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THE USE OF HIGH IMMUNE RESPONSE BULLS IN A HERD

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Introduction

The immune system is the body's natural defense system that provides protection from a wide range of pathogenic micro-organisms. The immune system is genetically regulated and can be enhanced by genetic selection. The University of Guelph's award-winning patented High Immune Response (HIR) technology is designed to identify cattle with optimized immune responsiveness and thereby greater resistance to a broad range of diseases. The Semex Alliance has an exclusive license to utilize this technology to identify high immune responder sires, marketed under the *Immunity+* trademark, which can be used to breed for improved immune responses. The HIR technology is comprised of novel management and selection methods which reduce the negative impacts of disease and improve overall herd health and reproductive fitness.

Immune Response Studies in Dairy Herds

Measuring & Categorizing Adaptive Immune Responses

In one research collaboration with the Canadian Bovine Mastitis Research Network (CBMRN), 690 cows from 58 herds across Canada were immunized using the HIR system to evaluate and determine breeding values on immune response traits. Blood samples and skin tests were used to measure specific Antibody-mediated Immune Responses (AMIR) and Cell-mediated Immune Responses (CMIR) respectively. Enhancing both of these immune response traits is particularly important to control complex diseases, such as mastitis, where there are multiple causative organisms that require various immunological mechanisms to control the disease. The AMIR and CMIR phenotypes were then included in a genetic model to estimate Estimated Breeding Values (EBV) for AMIR, CMIR and overall IR for all animals tested. Animals were categorized as High, Average or Low immune responders with approximately 15% of cows classified as High, 70% as Average and 15% as Low. These HIR tests have been performed on several thousand dairy

females to date as part of various research studies or test herds including 700 cows tested from a 3,000 cow dairy in Florida, USA.

In the CBMRN study, there were some notable differences in immune responses across provinces. For instance, cows in the province of Alberta had higher CMIR responses than those in other provinces and higher secondary antibody responses. Interestingly, cows in Alberta were also found to have the lowest incidence of *Escherichia coli* and *Staphylococcus aureus* mastitis compared to other regions again demonstrating the benefits of an appropriate and more robust immune response. This was the first study to evaluate adaptive immune response profiles and disease incidence of dairy cows on a national scale and provided the first evidence that the HIR test could be used across regions or countries (Thompson-Crispi et al 2011a,b).

Calves can be HIR tested as early as two months of age (Cartwright et al 2011). Calves with the highest immune response profiles as calves have the greatest chance of survival as cows. The ability to HIR test calves early and identify those with the greatest immunological potential provides an excellent opportunity to help dairy producers decide which calves to keep and which to cull, or to tailor management and breeding practices within calf groups.

Disease Resistance

Antibiotics may be the treatment of choice for diseases such as certain types of mastitis, but this is less than an ideal treatment in a world making every effort to restrain the use of antibiotics with a goal to limit the emergence of antibiotic resistant pathogens. Therefore there is an urgent need for alternative health strategies, both preventative and therapeutic, in which microbial drug resistance cannot develop. These may include a combination of vaccination, dietary supplementation (e.g. probiotics) and genetic strategies that can act safely and synergistically in the prevention of disease.

The immune system is the body's natural defense against infectious disease and cancer. This system has the ability to customize a protective response against a set of diverse pathogens. It can detect danger signals emitted from foreign agents or defective host cells and deliver defensive mechanisms to destroy bacteria, viruses, fungi, worms or cancer cells. These defensive mechanisms are delivered via a collection of genetically regulated cells and molecules that control invading micro-organisms, including those that cause bovine mastitis, metritis, pneumonia, Johne's disease, BLV and so on. The appeal with a genetic approach for immune response traits is that it makes use of the animal's own inherent ability to make appropriate immune responses to a given pathogen.

A study assessing immune response in comparison with disease incidence found a lower occurrence of mastitis in high immune responders in 2 out of 3 Canadian herds tested (Wagter et al 2000). A more recent study of 58 dairy herds that were part of the Canadian Bovine Mastitis Research Network (CBMRN) also indicated that cows identified as having high immune responses had lower mastitis incidence, and for mastitis that did occur, it had lower severity than in cows classified as average or low responders (Thompson-Crispi et al 2013). This included the incidence of both *E. coli* and *S. aureus* mastitis, as well as other causative bacteria. Another study in a 3,000 cow dairy in the US substantiated these findings by demonstrating reductions in the incidence of mastitis (27%), metritis (17%) and retained placenta (32%) of cows with both high antibody-mediated and cell-mediated immune responses compared

to average or low responders based on their EBVs for these traits (Thompson-Crispi et al 2012b). In general high responder cows have about half the disease occurrence of low responders (Figure 1).

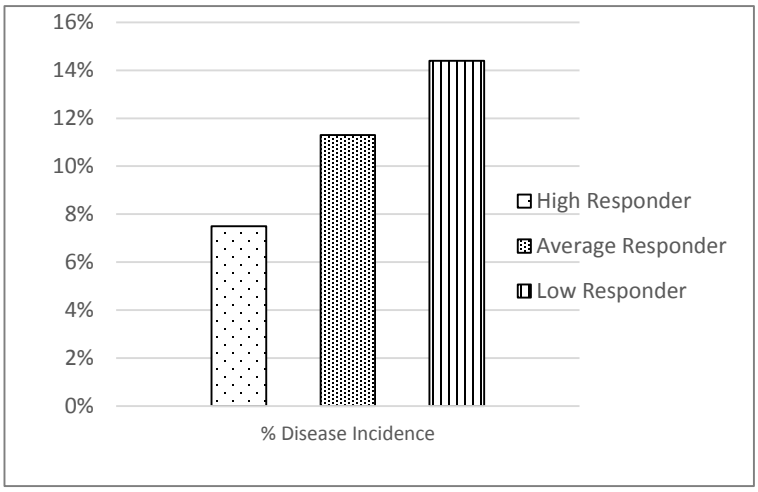

Figure 1. Disease Occurrence (%) in High, Average and Low Immune Responder Cows (Wagter et al 2000, Thompson-Crispi et al 2012b, 2013)

A recent study compared hoof health data, collected by a hoof trimmer who is part of larger hoof health project, on 190 Holstein cows with immune response for AMIR and CMIR (Cartwright et al, 2014). The data included first hoof trimming for each animal and multiple lesions per cow were reported if present. Lesions were analyzed both as individual lesion type and grouped into infectious and horn categories. The hoof trimmer scored each lesion for severity as: 1 = least, 2 = middle, 3 = most. Results showed that high AMIR cows had significantly less infectious digital dermatitis (23%) compared to average (60%), and less but non-significant (P = 0.19) compared to low responders (50%). Of the reported cases, the high responders had significantly less infectious digital dermatitis of the highest severity score (3%) compared to low responders (47%). High CMIR cows had a trend towards less interdigital hyperplasia (0%) compared to average responders (11%).

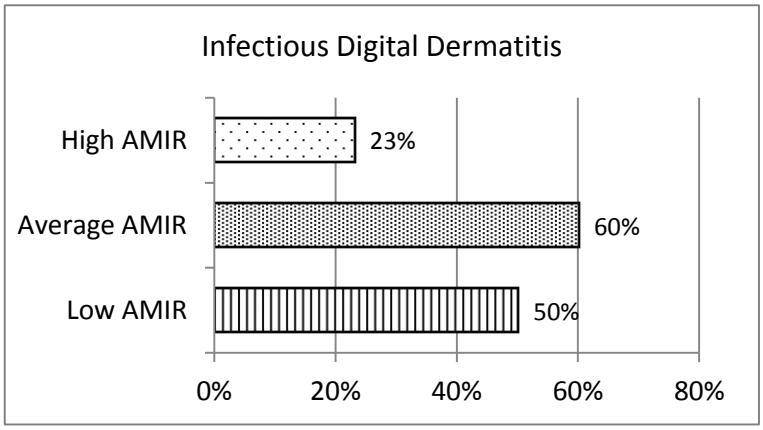

Figure 2. Percentage Incidence of Infectious Digital Dermatitis in High, Average and Low AMIR Cows (Cartwright, S.L. et al 2014)

Higher Colostrum Quality and Greater Response to Vaccination

Of substantial relevance to animal health is the fact that high immune responders produce higher quality colostrum. Initial experiments showed that colostrum from high responder cows contain more specific antibodies to a test antigen compared with average and low responders (Wagter et al., 2000). This finding was recently substantiated in experiments showing that antibody to a test antigen in blood was positively and significantly correlated with antibody in colostrum or milk during early (calving to 5 days postpartum) and late lactation (around 280 days in milk on average). It is worth noting the positive and significant correlation detected between antibody in colostrum of the mother and in the blood of their calves 2 days after administration of colostrum (Wagter et al., 2012). In addition, it recently has been shown that cows classified as high antibody- mediated immune responders have greater concentrations of total immunoglobulin (IgG) and β-lactoglobulin in colostrum compared with average and low responders (Fleming, 2014; Figure 1). It is well known that molecules such as IgG, and β-lactoglobulin exert anti-microbial activities against mastitis-causing pathogens (Butler 1983; Chaneton et al., 2011). The anti-microbial activity of molecules such as IgG, lactoferrin and beta-lactoglobulin may explain, at least in part, the lower incidence of mastitis in high immune responders and may provide a platform for future research into the plethora of immunologically-active components in colostrum from high immune responding cattle. Wagter et al. (2000) also reported that cows with high antibody responses responded better to a J5 mastitis vaccine. It is estimated that up to 20% of animals have little or no response to commercial vaccination in comparison to only 4% of high immune responders (Mallard, B. Personal communication).

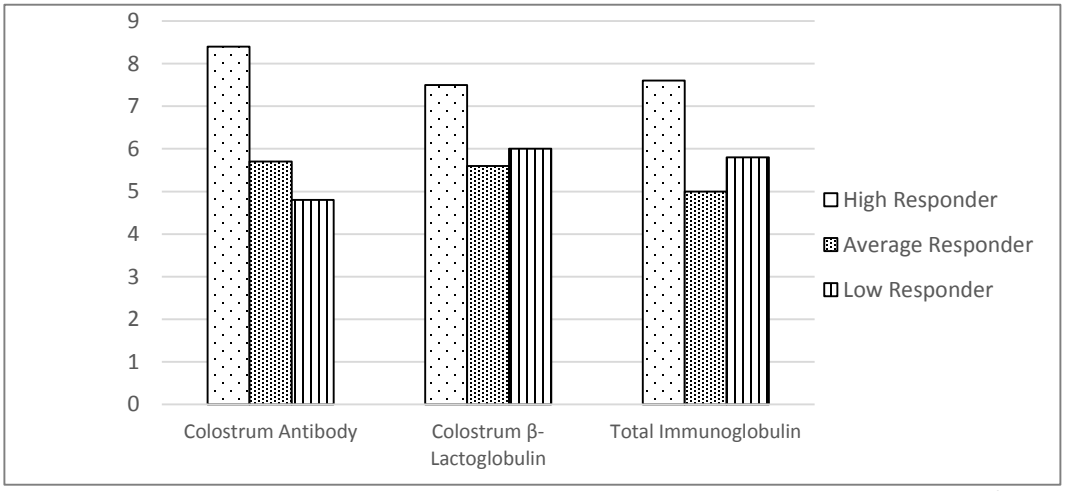

Figure 3. Colostral-specific antibody (ELISA Optical Density x 3), colostrum β-lactoglobulin (mg/ml) and total immunoglobulin (mg/dl x 1000) in high, average and low immune responder cows (Wagter et al 2000, and Fleming, 2014)

Genetic Components & Genomics of Immune Response

Immune response phenotypes are influenced of two major factors - genetics plus environment. By comparing the phenotypic response traits in related individuals the genetic components can be estimated. Knowledge of the genetic component allows computation of the heritable fraction (heritability) and the calculation of estimated breeding value (EBV) for the animals. Immune response traits have a high heritability (19 to 46%) which is very

comparable to the heritability for production traits (table 1 - Thompson-Crispi et al. 2012b and Thompson-Crispi et al. 2014a). This implies a tremendous opportunity for the dairy industry given that most health related traits have low heritability estimates that range between 2 and 9%. These levels are sufficiently high to allow for improvement via genetic selection (Abdel-Azim et al 2005, Thompson-Crispi et al 2012a). Utilizing EBVs for immune response to breed animals, it is feasible to make genetic gains in immune response relatively quickly in a similar manner to cattle populations that have been genetically improved for production and some conformation traits.

Table 1. Heritability Estimates for Antibody-mediated and Cell-mediated Immune Response for Holstein Cows and Sires (Thompson-Crispi et al. 2012b and Thompson-Crispi et al. 2014a)

Trait	Heritability	
	Holstein Cows	**Holstein Sires**
Antibody-mediated Immune Response (AMIR)	0.29 (+/- 0.10)	0.46 (+/- 0.08)
Cell-mediated Immune Response (CMIR)	0.19 (+/- 0.10)	0.22 (+/- 0.08)

Two separate genome wide association studies for immune response were conducted, one based on a selected population of tested females and another on a larger group of non-related tested sires. The animals were genomic tested using the Illumina 50K SNP chip and immune tested using the HIR technology. In the female study, 186 genetic markers that are part of 11 genetic pathways were shown to differ between these cows based on antibody responses, and 21 genetic markers were associated with cell-mediated responses (Thompson-Crispi et al 2012c). Genetic pathways included those within the bovine Major Histocompatibility Complex (BoLA), a complex gene region that is important in the initiation and regulation of adaptive immune responses.

In the male study, 266 unique genetic markers were shown to differ between these sires based on antibody responses and 46 unique genetic markers were significant in explaining differences between sires based on cell-mediated responses (Thompson-Crispi et al. 2014b). Though the proportion of significant SNPs on chromosome 23 associated with AMIR were higher on the female compared to the male study (Thompson-Crispi et al. 2012c, Thompson-Crispi et al. 2014b), they confirm chromosome 23 is a highly important gene region regulating antibody responses in dairy cattle.

Dr. Mallard's group at the University of Guelph are currently working to establish a large reference population of Holstein sires (~2,000) and cows (~3,000) with immune response phenotypes and 50K SNP genotypes. If successful, it may be possible in the near future to identify high or low immune responders from a simple hair sample.

Sires Tested for Immune Response

The Semex Alliance has an exclusive license to utilize the high HIR technology to identify high immune responder sires, marketed under the *Immunity+* trademark, which can be used to breed for improved immune responses. Since April 2012, Semex has been immune testing all young bulls coming out of isolation, older bulls approaching their first daughter proof, and selected young genomic sires at each of their four main locations housing bulls. As of December 2014, there have been 1789 Holstein, Jersey and Ayrshire sires tested for immune response. Similar to the testing of females, blood samples and skin tests were assessed for each sire to measure specific AMIR and CMIR, respectively. The AMIR and CMIR phenotypes were then included in a genetic model to determine EBV for AMIR, CMIR and overall IR for all animals tested. The top 10% of tested sires are categorized as being high immune responders, and marketed under the Immunity+ trademark (Shannon 2014).

With the high heritability of immune response traits (19 to 46% - Thompson-Crispi et al. 2012b and Thompson-Crispi et al. 2014)), there is a tremendous opportunity for dairy producers to use high immune response sires (Immunity+ sires) to reduce disease incidence in their herds through the improved immune response in their daughters. Based on the disease reduction for many diseases observed in high immune responder cows, and taking into account the high heritability estimates and the fact that a sire contributes half of the genetic profile of their daughters, Dr. Jacques Chesnais, Senior Geneticist at Boviteq/Semex, estimated that daughters of Immunity+ sires should be expected to have a 4 to 8% reduction in disease incidence compared to herd average. This reduction is additive by generation bred to an Immunity+ sire, so like breeding for production traits, greater resistance accumulates over time as well as the additional improvements in colostrum quality and response to commercial vaccines.

A comparison study using health recording data on three large US dairies was conducted to compare disease incidence of daughters of Immunity+ sires with daughters of other sires. With the theoretical expectations of 4-8% disease reduction, the Immunity+ daughters met or surpassed those expectation with 8 to 44% less disease and the most notable reductions observed with mastitis and calf pneumonia (Table 2).

Table 2. Disease Occurrence of Immunity+ Daughters on Three Large US Dairy Herds

Disease	Herd (No. of Milking Cows)	Cattle	Immunity+ Daughters	All Other Daughters	Disease Reduction *
Mastitis	Herd 1 (1500 cows)	1st lactation	8.8%	15.8%	44.3%
	Herd 2 (1700 cows)	1st & 2nd lactation	11.7%	14.5%	19.3%
Metritis	Herd 2	1st & 2nd lactation	4.2%	5.6%	25.0%
Retained Placenta	Herd 2	1st & 2nd lactation	0.0%	0.7%	100%
All Recorded Diseases	Herd 1	1st lactation	16.7%	18.2%	8.5%
Pneumonia	Herd 1	Heifers	6.8%	9.1%	25.3%
Pulmonary Treatment (regular/intensive)	Herd 3 (2500 cows)	Heifers	19.5% / 0.9%	27.4% / 1.6%	28.8% / 43.8%

* (Disease incidence for all others) – (Disease incidence for Immunity+ daughters) / (Disease incidence for all others)*100

Numerous studies over two decades of research have shown that breeding for enhanced disease resistance based on breeding values of immune response improves livestock health while not negatively impacting production traits (Mallard et al 1992, Wagter et al 2003; Thompson-Crispi et al 2014b). In fact, in dairy cattle this approach appears to improve overall herd life, as well as certain reproductive traits, including first service to conception and non-return rate (Thompson-Crispi et al 2012a, Mallard et al 2014). This relation can be further substantiated by comparing Immunity+ sires vs. non-Immunity+ sires among all sires tested over a one-year period, and analyzing proofs for traditional health traits with these two groupings of sires. The Immunity+ sires had a favorable advantage for all four health related traits compared to non-Immunity+ sires, thus showing an association between immune response and health, fitness and fertility traits (Table 3).

Table 3. August 2014 Sire Proofs (USDA) for Immunity+ Sires vs. Other Sires for All Bulls Tested from July 2013 to June 2014

Trait	Average Proof for Immunity+ Sires	Average Proof for Non-Immunity+ Sires	Difference
Productive Life	+4.8	+3.0	+1.8
Daughter Pregnancy Rate	+1.0	+0.1	+0.9
Somatic Cell Score	2.72	2.82	-0.10 favorable
Daughter Calving Ease	5.4%	6.0%	-0.6 favorable

Furthermore, for all sires tested, there were low but favorable correlations between sire EBV for immune response and the four health related traits. The correlations were 6% for Daughter Calving Ease, 14% for SCS, 16% for Productive Life and 27% for Daughter Pregnancy Rate. These correlations show a relation between immune response and animal fitness and fertility, however the low level confirms immune response as a unique trait that has previously not been selected for in the dairy cattle industry. Greater immunity and disease resistance can best be developed genetically via selection for immune response and Immunity+ sires.

Conclusions

HIR/*Immunity+* is an award winning patented technology that allows dairy producers to safely and effectively improve the health of their herd. High immune responder cows have about half the disease occurrence of low responders, along with improved colostrum quality and greater response to commercial vaccination. Immune response is highly heritable (19-46%). Early indications show that daughters of Immunity+ sires have a lower incidence of disease that surpasses the theoretical genetic expectations of the technology. And using Immunity+ bulls allows the beneficial disease resistance genes to be passed on to subsequent generations of offspring.

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