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Short communication: Variation in production parameters among Canadian Holstein cows classified as high, average, and low immune responders

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ABSTRACT

Dairy cattle evaluated for immune responses and identified as high responders are known to have a lower occurrence of economically important diseases, including mastitis, metritis, ketosis, and retained placenta. These high immune responders have also been shown to make more antibody following vaccination and to have improved milk and colostrum quality. Therefore, breeding for improved immune response is expected to have several benefits in the dairy industry. However, a concern of such an approach to improve animal health is the potential cost of lost production due to an allocation of host resources to mount a robust immune response. The objective of this study was to evaluate early- and late-lactation production parameters in cattle classified as having high, average, or low estimated breeding values (EBV) for cell-mediated (CMIR), antibody-mediated (AMIR), and overall immune responses. A total of 561 cows from 6 herds were phenotyped for immune response and ranked based on EBV for CMIR and AMIR. A linear animal model was used to evaluate differences in milk, fat, and protein yields among immune response groups, and a regression analysis was conducted based on immune response EBV. Overall, no difference in production parameters was found based on immune response rank; however, some positive relationships with immune response EBV were found, suggesting that breeding for enhanced immune responsiveness as a prophylactic approach to improve animal health would not come at the cost of lost production.
Key words: dairy cattle, immune response, milk, production

Short Communication

Although substantial genetic advancements are being made along with increasing milk production in the dairy industry, disease occurrence continues to be a prevalent problem as it incurs costs to both the producer and consumer (Oltenacu and Broom, 2010). A proposed solution to decrease disease is the incorporation of immune response (**IR**) traits into current selection indices to breed for broad-based disease resistance (Wilkie and Mallard, 1999; Abdel-Azim et al., 2005). The adaptive immune response phenotype can be evaluated using a patented protocol (US#7,258,858; Wagter-Lesperance and Mallard, 2007), allowing cows to be selected on both cell-mediated immune response (**CMIR**) and antibody-mediated immune response (**AMIR**) for an overall high immune response profile.

Various components allow the immune system to mount both innate responses, along with more specific adaptive responses (Kumar and Burns, 2008). The adaptive immune system is mediated by cells and cytokines and can be broadly categorized into CMIR and AMIR; it is capable of memory and mounting a superior response to subsequent exposure of an antigen through the proliferation of memory B and T cells (Ingvartsen et al., 2003; Crawley et al., 2005; Lippolis, 2008). The CMIR targets intracellular pathogens such as viruses and *Mycobacterium avium* ssp. *paratuberculosis*, which causes Johne's disease in dairy cattle (Koo et al., 2004). On the other hand, the AMIR targets extracellular pathogens, including mastitis-causing bacteria, through the production of antibodies (Thompson-Crispi et al., 2012b).

Multiple studies have shown that high-immune-responding (HIR) dairy cattle have a lower occurrence of infectious and metabolic diseases such as mastitis, metritis, ketosis, and retained fetal membranes (De La Paz, 2008; Thompson-Crispi et al., 2012a, 2013), along with better response to vaccines and higher milk and colostrum quality (Wagter et al., 2000; Fleming, 2014). Overall, breeding for immune response is expected to reduce disease and improve animal health and well-being (Thompson-Crispi et al., 2014; Mallard et al., 2015). Furthermore, immune response traits are heritable, with recent estimates of 0.29 and 0.19 for AMIR and CMIR, respectively, indicating that genetic progress is possible (Thompson-Crispi et al., 2012b).

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A previous study that classified animals based solely on their antibody response demonstrated no adverse effects on 305-d milk yield. Wagter et al. (2003) observed higher fat and protein yields in low-responder, first-parity cows compared with average or high responders when ranked based on AMIR; however, these differences for protein and fat did not carry over into older cows. Additionally, the high-AMIR cows in third or greater parity had a higher milk yield than the average and low groups (Wagter et al., 2003). Another study examined correlations of sire EBV for AMIR and CMIR with 45 traits from the lifetime profit index. Of the production traits, significant correlations were observed only between AMIR and milk fat (0.184; $P = 0.011$) and CMIR and milk protein (−0.147; $P = 0.042$) (Heriazon et al., 2013). On the other hand, positive genetic correlations for CMIR with milk yield have been reported at 0.16 ($P \leq 0.01$) (Thompson-Crispi et al., 2012b). These previous studies used 305-d projected milk, fat, and protein yields; however, associations with early-lactation production parameters remain unknown. The objectives of this study were to (1) use standardized breeding values to classify cattle as high, average, or low immune responders for CMIR, AMIR, and overall IR; (2) evaluate the association of AMIR, CMIR, and overall IR rank with milk and production parameters from the first 60 DIM; and (3) evaluate the association of AMIR, CMIR, and overall IR with complete 305-d production records.

All experimental procedures were approved by the Animal Care Committee of the University of Guelph under guidelines of the Canadian Council of Animal Care (1993). A total of 561 Holstein cows and heifers from 6 commercial herds in southern Ontario were evaluated for immune response using the patented HIR test protocol (Wagter-Lesperance and Mallard, 2007). Briefly, all cattle were immunized intramuscularly on d 0 (study start day) with 0.5 mg of type-I antigen, 0.5 mg of type-II antigen, and 0.5 mg of Quil-A adjuvant (Cedarlane Laboratories Ltd., Hornby, ON, Canada) dissolved in 1.0 mL of PBS. A blood sample was taken on d 0 as a measure of baseline antibody response and at d 14 to measure a primary antibody response. A delayed-type hypersensitivity (DTH) to the type-I antigen and a PBS control was used as a measure of CMIR and was initiated at d 14 postimmunization (Hernández et al., 2005; Heriazon et al., 2009). Skin thickness measurements were taken on both tail folds, and cows then received an intradermal injection of the type-I antigen on the right tail fold and a PBS control on the left tail fold. Twenty-four hours later, skin thickness measurements were repeated. The ratio of skin thickness measurements at 24 h to 0 h relative to intradermal injection was used for both the test site and control sites. The AMIR was evaluated by antibody production in response to the type-II antigen (Heriazon et al., 2009). Serum antibody was quantified using a modified ELISA protocol as described by Hine et al. (2011) from blood collected on d 0 and 14 of the test protocol.

Complete immune response phenotypes and registration numbers were available for 561 dairy cattle from 6 herds (herd 1, n = 112; herd 2, n = 60; herd 3, n = 94; herd 4, n = 118; herd 5, n = 95; herd 6, n = 82). The full pedigree included 26,673 animals and was provided by the Canadian Dairy Network (Guelph, Ontario). For CMIR, the response variable was the log ratio of the 24-h skin thickness measurement at the test site to the 0-h test site measurement, with the control site as a covariate. For AMIR, the d 14 value, indicative of a primary antibody response, was the response variable and the d 0 value was the covariate. ASREML software (Gilmour et al., 1995) was used to estimate heritability and breeding values to rank animals for CMIR or AMIR based on the following univariate linear animal model:

$$y_{ijklmn} = \mu + \alpha \times d_i + h_j + p_k + sl_l + ps_m + a_n + e_{ijklmn},$$

where y_{ijklmn} = CMIR or AMIR; μ = population mean; d_i = control site of CMIR or AMIR at d-0 as fixed regressions; α is a regression coefficient; h_j = fixed effect of herd (1–6); p_k = fixed effect of parity (0, 1, 2, 3, ≥4); sl_l = fixed effect of stage of lactation group (not lactating, 1–20, 21–105, 106–235, >235 DIM); ps_m = fixed effect of pregnancy status (not pregnant, <100, 100–200, >200 d pregnant); a_n = random animal effect; and e_{ijklmn} = residual error. Variables with $P > 0.1$ were removed from the model and results were considered to be statistically significant if $P \leq 0.05$. Interactions were tested and remained in the model if $P < 0.1$. The EBV were standardized to a mean of 0 and a standard deviation of 1; animals with an EBV ≥ +1 standard deviation (**SD**) from the mean were classified as high responders, those with an EBV ≤ −1 SD were classified as low immune responders, and those with an EBV between −1 and +1 SD from the mean were average immune responders. To have an EBV for overall IR, standardized breeding values for CMIR and AMIR were averaged as described previously (Thompson-Crispi et al., 2012b).

Milk records within the first 60 DIM of lactation were available for 442 of the tested animals, and complete 305-d records for 402 cows, through the Ontario Dairy Herd Improvement Corporation and the Canadian Dairy Network. Records were obtained from the lactation in which animals were IR tested, or in the

first lactation if data were available for heifers. Milk parameters obtained during the first 60 DIM included test-day milk, fat, and protein yields. If more than one record was available within the first 60 DIM, an average was used. The total 305-d milk, fat, and protein yields were obtained once the animal completed their lactation.

Milk parameters were analyzed independently using a SAS (SAS Institute Inc., Cary, NC) mixed linear model, as follows:

$$y_{ijklm} = \mu + ar_i + cr_j + ir_k + h_l + p_m + e_{ijklm},$$

where y_{ijklm} = 60-d milk parameter (daily fat, protein, or milk yield within the first 60 DIM) or 305-d milk parameter (fat, protein, or milk); μ = population mean; ar_i = AMIR rank (high, average, low); cr_j = CMIR rank (high, average, low); ir_k = overall IR rank (high, average, low); h_l = fixed herd effect (6 herds); p_m = fixed effect of parity (0, 1, 2, 3, $\geq$4); and e_{ijklm} = residual error. Results were considered statistically significant at $P \leq 0.05$. Herd × parity interactions were tested along with a combined AMIR-CMIR rank (high-high, average-average, low-low) and remained in the model if $P < 0.1$. A regression analysis was performed using CMIR, AMIR, or IR breeding values and included the effects of herd and parity. As in the above model, the y variable was the production parameter in the regression analysis.

Results showed significant variation in breeding values for IR traits, which allowed the animals to be ranked, as previously demonstrated (Thompson-Crispi et al., 2012b), as high, average, or low for CMIR and AMIR. Cell-mediated IR and AMIR were found to be heritable, with estimates of 0.24 (SE = 0.09) and 0.52 (SE = 0.09), respectively. For AMIR, there were n = 49 high, n = 301 average, and n = 52 low responders and for CMIR, there were n = 75 high, n = 249 average, and n = 78 low responders. When cows were ranked for combined overall IR, there were 54 high, 283 average, and 65 low responders.

For all production parameters, herd was significant ($P < 0.0001$) and parity was significant for 60-d milk yield and 305-d milk, fat, and protein yields ($P < 0.05$; Table 1). Herd × parity interactions were tested but only significant for 305-d fat yield. Herd effects are generally accepted in the industry as large variation in nutrition and management practices between farms; frequency of feeding, bunk space, feed composition, housing, and health status are just a few of the environmental factors largely affecting milk production (Rauw et al., 1998; Sova et al., 2013). Least squares means of 305-d milk parameters by rank are presented in Table 2. Overall, the results of this study found that immune response rank had no effect on milk production when examining both early-production and full-lactation parameters (Table 1). A regression analysis using EBV for AMIR, CMIR, and IR revealed some significant relationships with production parameters. The AMIR was beneficially associated with 60-d protein (r = 0.04, $P = 0.0239$), 305-d milk (r = 0.33, $P = 0.0157$), 305-d fat (r = 0.32, $P = 0.0040$), and 305-d protein (r = 0.32, $P = 0.031$). The regression analysis found overall IR to be associated with 305-d fat (r = 0.31, $P = 0.0489$); however, no associations with CMIR were found.

Correlations between immune response and 305-d milk production have been observed in previous studies, including positive associations for CMIR with milk yield (0.16) and milk yield in the first parity (0.15; Thompson-Crispi et al., 2012b). Wagter et al. (2003) noted the largest overall milk yields in high AMIR cows with a parity of 3 or greater. Positive genetic correlations have also been found between sire EBV for AMIR and milk fat (0.184; $P = 0.011$; Heriazon et al., 2013); however, this trend is opposite to that observed by Wagter et al. (2003) for first-parity fat yields, although these differences did not carry over into subsequent parities.

Some have speculated that a more balanced and robust immune system may be associated with decreased production as a cow allocates more resources, such as the partitioning of nutrients, to the immune system. The

Table 1. Probabilities of significance (*P*-values) for the effects of antibody-mediated (AMIR), cell-mediated (CMIR), and overall immune response (IR) rank, herd and parity of 60-d and 305-d production parameters of Holstein cows

	60 d			305 d		
Item	Fat (%)	Protein (%)	Milk (kg)	Fat (kg)	Protein (kg)	Milk (kg)
AMIR rank	0.7342	0.5544	0.4568	0.8545	0.6012	0.3217
CMIR rank	0.7804	0.3669	0.7094	0.3609	0.2469	0.4868
IR rank	0.2028	0.2464	0.6925	0.3786	0.6833	0.8751
Herd	<0.0001	0.0287	<0.0001	<0.0001	<0.0001	<0.0001
Parity	0.1270	0.1396	<0.0001	<0.0001	<0.0001	<0.0001
Herd × parity	NA[1]	NA	0.2608	0.0401	0.2839	0.3376

[1]Interaction not tested.

Table 2. Production parameters at 305 d among Holstein cattle classified as high, average, and low responders for antibody-mediated (AMIR), cell-mediated (CMIR), and overall immune response (IR) classified based on EBV[1]

Trait	Rank	Fat (kg)	Protein (kg)	Milk (kg)
AMIR	High (n = 49)	435.45 (7.04)	351.91 (5.02)	11,348 (172)
	Average (n = 301)	431.26 (4.07)	347.33 (2.86)	11,208 (98.0)
	Low (n = 52)	421.07 (6.92)	339.94 (4.91)	10,937 (168)
CMIR	High (n = 75)	429.84 (8.96)	347.14 (6.32)	11,143 (216)
	Average (n = 249)	429.21 (3.70)	345.35 (2.61)	11,147 (89.0)
	Low (n = 78)	436.75 (8.54)	355.51 (6.08)	11,442 (208)
IR	High (n = 54)	438.82 (8.34)	351.81 (5.93)	11,258 (203)
	Average (n = 283)	430.15 (3.82)	346.58 (2.71)	11,183 (93.0)
	Low (n = 65)	422.15 (7.62)	343.31 (5.38)	11,111 (184)

[1]Data represent least squares means with SEM in parentheses.

results of the current study suggest that this may not be the case. Feed intake and thus resources are limited, and must be utilized for growth, reproduction, maintenance, and lactation; once a resource has been used for one requirement, it is no longer available for another, making trade-offs unavoidable (Rauw, 2012). Previous studies in various species demonstrate reduced performance due to energy and nutrient costs of a superior immune system (Klasing and Leshchinsky, 1999; Soler et al., 2003). In contrast, greater growth performance has been observed in high-immune-responding swine, where animals were bred for high and low immune responsiveness over 8 generations (Mallard et al., 1998). Another study in wild white-footed mice, which quantified resting and daily metabolic rates along with masses of body organs, determined that although mounting an immune response requires a significant amount of energy, maintaining the immune system has a minimal cost (Derting and Compton, 2003). Nonetheless, mounting efficacious immune responses are essential to life and our results show that a robust immune response may not translate to decreased production in the dairy cow.

Previous research has found that cows classified as high immune responders based on their EBV of AMIR and CMIR have decreased incidence of disease compared with average or low responders (Thompson-Crispi et al., 2012a, 2013). In conclusion, the results of the current study suggest that this ability to mount a superior immune response does not come at the cost of early lactation or complete 305-d production parameters; therefore, speculations of the milk production costs that could potentially accompany breeding and selecting for improved immune response traits may be relieved.

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