Antibody and cell-mediated immune responses and survival between Holstein and Norwegian Red × Holstein Canadian calves

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ABSTRACT

As an extension of a former study, the objectives of this study were to evaluate purebred Holstein (HO; $n = 140$) and crossbred Norwegian Red × Holstein (NRFX; $n = 142$) calves for antibody (AMIR) and cell-mediated immune responses (CMIR) as well as survival. Blood was collected on d 0, 14, and 21, and calves were immunized on d 0 and 14 with type 1 (*Candida albicans*) and type 2 (hen egg white lysozyme) antigens, which have been shown to induce CMIR and AMIR, respectively. Day 21 background skin-fold measurements of either side of the tail-fold were taken and intradermal injections of test (type 1 antigen) and control (phosphate saline buffer) were administered. Day 23 final skin-fold measurements were taken to assess delayed type hypersensitivity as an indicator of CMIR. Survival data were obtained from CanWest Dairy Herd Improvement. Statistical Analysis System general linear models were used to analyze all immune response and survival data and to determine statistical significance between breeds. Results showed that NRFX had greater primary IgM, IgG, IgG1, and secondary IgG1 antibody response, as well as greater primary IgG1:IgG2 ratio to the type 2 antigen compared with HO. The NRFX also had greater primary IgG1 and IgG2, and secondary IgG2 antibody response as well as greater primary IgG1:IgG2 ratio to the type 1 antigen. The NRFX calves had a tendency toward greater survival from age at immune response testing to calving. No difference was observed between breeds for other secondary antibody response traits or delayed type hypersensitivity. Results indicate NRFX have greater AMIR and therefore may have enhanced defense against extracellular pathogens. This may contribute to increased survival compared with HO. Both breeds, however, likely have similar defense against intracellular pathogens, because no differences in CMIR were observed. In general, these results may suggest

that crossbreeding could improve resistance to certain diseases in dairy calves, resulting in decreased input costs to producers for crossbred calves compared with purebred calves. However, more research with larger sample sizes and different breeds should be conducted to confirm these results and obtain a complete picture of the benefits of crossbreeding on immune response traits in calves.

Key words: immune response, crossbreeding, calf

INTRODUCTION

Inbreeding in the Canadian Holstein (HO) population has been steadily increasing, with the average inbreeding level reported at 5.75% for 2007, with an average annual increase of 0.08% for the period from 2000 to 2007 (Canadian Dairy Network, 2008). Increases in inbreeding have been associated with fitness problems including increased risk of disease occurrence and reduced survival (Sorensen et al., 2006; Mc Parland et al., 2007). This reduced performance, also known as inbreeding depression, can be attributed to increased homozygosity caused by inbreeding and therefore increasing the chance of homozygosity for deleterious recessive alleles. One study reported that with each 1% increase in inbreeding, SCS increased by 0.012, which could be related to weaker host defense in more inbred animals and therefore could lead to increased mastitis prevalence (Miglior et al., 1995). Similarly, another study showed cows that were 5% inbred compared with those that were 2% inbred had higher SCC and a greater incidence of mastitis (Sorensen et al., 2006). Increases in inbreeding have also been associated with reduced survival, with one study finding that as inbreeding levels increased survival decreased, and this was the case across all lactations (Thompson et al., 2000).

Increased inbreeding of the HO breed may also relate to increased disease occurrence in HO calves, which could have downstream effects on both productivity and survival of the dairy cow. Studies have shown that increases in inbreeding can increase the occurrence of dystocia (Adamec et al., 2006). Calves born to dams

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with severe dystocia have greater odds of disease occurrence and decreased survival (Lombard et al., 2007). Disease occurrence in calves has also been associated with problems as the calf matures. Studies have shown that disease occurrence early in calthood has been associated with decreased growth in the first 3 mo of life (Virtala et al., 1996). Calves experiencing disease also have reduced survival to first calving. An early study evaluating calves from different farms in southwestern Ontario found that calves with disease occurrence in the first 90 d of life were 2.5 times more likely to die or be culled before first calving (Waltner-Toews et al., 1986).

The decreased fitness associated with inbreeding may be solved by crossbreeding with more robust breeds such as the Norwegian Red (NR), which have been selected for improved disease resistance and fertility since the 1970s (Heringstad et al., 2000). Crossbreeding increases heterozygosity by introducing favorable genes from other breeds; this alleviates inbreeding depression, and therefore increases performance of dairy cattle (VanRaden and Sanders, 2003). Many studies have demonstrated that crossbreds have increased survival compared with purebreds, which may be related to enhanced health in crossbreds (Touchberry, 1992; Heins et al., 2006). One study evaluating calves sired by Jersey $\times$ HO mated to HO dams compared with purebred HO calves found that the calves with crossbred sires had higher serum protein and serum IgG levels, as well as increased survival and decreased incidence of scours compared with calves with purebred sires (Maltecca et al., 2006). Similarly, another preliminary study evaluating immune response in purebred HO and crossbred Norwegian Red $\times$ Holstein (NRFX) calves found that crossbred calves had significantly greater primary IgG antibody response compared with purebreds (Begley et al., 2009a).

Many studies have been conducted to evaluate whether enhanced immune response is associated with decreased disease occurrence. Initial studies done in mice showed that mice selected for high antibody-mediated immune response (AMIR) had better resistance to extracellular pathogens compared with low responders (Biozzi et al., 1979). Subsequent studies in livestock demonstrated the ability to identify both high and low AMIR and cell-mediated immune responses (CMIR) in chickens (Pinard et al., 1992), pigs (Mallard et al., 1992), and cattle (Hernandez et al., 2003). Studies in cattle indicated that animals with high AMIR have decreased SCC (Rupp et al., 2007) and decreased occurrence of mastitis in 2 out of 3 herds evaluated (Wagter et al., 2000) compared with low responders. Another study found that HO cattle identified as low CMIR or low AMIR had increased risk of disease occurrence for a

number of diseases, including mastitis, metritis, ketosis, and retained fetal membranes, compared with cattle identified as high CMIR or high AMIR (De La Paz, 2008). This suggests that animals identified as having an enhanced immune response may also have decreased disease occurrence and that these traits would be well suited to examine potential health differences between purebred and crossbred cattle.

To test the hypothesis that crossbred calves will have greater immune responsiveness and increased survival compared with purebreds, the preliminary study previously done by Begley et al. (2009a) was extended to evaluate a larger sample size of purebred HO and crossbred NRFX calves for AMIR and CMIR. Furthermore, new traits including survival to first calving and other immune response traits including IgM antibody response and IgG1 and IgG2 responses to a type 1 antigen were also evaluated. The test antigen *Candida albicans*, used in this study to evaluate CMIR (type 1 response), has been shown in several previous studies to preferentially induce a type 1 immune response bias (Romani, 2000; Heriazon et al., 2009a,b), whereas the test antigen hen egg white lysozyme (HEWL), used to evaluate AMIR (type 2 response), has been shown to preferentially induce a type 2 immune response bias (Raymond and Wilkie, 2004; Raymond et al., 2006; Hine et al., 2011).

MATERIALS AND METHODS

Experimental Design: Animals and Immunization

Purebred HO (n = 140; female n = 123, male n = 17) and crossbred NRFX (n = 142; female n = 122, male n = 20) calves, 2 to 6 mo old (mean age HO = 4.24 mo, mean age NRFX = 4.11 mo) from various sires (NR n = 6, with total merit index having 21% emphasis on mastitis and 2% emphasis on other diseases; European Red Dairy Breed Association, 2009; and HO n = 72, with lifetime profit index having 15% emphasis on health and fertility; Canadian Dairy Network, 2010) on 26 different commercial dairy farms in southwestern Ontario were evaluated for both AMIR and CMIR. On all farms, similar numbers and similar ages of both HO and NRFX calves were evaluated. The 67 NRFX and 68 HO previously evaluated by Begley et al. (2009a) were included in this study as part of the 142 NRFX and 140 HO. However, raw data from the previous calves were used and reanalyzed using the statistical models described in the statistical analysis section. Moreover, these calves were evaluated for additional traits added to this study, which included survival, IgM antibody response, and IgG1 and IgG2 responses to the type 1 antigen (*C. albicans*). Calves were immunized using a

patented and previously described protocol (Wagter et al., 2000; Begley et al., 2009a; Heriazon et al., 2009b) with type 1 (*C. albicans*) and type 2 (HEWL) antigens known to preferentially induce CMIR and AMIR biases, respectively. Even though CMIR and AMIR work together, type 1 responses are generally associated with CMIR, which predominate in the control of intracellular pathogens and are of the IgG2 antibody isotype, whereas type 2 responses are associated with AMIR and are of the IgG1 isotype predominant in control of extracellular pathogens (Furesz et al., 1998; Crawley et al., 2003). Briefly, d 0 calves were bled via the tail vein and immunized intramuscularly in the neck with 0.5 mg of *C. albicans* (type 1 antigen; Greer Laboratories Inc., Lenoir, NC), 0.5 mg of HEWL (type 2 antigen; Sigma-Aldrich, St. Louis, MO), and 0.5 mg of adjuvant (Quil A, Brenntag, Toronto, Ontario, Canada) dissolved in 1.0 mL of PBS. On d 14, calves were again bled and immunized intramuscularly as described previously with type 1 and type 2 antigens. On d 21, calves were bled and background skin-fold measurements were taken, in triplicate, of either side of the tail-folds using Harpenden skin calipers (Creative Health Products Inc., Ann Arbor, MI). Intradermal injections of control (PBS) and test (0.1 mg of type 1 antigen dissolved in 0.1 mL of PBS) were administered on either side of the tail-folds. On d 23, 48 h later, final skin-fold measurements of the tail-folds were taken in triplicate.

Evaluation of AMIR

Sera were obtained from blood samples collected on d 0, 14, and 21 by centrifugation ($700 \times g$ for 20 min) and stored at -20°C until time of analysis. Antibody activity to type 1 and type 2 test antigens was evaluated by ELISA, as an indicator of AMIR, using a previously described protocol (Wagter et al., 2000; Begley et al., 2009a). Briefly, 96-well plates were coated with either type 1 or type 2 antigen dissolved in carbonate buffer and all plates were blocked with 3% Tween 20, 1.5% BSA, and 1.5% fetal calf serum in PBS. Sera samples and controls, respectively, were diluted to 1:50 and 1:300 for plates coated with the type 2 antigen and 1:200 and 1:400 for plates coated with the type 1 antigen. Positive and negative controls were obtained by pooling sera samples preimmunization (negative control) and sera samples obtained on d 21 (positive control). All controls and samples were added to the plate in quadruplicate, allowing 10 samples to be run per plate. Sheep anti-bovine IgG, IgG1, and IgG2 conjugated to alkaline phosphatase (Cedarlane, Burlington, Ontario, Canada) were diluted 1:250, 1:500, and 1:100, and mouse anti-bovine IgM conjugated to horseradish peroxidase (AbD Serotec, Raleigh, NC)

was diluted 1:10,000 and added to plates coated with the type 2 antigen. Sheep anti-bovine IgG1 and IgG2 conjugated to alkaline phosphatase were diluted 1:500 and 1:100 and added to plates coated with the type 1 antigen. Plates were read using an EL311 automatic ELISA plate reader (Bio-Tek Instruments, Highland Park, VT) at a wavelength of 405 nm until positive controls reached an optical density (OD) ≥ 0.999 for those plates in which antibody conjugated to alkaline phosphatase was added. Plates evaluating IgM antibody activity were initially read at a wavelength of 630 nm and then stopped once positive controls reached an OD of 0.45 using stop solution (1 M H_2SO_4 , 5.4 mL of concentrated H_2SO_4 added to 94.6 mL of Milli-Q water; Millipore, Etobicoke, Ontario, Canada) and re-read at a wavelength of 450 nm to obtain final OD values. All OD values of samples were corrected to the positive controls to compare samples run on different plates and different days. Corrected means of each dilution for samples were added together to obtain an additive OD value. To minimize variation, if a plate had a CV $>10\%$ for positive controls, the entire plate was repeated, and in the case of a sample with a CV $>10\%$, the sample was repeated.

Evaluation of CMIR

Triplicate skin-fold measurements taken on d 21 and 23 were averaged and used to determine delayed type hypersensitivity (DTH) as an indicator of CMIR (Hernandez et al., 2005).

Evaluation of Survival

Data on calf death and culling events were obtained from CanWest DHI (Guelph, Ontario, Canada). These data included the dates the animals left the herd and the reasons for leaving, which included death and culling for reproduction and health (illness). Only data on heifer calves ($n = 245$) were included in the analysis, because male dairy calves are always culled. Survival was evaluated at 3 time points, which included survival from age at immune response testing to first breeding, from first breeding to calving, and overall from age at immune response testing to calving. If no culling or death event was recorded from age at immune response testing to first recorded breeding, the calf was assumed to survive to first breeding. Only calves that survived to first breeding were included in the analysis for survival from first breeding to calving. If no culling or death event was recorded from first recorded breeding to time of calving, the calf was assumed to survive to calving. All calves were included in the analysis for overall survival from age at immune response testing to calving,

and those calves that did not have a recorded death or culling event were assumed to have survived from age at immune response testing to calving.

Statistical Analysis

A SAS general linear model was used to analyze all immune response data and determine statistical significance between breeds. A P -value of ≤ 0.05 was considered statistically significant and trends were reported at $P \leq 0.10$. All data except for primary antibody response to the type 2 antigen were initially analyzed using the following model:

$$Y_{ijklmno} = \mu + \text{year}_j + \text{farm} \times \text{breed}_{kl} + \text{breed} \times \text{sex}_{lm} \\ + \text{breed} \times \text{age}_n + \text{breed} \times \text{time } 0_{ijklmno} + e_{ijklmno},$$

which differed from the previous model described by Begley et al. (2009a). In this model, $Y_{ijklmno}$ = the immune response trait being evaluated for the i th calf during the j th year in the k th herd of the l th breed, m th sex, and n th age with an o th baseline measurement of immune response trait; year_j = the year of testing (where j = 2006 or 2007); farm_k = herd of origin (where k = farm A through Z); breed_l = fixed effect of breed (where l = HO or NRFX); sex_m = sex of calf (where m = male or female); age_n = age of calf in months at date of testing (where n = 2 through 6 mo); $\text{time } 0_o$ = d 0 (for primary antibody response traits) or d 14 (for secondary antibody response traits) or 0 h (for DTH response trait) value of immune response trait (where o = value of baseline measurement fitted as covariate); and e = residual error. Primary antibody response to the type 2 antigen was analyzed using a similar model; however, it did not include the term $\text{time } 0$, because the baseline measurements are similar to background OD values. Interaction terms remained in the model, if significant ($P \leq 0.05$); however, if the term was nonsignificant, it was dropped from the model and replaced with the single terms in the interaction. All single terms were left in the model, whether significant or not. If the interaction term was found to be significant, the Bonferroni test was applied to correct for multiple comparisons.

AMIR. To analyze primary IgG, IgG1, IgG2, and IgM antibody response to the type 2 antigen and primary IgG1 and IgG2 antibody response to the type 1 antigen, log-transformed d 14 OD values were tested as the response variable. For primary IgG, IgG1, and IgG2 antibody responses to the type 2 antigen, the log of d 0 OD values were subtracted from the log of d 14 OD values before analysis, but for all other primary antibody response traits, the log of d 0 OD values were fitted as

a covariate. Similarly, to analyze primary IgG1:IgG2 ratios to type 1 and type 2 antigens, the transformed IgG1:IgG2 ratios at d 14 were tested as response variables, with transformed IgG1:IgG2 ratio at d 0 fitted as a covariate for analysis to the type 1 antigen only. To evaluate secondary IgG, IgG1, and IgG2 response to the type 2 antigen and secondary IgG1 and IgG2 response to the type 1 antigen, transformed d 21 OD values were tested as response variables, with transformed d 14 OD values fitted as a covariate. Similarly, to analyze secondary IgG1:IgG2 ratios to both type 1 and type 2 antigens, transformed ratios at d 21 were tested as the response variable and transformed ratios at d 14 fitted as a covariate.

DTH. To evaluate DTH as an indicator of CMIR, the log of the ratio of test at 48 h/control at 48 h was tested as a response variable, with the transformed ratio of baseline measurements (test at 0 h/control at 0 h) fitted as a covariate in the model (Heriazon et al., 2009a; Hine et al., 2011).

A SAS general linear model was also used to analyze survival data, with the following model being used: $Y_{ijklm} = \text{farm} \times \text{year}_{kl} + \text{breed}_m + e_{ijklm}$ (Heins et al., 2006), where Y_{ijklm} = survival _{i} for the j th cow from the k th farm born in the l th year of the m th breed (where i = survival from age at immune response testing to first breeding or first breeding to calving or age at immune response testing to calving); farm_k = herd of origin (where k = A to Z), year_l = birth year (where l = 2006 or 2007); breed_m = fixed effect of breed (where m = HO or NRFX); and e = residual error term. All terms were left in the model whether significant or not. Least squares means (LSM), obtained from the model, for each breed were converted to a percentage for clarity by multiplying LSM by 100.

The PROC UNIVARIATE procedure was used to test residuals for normality (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests). All data were log-transformed, because this best satisfied the assumptions of ANOVA, except for survival data, because this is a binary trait and therefore each calf would be assigned a value of 1 (calf survived) or 0 (calf was culled or died). However, for clarity, LSM for untransformed data will be presented in all figures and tables.

RESULTS

AMIR

Results show that NRFX calves had both significantly greater IgM (LSM = 0.987 OD; P = 0.036) and IgG (LSM = 0.376 OD; P = 0.019) antibody response to the type 2 antigen compared with HO calves (LSM

Table 1. Least squares means and standard error of primary and secondary antibody isotype responses expressed in optical density values (OD) and IgG1:IgG2 ratios to type 2 antigen between Holstein (HO) and Norwegian Red $\times$ Holstein (NRFX) calves

Trait	Breed		SE	P-value
	HO	NRFX		
Primary IgG1 (OD)	0.248	0.333	0.042	0.005
Primary IgG2 (OD)	0.244	0.212	0.052	0.391
Primary IgG1:IgG2 ratio	1.002	1.360	0.131	0.003
Secondary IgG1 (OD)	1.193	1.331	0.085	0.005
Secondary IgG2 (OD)	0.988	1.103	0.102	0.070
Secondary IgG1:IgG2 ratio	1.166	1.356	0.352	0.552

for IgM = 0.736 OD, LSM for IgG = 0.264 OD; Figure 1). Conversely to what was observed for primary IgG antibody response to the type 2 antigen, initially a significant farm $\times$ breed effect ($P = 0.002$) was observed for secondary IgG antibody response to the type 2 antigen. Results showed that 3 out of 26 farms displayed a significant breed effect, 1 of 26 farms showed a trend ($P = 0.075$), and another 1 of 26 farms showed a tendency toward a breed effect ($P = 0.124$). On all farms where a significant breed effect was observed, NRFX had the greater secondary IgG antibody response. Similarly, on the farm, where a trend was observed, NRFX also had a higher secondary IgG antibody response, although HO had higher antibody response at the farm, where a tendency toward a breed effect was observed. However, when the Bonferroni test was applied to correct for multiple comparisons, only 1 out of 26 farms still displayed a significant breed effect ($P = 0.031$), with the breed effect on the other 25 farms being nonsignificant. The overall effect of breed for secondary IgG antibody

response to the type 2 was also nonsignificant (LSM for NRFX = 1.08 OD, LSM for HO = 1.01 OD).

Table 1 depicts ELISA results for primary and secondary IgG1 and IgG2 antibody isotypes as well as IgG1:IgG2 ratios to the type 2 antigen. Results show that NRFX had a primary IgG1:IgG2 ratio >1.00 indicative of a type 2 bias compared with HO, which had a primary IgG1:IgG2 ratio of 1.00 indicative of a balance in isotype production (Table 1). Both breeds had secondary IgG1:IgG2 ratios >1.00 . The NRFX calves had significantly greater primary IgG1 antibody response ($P = 0.005$), secondary IgG1 antibody response ($P = 0.005$) and primary IgG1:IgG2 ratio ($P = 0.003$) to the type 2 antigen compared with HO. A trend toward NRFX having greater secondary IgG2 antibody response ($P = 0.070$) was also observed. No significant difference was observed between breeds for primary IgG2 antibody response and secondary IgG1:IgG2 ratio.

Table 2 shows primary and secondary IgG1 and IgG2 antibody response and primary and secondary IgG1:IgG2 ratios to the type 1 antigen. Both breeds had primary and secondary IgG1:IgG2 ratios >1.00 (Table 2). The NRFX calves had significantly greater primary IgG1 ($P = 0.050$) and IgG2 ($P = 0.050$) antibody response and greater primary IgG1:IgG2 ratio ($P = 0.029$) to the type 1 antigen compared with HO. Initially, a significant breed $\times$ age ($P = 0.034$) interaction was observed for secondary IgG2 antibody response to the type 1 antigen. A significant breed effect was observed for 2 out of the 5 age groups (5 mo, $P = 0.026$; and 6 mo, $P = 0.014$) and, although not significant, a tendency toward a breed effect was also observed in 1 out of the 5 age groups (4 mo, $P = 0.115$). In those age groups, where a significant breed effect was observed, NRFX had a greater response compared with HO, and in the age group, where a tendency toward a breed effect was observed, HO had a greater response. When the Bonferroni correction was applied, the breed effect at each age group was no longer significant. However, the overall breed effect for secondary IgG2 antibody response was

Figure 1. Least squares means (LSM) and standard errors (SE) of primary IgM and IgG antibody response to type 2 antigen for Holstein (HO) and Norwegian Red $\times$ Holstein (NRFX) calves expressed in optical density (OD) values. Different symbols above SE bars represent statistical significance ($P \leq 0.05$) between breeds for each class of antibody.

Table 2. Least squares means (LSM) and standard error of primary and secondary antibody isotype responses expressed in optical density values (OD) and IgG1:IgG2 ratios to type 1 antigen for Holstein (HO) and Norwegian Red $\times$ Holstein (NRFX) calves

Trait	Breed		SE	P-value
	HO	NRFX		
Primary IgG1 (OD)	0.997	1.593	0.165	0.050
Primary IgG2 (OD)	0.311	0.400	0.028	0.050
Primary IgG1:IgG2 ratio	3.446	4.263	0.118	0.029
Secondary IgG1 (OD)	1.377	1.324	0.100	0.418
Secondary IgG2 (OD)	0.381	0.447	0.053	0.033
Secondary IgG1:IgG2 ratio	3.353	2.750	0.182	0.322

significant ($P = 0.033$; Table 2). No significant difference was observed between breeds for secondary IgG1 antibody response and secondary IgG1:IgG2 ratio.

CMIR

No significant difference was observed for DTH response at 48 h to the type 1 antigen between NRFX (LSM = 2.45) and HO (LSM = 2.50).

Survival

The NRFX calves had a trend toward a greater proportion of calves that survived from age at immune response testing (LSM = 93.39%; $P = 0.079$) to calving compared with HO (LSM = 86.71%; Table 3). Similarly, although not significant, a tendency toward greater proportions of NRFX calves surviving from age at immune response testing to first breeding (NRFX LSM = 98.35%, HO LSM = 94.59%) and first breeding to calving (NRFX LSM = 94.35%, HO LSM = 91.99%; Table 3) was observed. Raw data showed that NRFX had 3 out of 122 calves tested that were culled or died between age at testing to first breeding, 6 out of the 119 calves that survived to first breeding that were culled or died between first breeding to calving and, therefore, 9 out of 122 calves tested that were culled or died between age of testing to calving. Purebreds (HO) had 7 out of 123 calves tested that were culled or died between age at testing to first breeding, 10 out of 116

calves that survived to first breeding that were culled or died between first breeding to calving and, therefore, 17 out of 123 calves tested that were culled or died between age at testing to calving. The effect of farm $\times$ year on all 3 survival traits was significant.

Effects of Variables Fit to the Model

Results for effects of each variable fitted to the model on immune response traits evaluated to the type 2 antigen are summarized in Table 4. The effects of each variable in the model on immune response traits evaluated to the type 1 antigen are summarized in Table 5. The numbers in both Tables 4 and 5 represent the P -values of each variable fitted in the different models used to analyze the immune response traits evaluated.

DISCUSSION

Although limited studies have been done to assess calf health and even fewer studies on the effects of inbreeding on calves, many studies in lactating cows have demonstrated that inbreeding is associated with increased disease occurrence (Miglior et al., 1995; Sorensen et al., 2006). Therefore, inbred calves are likely to have increased disease occurrence. Studies that have evaluated calf health show that calves with disease incidences early in calthood have problems later in life, including reduced survival (Waltner-Toews et al., 1986; Virtala et al., 1996). This signifies the importance of

Table 3. Least squares means (LSM) and standard error of proportion of Holstein (HO) and Norwegian Red $\times$ Holstein (NRFX) calves that survived from age at immune response testing to first breeding, first breeding to calving, and overall from age at immune response testing to calving expressed in percentage (%)¹

Period	Breed		SE	P-value
	HO	NRFX		
Age at immune response testing to first breeding (%)	94.59	98.35	1.98	0.137
First breeding to calving (%)	91.99	94.35	2.55	0.369
Age at immune response testing to calving (%)	86.71	93.39	2.97	0.079

¹Numbers for each breed represent the proportion of individuals that survived for that particular period.

Table 4. Effects of farm, year, age, sex, and baseline measurement of immune response trait (time 0) on the immune response traits to type 2 antigen¹

Trait	Farm	Breed	Year	Age	Sex	Time 0	Farm × breed	Breed × age	Breed × sex	Breed × time 0
Primary IgG type 2 antigen	0.100	0.019	NS	NS	NS	NA	NS	NS	NS	NA
Primary IgM type 2 antigen	NS	0.036	NS	0.109	NS	NS	NS	NS	NS	NS
Secondary IgG type 2 antigen	<0.001	NS	NS	0.078	NS	0.032	0.002	NS	NS	NS
Primary IgG1 type 2 antigen	0.099	0.005	NS	NS	NS	NA	NS	NS	NS	NA
Primary IgG2 type 2 antigen	NS	NS	NS	0.010	NS	NA	NS	NS	NS	NS
Primary IgG1:IgG2 ratio type 2 antigen	NS	0.003	NS	NS	NS	NA	NS	NS	NS	NA
Secondary IgG1 type 2 antigen	0.089	0.005	NS	0.108	NS	0.092	NS	NS	NS	NS
Secondary IgG2 type 2 antigen	NS	0.070	NS	NS	NS	0.048	NS	NS	NS	NS
Secondary IgG1:IgG2 ratio type 2 antigen	NS	NS	NS	NS	NS	0.011	NS	NS	NS	NS

¹Numbers in table represent *P*-values for each effect; NA = not applicable

calves being able to defend themselves against a wide variety of pathogens (both intracellular and extracellular). Early studies in mice showed that enhanced CMIR favors defense against intracellular pathogens, and enhanced AMIR favors defense against extracellular pathogens (Biozzi et al., 1984). Therefore, the objectives of this study were to evaluate both AMIR and CMIR between purebred HO and crossbred NRFX 2- to 6-mo-old calves and determine survival of these calves to parturition.

The NRFX calves had both a significantly greater primary IgM and a higher IgG antibody response to the type 2 antigen compared with HO. However, the overall breed effect for secondary IgG antibody response to the type 2 antigen was nonsignificant. These results are similar to those in previous studies, where crossbred calves had greater primary antibody responses (Begley et al., 2009a) or greater serum IgG and reduced incidence of scours (Maltecca et al., 2006) compared with purebred calves. These results are also similar to those observed in second-lactation cows, where crossbreds had a tendency to have greater primary antibody response, expressed as the percentage increase from d 0 to d 14, compared with purebred HO cattle (Begley et al., 2009b). Other studies have shown that IgM plays a significant role in resistance to bacterial and protozoal

parasites (Butler, 1983). Therefore, these results could indicate that upon initial infection with an extracellular pathogen, NRFX may have enhanced defense compared with HO, which may result in increased resistance to diseases caused by these pathogens. However, upon repeated exposure to the same extracellular pathogen, both breeds would have similar defense.

As was previously mentioned, NRFX calves initially had greater type 2 bias (AMIR) compared with purebreds, and this was confirmed by the evaluation of IgG1 and IgG2 antibody isotype responses. Typically, IgG1 and IgG2 antibody isotypes and IgG1:IgG2 ratios are measured to determine type 2 or type 1 bias (Furesz et al., 1998; Crawley et al., 2003), which tends to dominate antibody and cell-mediated immunity, respectively. Studies in cattle have demonstrated that the cytokine IL-4, which is typically produced by T-helper 2 cells, preferentially induces IgG1, IgM, and IgE (Estes et al., 1995), whereas the cytokine IFN- γ , which is typically produced by T-helper 1 cells, preferentially induces IgG2 production (Estes et al., 1994). The NRFX calves had significantly greater primary and secondary IgG1 antibody response to the type 2 antigen as well as significantly greater primary IgG1 antibody response to the type 1 antigen compared with HO. The NRFX calves also had significantly greater primary IgG1:IgG2

Table 5. Effects of farm, year, age, sex, and baseline measurement of immune response trait (time 0) on the immune response traits to type 1 antigen

Trait	Farm	Breed	Year	Age	Sex	Time 0	Farm × breed	Breed × age	Breed × sex	Breed × time 0
Primary IgG1 type 1 antigen	0.105	0.050	NS	0.083	0.109	0.104	NS	NS	NS	NS
Primary IgG2 type 1 antigen	0.086	0.050	NS	0.093	NS	0.082	NS	NS	NS	NS
Primary IgG1:IgG2 ratio type 1 antigen	0.104	0.029	0.063	0.078	NS	0.082	NS	NS	NS	NS
Secondary IgG1 type 1 antigen	0.001	NS	NS	<0.001	NS	<0.001	NS	NS	NS	NS
Secondary IgG2 type 1 antigen	0.109	0.033	NS	<0.001	NS	0.105	NS	0.0343	NS	NS
Secondary IgG1:IgG2 ratio to type 1 antigen	0.007	NS	NS	<0.001	NS	<0.001	NS	NS	NS	NS
Delayed type hypersensitivity	<0.001	NS	<0.001	NS	0.018	<0.001	NS	NS	NS	NS

¹Numbers in table represent *P*-values for each effect.

ratio to both type 2 and type 1 antigens. These results suggest that, in general, NRFX have a greater type 2 bias upon initial exposure to either the type 2 or type 1 antigen. In the study by Begley et al. (2009a), no significant difference was observed between breeds for IgG1 and IgG2 antibody response and IgG1:IgG2 ratios on both d 14 and 21. The reason for the difference between the current study versus the previous study could be the larger sample size in this study and the different years of testing between some of the calves.

Results for IgG1:IgG2 ratios showed that both breeds tend to display type 2 bias to either type 2 and type 1 antigens, which was indicated by an IgG1:IgG2 ratio >1.00 . This would typically be the type of isotype response expected to a type 2 antigen in cattle; whereas typically it would be expected that the IgG1:IgG2 ratio to a type 1 antigen would be <1.00 (type 1 bias) (Estes and Brown, 2002). However, the nature of the antigen, genetics of the host, and age of the test animal all contribute to immune response bias. Studies have shown that neonates and young animals tend to have a type 2 bias regardless of the test antigen (Chen et al., 1995; Chase et al., 2008). Specifically, young calves tend to remain in a state of type 2 bias until their immune system has fully developed and this likely explains the results of the current study (Chase et al., 2008). Previous studies in mice have shown that neonates tend to be type 2 biased in their immune responsiveness irrespective of the antigen or pathogen (type 1 or type 2; Barrios et al., 1996). Similarly, a study looking at the effect of age and pregnancy status on type 1 and type 2 immune responses in replacement heifers found that the 2- to 6-mo-old replacement heifer calves had IgG1:IgG2 ratios to a type 1 antigen >1.00 , thereby displaying type 2 biased to the type 1 antigen (Hine et al., 2011). This type 2 bias of replacement heifer calves, regardless of antigen type, gradually diminished by 1 yr of age, at which time the immune response was more influenced by the nature of the antigen than by age (Hine et al., 2011).

No significant difference was observed between breeds for DTH response (an indicator of CMIR). This could indicate that both breeds have a similar defense against intracellular pathogens that are mainly eliminated by a CMIR. However, NRFX had both a significantly greater primary and secondary IgG2 (which is typically thought of as a type 1 isotype; Estes et al., 1994) antibody response to the type 1 antigen. In a study evaluating immune mechanisms involved in bovine respiratory syncytial virus in calves, clinical scores for disease were lowest when IgG2 antibody activity peaked (Gershwin et al., 2005). These results provide evidence that IgG2 antibodies can be protective against certain intracellular pathogens. Therefore, results of this study may

suggest that NRFX have enhanced protection against certain intracellular pathogens and diseases caused by these pathogens when IgG2 antibody activity is involved compared with HO.

A greater proportion of crossbreds survived from age at immune response testing to calving compared with purebreds, which may be due, at least in part, to NRFX having greater primary AMIR than HO. Disease incidence early in calthood results in decreased survival to first calving (Waltner-Toews et al. 1986). Therefore, these results seem to indicate that HO may experience more disease incidence early in calthood, which could be due in part to increased inbreeding and lower AMIR. This study also showed a tendency toward a greater proportion of NRFX surviving from age at immune response testing to first breeding and from first breeding to calving. This is similar to results observed in another study comparing purebred HO and crossbred NRFX first-generation heifers, where crossbreds had significantly greater survival to first breeding and first lactation (Glover, 2010). Results of this study are also similar to those observed in further previous studies. Weigel and Barlass (2003) reported crossbred dairy calves having a decreased mortality and, therefore, increased survival compared with purebreds. Similarly, it has also been shown that crossbred beef cattle had increased survival and longevity and decreased culling and death due to health events compared with purebreds (Núñez-Dominguez et al., 1991). These previous studies as well as the current study provide evidence that survival decreases as inbreeding increases (Thompson et al., 2000). Overall, it seems that crossbreeding may be an effective way to improve resistance to certain diseases in dairy calves, which likely also leads to increased survival. Further studies with larger sample sizes and different breeds are required to gain a more comprehensive knowledge of the benefits of crossbreeding on immune response traits and survival in Canadian dairy calves.

CONCLUSIONS

Crossbred calves had significantly greater primary antibody response and primary type 2 immune response bias to both type 1 and type 2 antigens compared with HO. Crossbreds also had greater IgG2 response to the type 1 antigen. Overall, the proportion of NRFX that survived was greater than the proportion of HO. However, it is difficult to determine whether the differences in immune response traits and survival observed between crossbreds and purebreds were due to increased heterosis or to the effect of breed of sire, because purebred Norwegian Red calves were not included in this study. No significant difference in DTH response was

observed between breeds. Our results could indicate that compared with HO, NRFX calves may have better defense upon initial infection with a pathogen (IgM production) and also when secondary IgG2 antibody responses are required to defend against infections with intracellular pathogens. This could translate into increased resistance to certain diseases in NRFX calves compared with HO, resulting in reduced treatments to producers with crossbred calves compared with purebred calves.

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