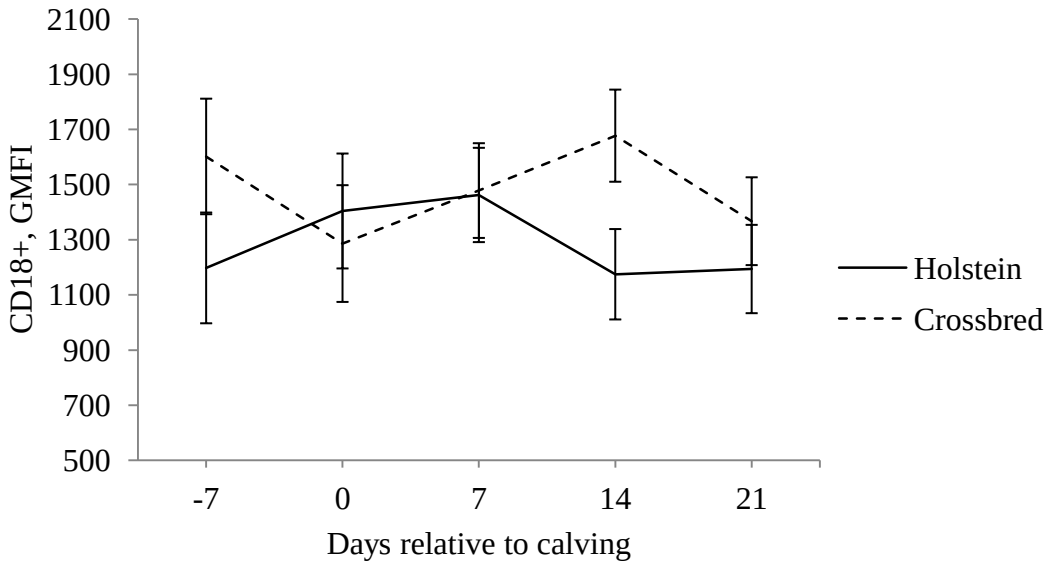

Figure 9. Intensity of CD18 expression in CD18+ PMNL from 7 d before calving to 21 d after calving in Holstein and Montbéliarde-sired crossbred cows. Breed – $P = 0.04$; study day – $P = 0.83$; and, breed by study day – $P = 0.45$.

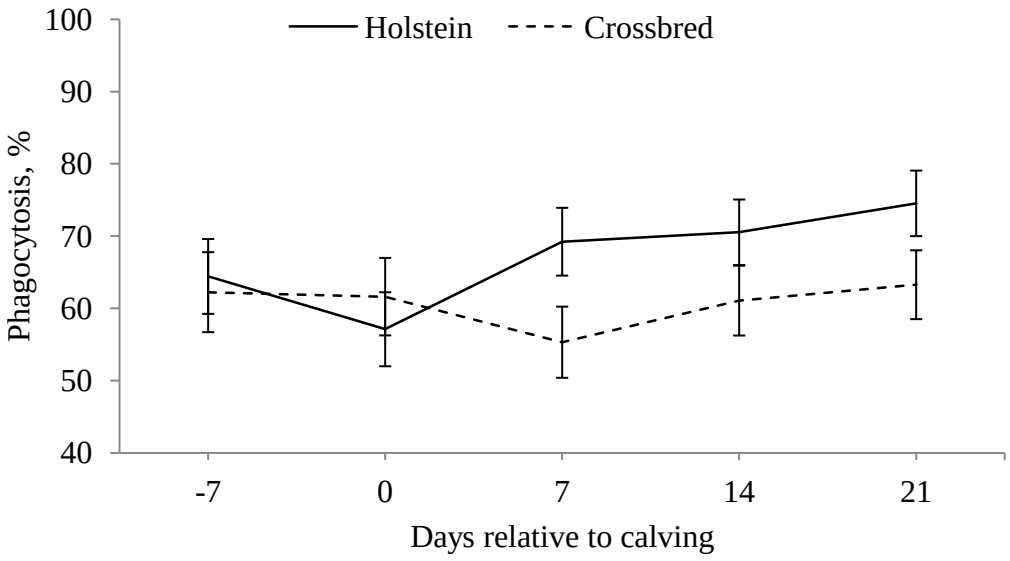

Figure 10. Percentage of PA+ PMNL from 7 d before calving to 21 d after calving in Holstein and Montbéliarde-sired crossbred cows. Breed – $P = 0.14$; study day – $P = 0.10$; and, breed by study day – $P = 0.08$.

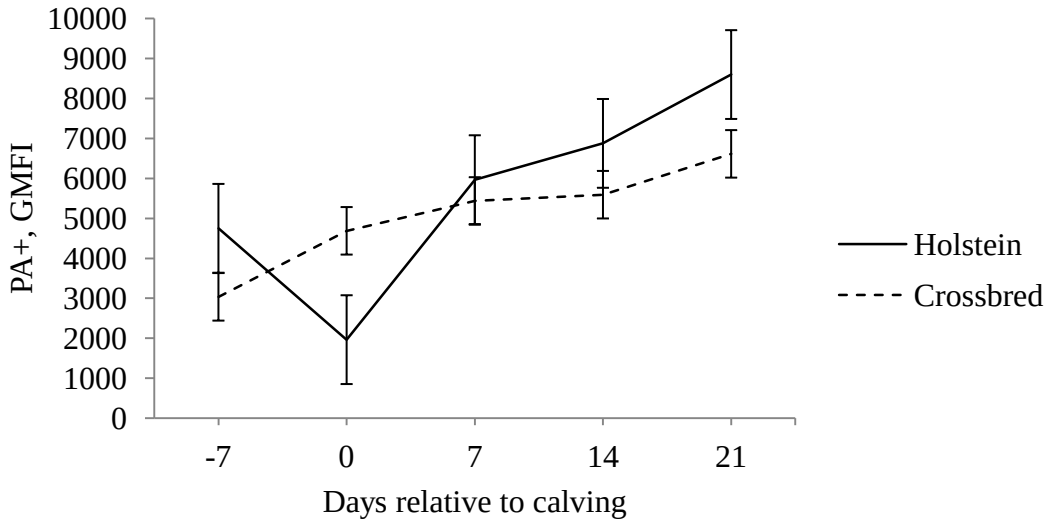

Figure 11. Intensity of PA of PA+ PMNL from 7 d before calving to 21 d after calving in Holstein and Montbéliarde-sired crossbred cows. Breed – $P = 0.54$; study day – $P < 0.01$; and, breed by study day – $P = 0.09$.

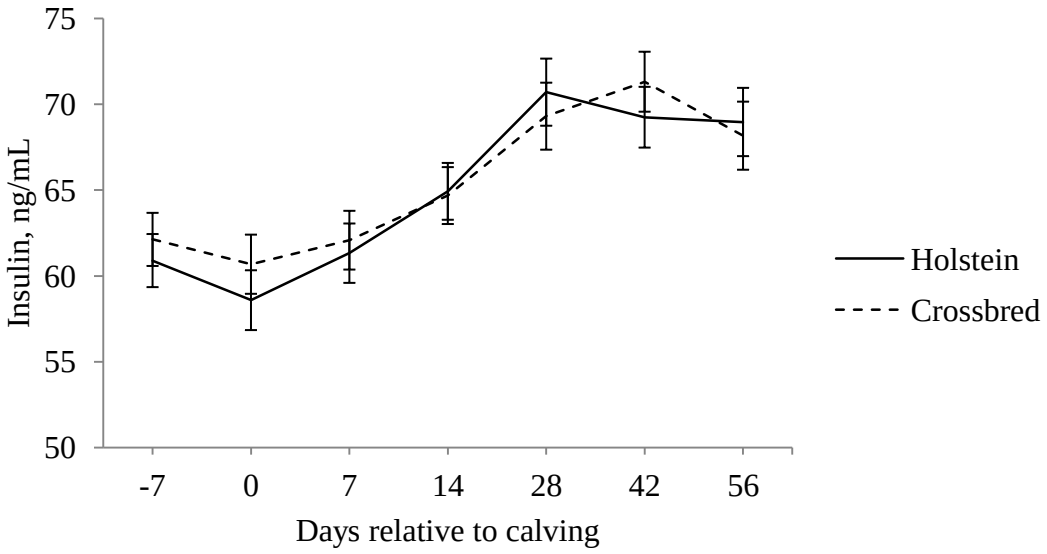

Figure 12A. Concentrations of somatotropic axis hormones from 7 d before calving to 56 d after calving in Holstein and Montbéliarde-sired crossbred cows. Insulin concentration: breed – $P = 0.69$; day – $P < 0.01$; and, breed by study day– $P = 0.91$.

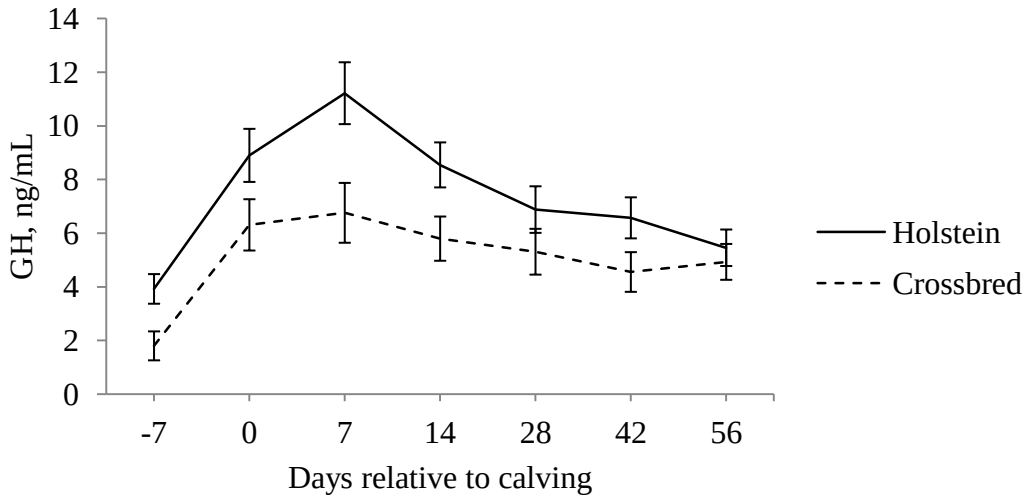

Figure 12B. Concentrations of somatotropic axis hormones from 7 d before calving to 56 d after calving in Holstein and Montbéliarde-sired crossbred cows. Growth hormone (GH) concentration: breed – $P < 0.01$; day – $P < 0.01$; and, breed by study day– $P = 0.47$.

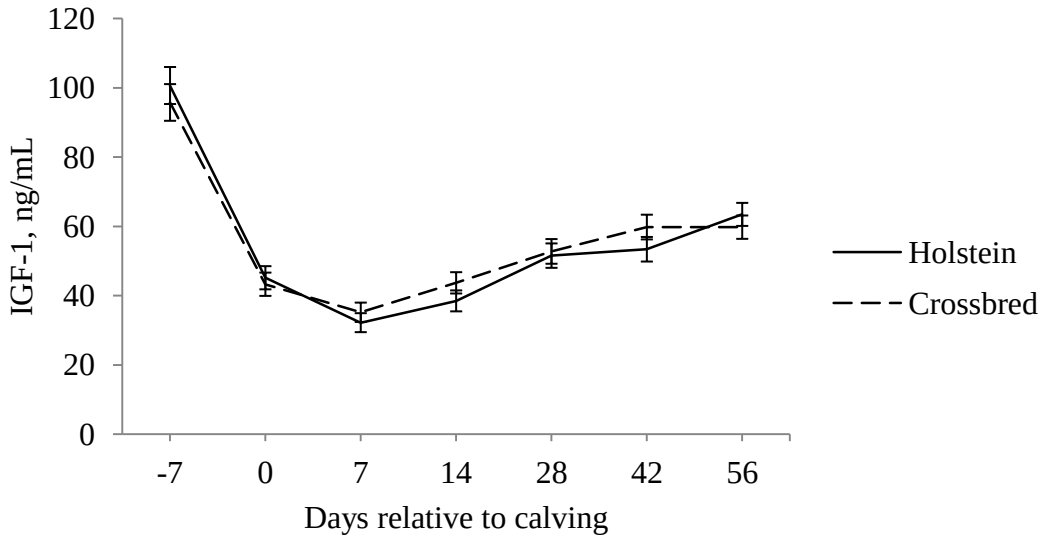

Figure 12C. Concentrations of somatotropic axis hormones from 7 d before calving to 56 d after calving in Holstein and Montbéliarde-sired crossbred cows. Insulin like growth factor (IGF)-1 concentration: breed – $P = 0.82$; day – $P < 0.01$; and, breed by study day– $P = 0.29$.

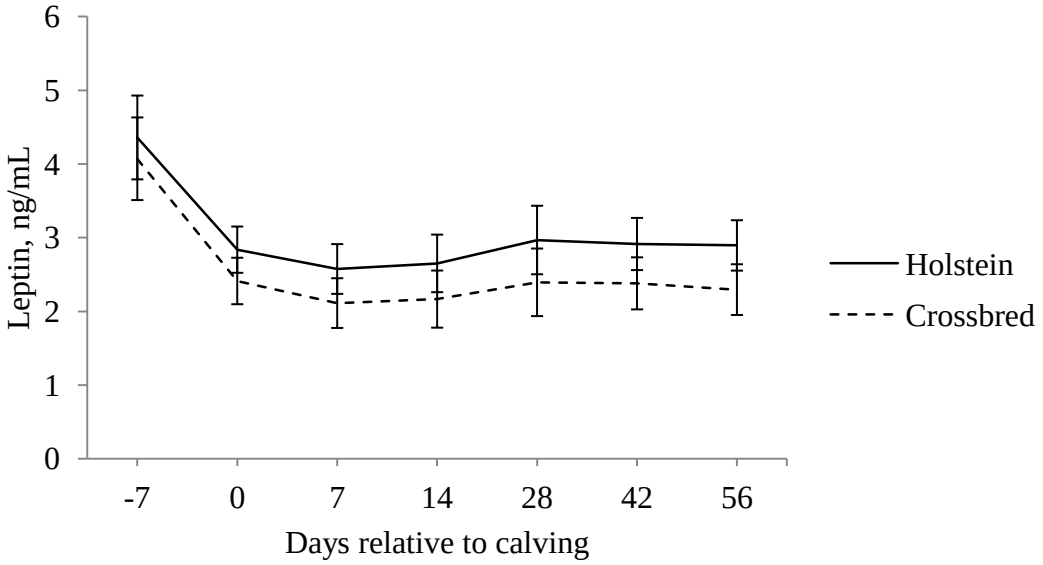

Figure 13. Leptin concentration from 7 d before calving to 56 d after calving in Holstein and Montbéliarde-sired crossbred cows. Breed – $P = 0.30$; day – $P < 0.01$; and, breed by study day– $P = 1.00$.

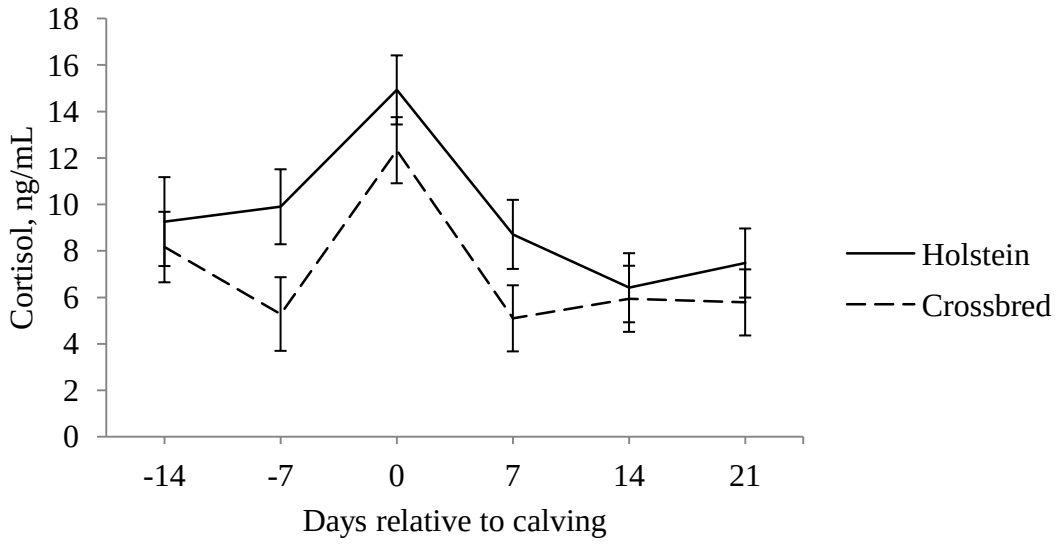

Figure 14. Cortisol concentration of Holstein and crossbred cows from 14 d before calving to 21 d after calving. Effect: breed – $P < 0.01$; day – $P < 0.01$; and, breed by study day– $P = 0.70$.

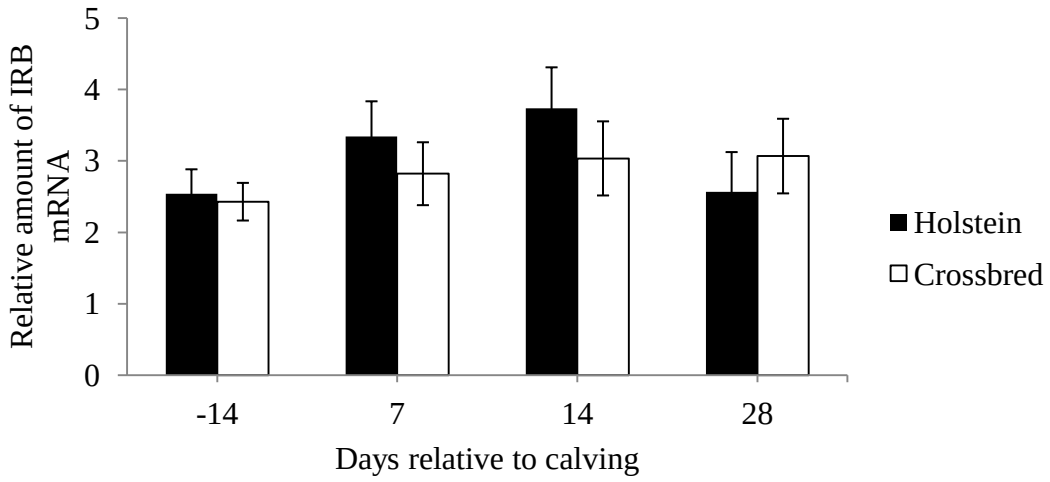

Figure 15A. Relative amount of liver mRNA of genes related to the somatotropic axis. Insulin receptor (IR) B mRNA: breed – $P = 0.69$; day – $P = 0.02$; and, breed by study day – $P = 0.02$.

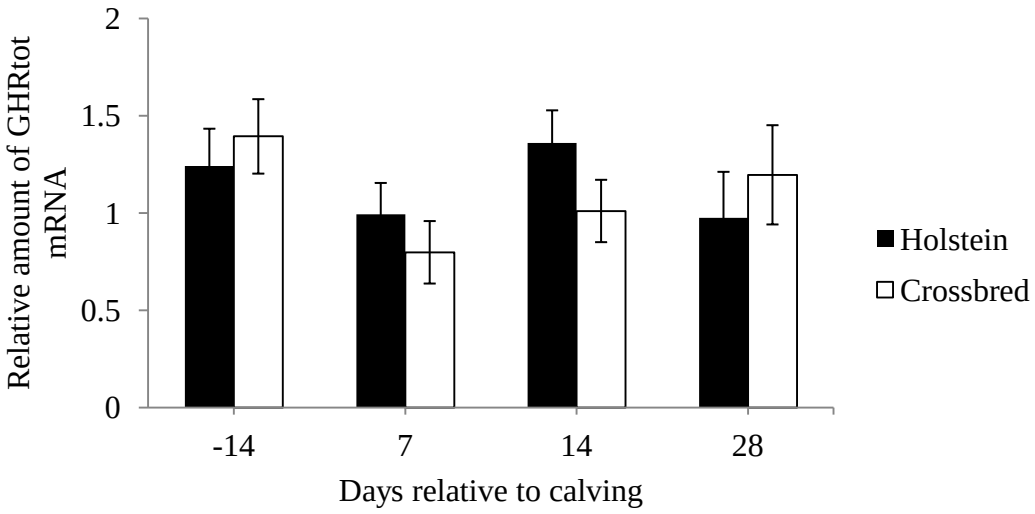

Figure 15B. Relative amount of liver mRNA of genes related to the somatotropic axis. Total growth hormone receptor (GHRtot) mRNA: breed – $P = 0.81$; day – $P = 0.08$; and, breed by study day – $P = 0.18$.

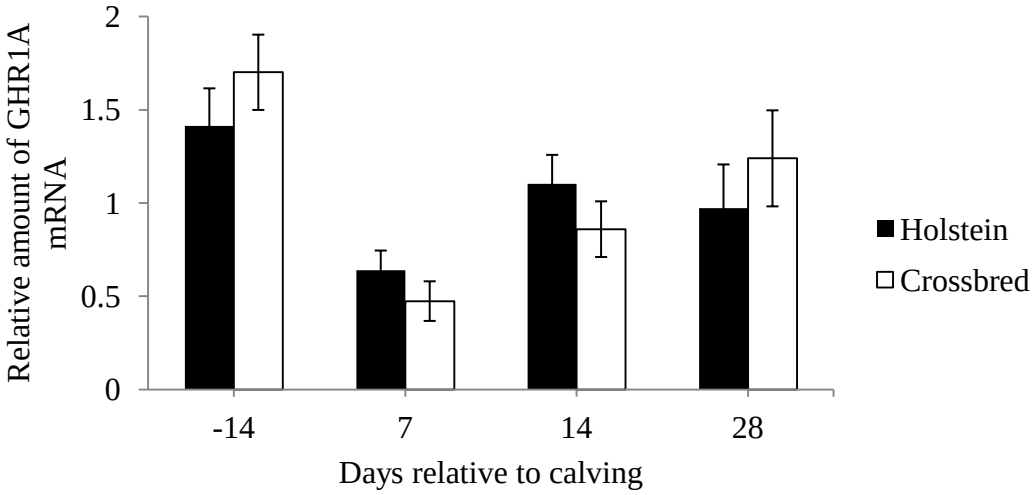

Figure 15C. Relative amount of liver mRNA of genes related to the somatotropic axis. Growth hormone receptor variant 1A (GHR1A) mRNA: breed – $P = 0.83$; day – $P < 0.01$; and, breed by study day – $P = 0.14$.

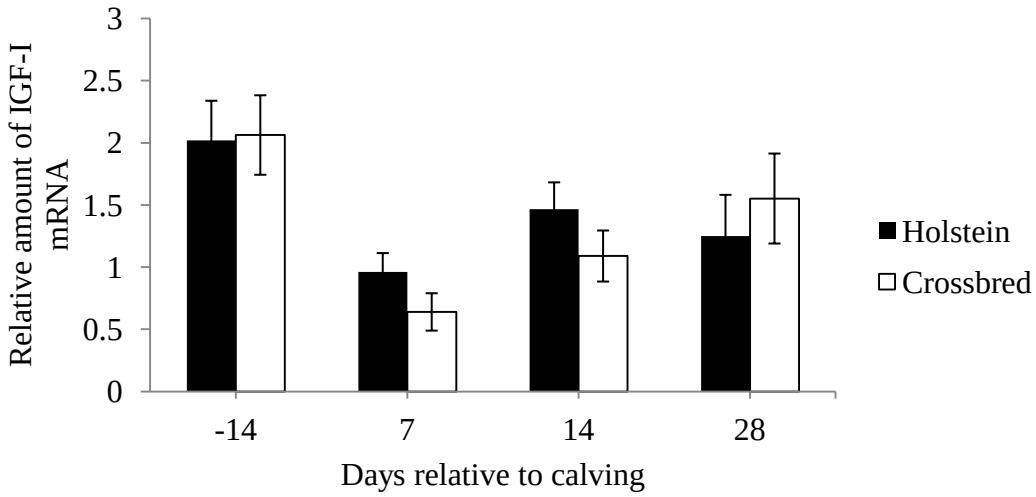

Figure 15D. Relative amount of liver mRNA of genes related to the somatotropic axis. Insulin like growth factor (IGF)-1 mRNA: breed – $P = 0.70$; day – $P < 0.01$; and, breed by study day – $P = 0.40$.

CHAPTER 4: Summary

The main goals of the studies presented herein were to determine the associations between heterosis and innate immune responses, health and ovarian function during the transition period of dairy cows. In these studies, such an association was evaluated by comparing purebred Holstein and Montbéliarde-sired crossbred dairy cows.

In chapter 2, it was demonstrated that Montbéliarde-sired crossbred and Holstein cows had a similar body weight (**BW**), energy corrected milk (**ECM**) yield and metabolites related to energy balance throughout the study. These similarities between breeds reduce the risk of confounding effect of these variables on outcomes such as dry matter intake (**DMI**), innate immune responses and health. Additionally, in chapter 3, it was demonstrated that both breeds had uncoupling of the somatotropic axis after calving. Thus, the differences in postpartum health and immune response between breeds reported in the studies were not confounded by differences in the degree of uncoupling of the somatotropic axis. Based on the results from this thesis we concluded that heterosis was likely the main factor that caused crossbred cows to have improved periparturient immunity compared with Holstein cows.

Although one could argue that the difference in prepartum DMI between breeds was one of the culprits for the impaired periparturient innate immune function and postpartum health of Holstein cows, the lack of differences between breeds in metabolites related to energy balance does not support the latter explanation.

Crossbreeding of dairy cows is expected to produce increased immune function of peripartum cows through heterosis and through complementarity of breeds. Interestingly,

however, the benefits of crossbreeding on immune function were mainly identified on the day of calving because of the considerably larger challenges to the immune system during the peripartum period. This probably led to differences in PMNL activity on the day of calving, which was likely associated with the greater likelihood of postpartum uterine disorders. Furthermore, the compromised uterine health and increased cortisol concentration likely influenced negatively the postpartum ovarian activity of Holstein cows, as explained in chapter 2.

In conclusion, this thesis demonstrates that crossbred cows had improved periparturient innate immune response, postpartum health and ovarian function compared with Holstein cows (Figure 16). Given the increased public pressure for strict rules on the use of antibiotics in livestock production, this research demonstrates that crossbreeding can be an alternative for dairy producers to consider in an attempt to reduce antibiotic usage in the postpartum period of dairy cows. The economic aspect of crossbreeding in dairy cattle, however, should be carefully studied by producers considering the possible differences in milk yield and components between Holstein and crossbred cows, and also the milk pricing system. Thus, dairy crossbreeding programs should have a systematic breeding strategy to produce offspring with comparable milk production and improved health performance compared to purebred counterparts.

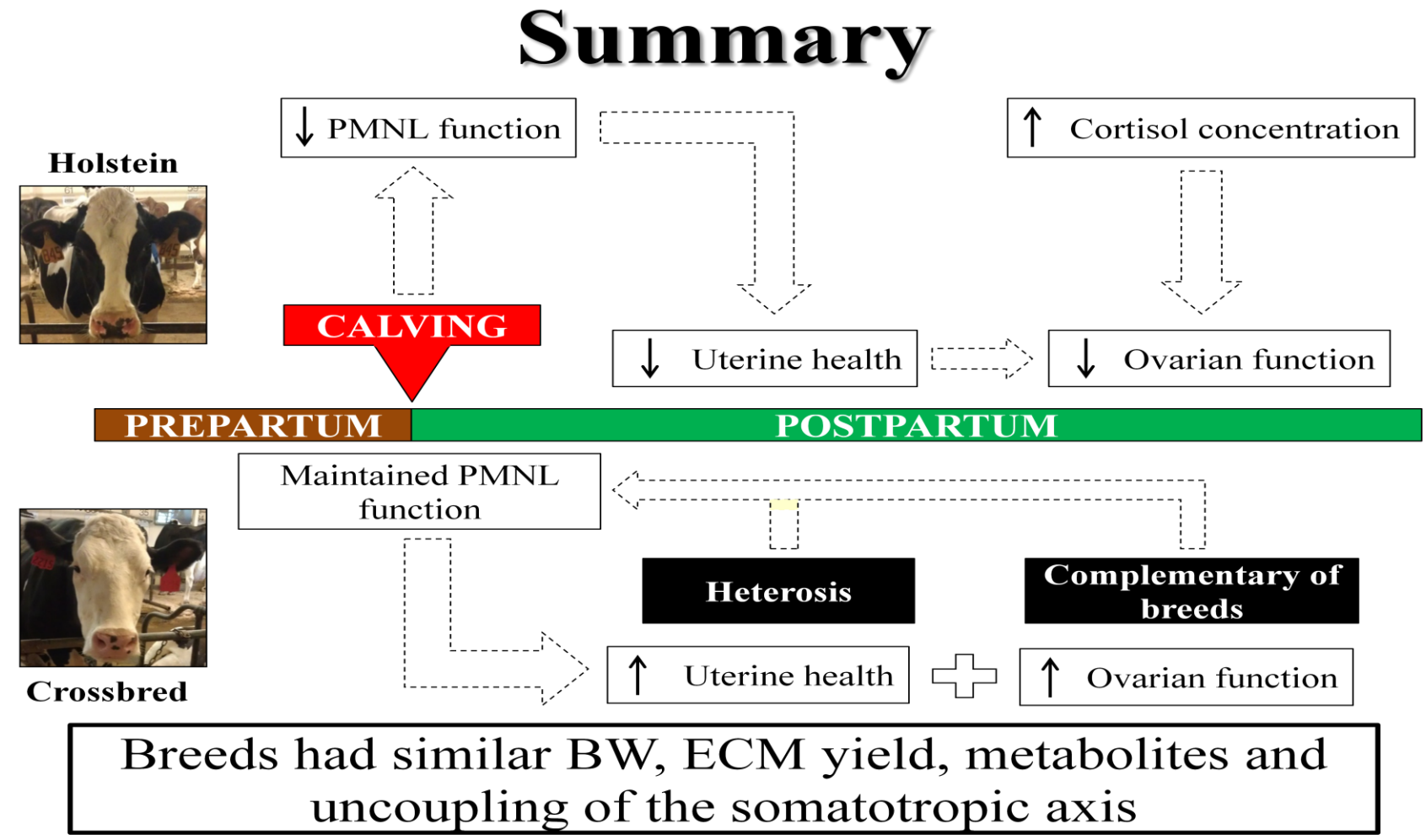

Figure 16. Summary

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