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GENETIC REGULATION OF THE BOVINE IMMUNE SYSTEM AND THE PRACTICAL APPLICATION OF THE HIGH IMMUNE RESPONSE TECHNOLOGY IN DAIRY HEALTH MANAGEMENT

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Identifying dairy cows with superior breeding values for immune response (IR) traits reduces disease, increases farm profit, improves milk quality and increases animal well-being. In Canada, it can cost the dairy producer as much as $320 to treat a single case of mastitis, and 1 out of every 5 dairy quarters in Canada is infected with a mastitis-causing pathogen at any given point in time (**Canadian Bovine Mastitis Research Network, "What's New in the World of Mastitis Research?"** http://www.medvet.umontreal.ca/rcrmb/dynamiques/PDF_AN/Results/NewspaperWhatsNew.pdf. 2009). Therefore in keeping with the European Unions' proactive thinking that "prevention is better than cure", genetic methods to identify animals at lower risk of disease are being deliberately sought. One of the most appealing options available is to take advantage of the animal's own immunogenetic potential by selecting the healthiest animals with the most robust immune system. To this end, we have identified and ranked dairy cows, calves and bulls as high, average or low immune responders using a University of Guelph (Ontario, Canada) patented testing protocol. Individuals with both higher and more optimally balanced antibody (AMIR) and cell-mediated immune responses (CMIR), are referred to as High Immune Responders, and this method of testing is referred to as the *High Immune Response* (HIR) technology. Heritability (h^2) estimates for AMIR and CMIR traits used in the HIR testing are ~25% allowing for improvement via genetic selection. Most importantly, health and production benefits of the *High Immune Responders* include lower occurrence of mastitis, metritis and retained placenta, as well as improvement in some reproductive traits, response to vaccination and colostrum quality. This approach is ideal for both conventional and organic dairy farms since it involves identifying individuals with the best natural immune-competency and breeding them in future generations. It does not involve GMOs and reduces the use of on-farm disease treatments. DNA collected from high, average and low immune responder cows in Canada and the US are now being tested on the Illumina 50K Chip platform to determine if there are specific SNP patterns associated with these diverse immune response phenotypes. This may allow identification of beneficial phenotypes, not only based on immune response breeding values, but also by genetic SNP chip analysis.

Genetic Regulation of the Immune System

It is estimated that at least 2000 of the approximately 23,000 genes in the mammalian genome govern host defence. This means that about 9% of the genome is involved with disease resistance. This may not be surprising given the importance of maintaining health. The immune system is the body's defence system and it is composed of integrated, genetically regulated sets of cells and molecules that control the response to external and internal stimuli, including pathogenic micro-organisms (Delves and Roitt, 2000; Mallard and Wilkie 2007). Understanding the biological and genetic relationships within the immune system, particularly during periods of stress, including the peripartum period and following vaccination or disease challenge, will help to facilitate the implementation of new genetic approaches to improve livestock health (Glazier et al 2002, Mallard 2010, Hine et al 2010). Studies show that certain genes, such as those within the Major Histocompatibility (MHC) locus, play a larger role than others in immune response (Ellis and Codner 2011); however, this varies depending on the disease of interest and therefore it is important to evaluate many genes when trying to improve broad-based disease resistance (Berry et al 2011). This may be accomplished by evaluating useful health phenotypes, such as immune response capacity, and determining the breeding values of animals for these traits. It may also be possible to use whole genome scanning once useful health phenotypes have been more fully characterized. Nonetheless, tools such as the Illumina SNP Chip analysis may not come on line as quickly for health as those for production traits. This is due to the complexity of disease resistance and the ever changing relationships between the host and pathogen. Nonetheless, breeding for improved disease resistance has a bright future with the many new tools on the horizon that will no doubt become an integral part of animal health maintenance programs.

Over the last several decades, many studies have focussed on evaluating host defence mechanisms as indicators of specific and broad-based inherent disease resistance yielding solid evidence that genetic solutions to improve animal health are useful (reviewed by Kelm et al 2001). In a number of species, including cattle it is possible to selectively breeding for high (H), average (A) or low (L) – immune responsiveness, and H-responders can positively influence resistance to infectious disease (reviewed by Stear et al 2001). The heritability (h^2) estimates of immune response traits tend to be moderate to high. For example, the h^2 of antibody and cell-mediated immune responses used to select H-responders are about 25% which is sufficient to allow for improvement via genetic selection (reviewed by Mallard 2007; Abdel-Azim et al 2005). Health and production benefits are clear following genetic identification of cattle and pigs for enhanced IR. In cattle, this includes lower occurrence of mastitis, ketosis, metritis and retained placenta, as well as improvements in number of services and first service to conception rates of cows with both high antibody and cell-mediated immune responses (DeLaPaz 2008, Wagter et al 2000, Thompson-Crispi et al 2011a,b). These individuals with both higher and balanced AMIR and CMIR are referred to as High Immune Responders, and we have developed a patented test system to quickly identify these animals within dairy herds and bull studs. This method is termed the *High Immune Response* (HIR) technology.

Evaluating Immune Response in Herds across Canada

Recently, in 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 (Figure 1). Blood samples and a simple skin test are used to measure specific AMIR and 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.

High, average and low immune responders have been found within all herds tested in the various dairy regions across Canada. Ranking of cows can be compared within herd, within province and across regions based on either their phenotype or immune response breeding values. In the CBMRN study approximately 15% of cows were high, 15% were low, and 70% were average immune responders with some slight differences between provinces (Thompson-Crispi et al 2010). In addition to cows that are high or low for both AMIR and CMIR, there are also animals that are high for AMIR and low for CMIR, and vice versa. These animals may benefit for specific management practices to optimize their health performance. On the other hand, it may be ideal, where possible to cull low responders. In the CBMRN study, there were also some differences across the various provinces. For instance, cows in 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 nation wide (Thompson-Crispi et al 2011a,b).

It is also possible to HIR test calves as young as 2 months of age (Cartwright et al 2011). This study compared immune response profiles in purebred Holstein and crossbred Norwegian Red x Holstein calves. Again calves with the highest immune response profiles had the greatest chance of survival. The ability to HIR test calves early and identify those with the greatest immunological potential provides a new tool to help dairy producers decide which calves to keep and which to cull, as well as how to tailor management practices to the various calf groups.

Immune Response Testing of Sires

Additionally, we have also begun HIR testing of AI sires and it is clear that some sires are high immune responders while others have lower immune responsiveness. With the ability to identify the immune response profiles of both sires and dams it is possible to select the most ideal pairs for mating in order to maintain and improve dairy herd health. Nonetheless, it is critically important for a producer not to solely rely on HIR sire testing

since immune response traits will only be one trait in the LPI and may only be used on a select set of sires. This means that it can take a substantial amount of time for the full benefits of HIR to reach future generations of cows; whereas, HIR testing of cows and calves allows the producer to make immediate culling and management decisions that will immediately result in lower disease.

Immune Response and Production

Results to date have shown that breeding for optimal high immune response based on both antibody and cell-mediated immune responses would not compromise production. There is evidence that some cows with only high AMIR have lower milk production but cows with high CMIR have higher milk production (Wagter et al 2003; DeLaPaz 2008; Mallard 2007). Therefore when both traits are used in a selection index there is no adverse affects on milk yield, fat or protein. Similarly in the CBMRN study there were no differences in 305 day milk yield, protein yield, fat yield or overall lifetime profitability in HIR cows compared to low or average IR cows (Thompson-Crispi et al 2010).

Practical Implication of the High Immune Response (HIR) Technology

Breeding companies distribute sire proofs (breeding values) to improve mastitis that include Somatic Cell Score (SCS) as an indicator of udder health. SCS, however focuses only on one disease, whereas HIR focuses on broad-based disease resistance. Breeding companies in Canada, including the Semex Alliance, are also beginning to distribute semen from various breeds that are more resistant to disease; for example, Norwegian Red cattle. Dairy Herd Improvement (DHI) companies, such as CanWest DHI provide information on SCS and bacterial colony forming units in individual milk samples, as well as offer diagnostic milk ELISA tests for *S. aureus* mastitis, Johne's Disease, and Bovine Leukosis Virus. Nutrition companies are offering rations that support optimal health and may enhance immune response while pharmaceutical companies market and distribute vaccines that prevent respiratory and gastrointestinal infections in cattle, and Gram negative intramammary infections in lactating cows. However, no company as of yet has attempted to produce a product or service that provides the producer or breeding company with an indication of how well their cattle may respond and/or resist infection to many different pathogens, and HIR technology is designed to meet that need. Further this technology offers a solution that results in a reduction in disease occurrence, a reduced use of antibiotics, a reduction in the cost of food animal production, and this translates into an increased quality of food for the consumer.

Qualitative market research was conducted by an independent firm, Agri-Studies (Guelph, Ontario), using 3 focus groups to assess interest in the HIR technology among dairy producers and the dairy support industry, including pharmaceutical companies, dairy herd improvement organizations, veterinarians, breeding and feed companies, and government. Results showed significant interest among dairy producers to use HIR to identify calves or cows with *High Immune Response* (75% of producers). They acknowledged that the technology would provide beneficial information for culling

decisions, grouping, breeding, and/or treating animals, but the key benefit they saw was the ability to cull animals as calves and save the cost of raising animal that later may have significant health issues (http://www.youtube.com/watch?v=4yjackdrQLI). They also saw the value of using sires that were classified as HIR to improve the health of their herds. Among participants from the dairy support industry, the most common benefits cited included the use of HIR technology as a diagnostic tool to target therapeutic drugs or vaccines toward the various IR phenotypes, to improve genetics, and to increase business opportunities with dairy producers. Further market assessment and beta testing of dairy herds is now underway to finalize the transferability of the technology to the marketplace.

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HIGH IMMUNE RESPONSE (HIR) ANIMALS ARE NATURALLY IMMUNE

HIR is a patented evaluation technology developed to identify dairy cattle with high adaptive immune response capability.

Identification is safe, fast and effective.

Benefits include:

- Lower disease occurrence and severity
- Reduced treatment and veterinary costs
- Increased response to vaccines
- Increased colostrum quality
- Cows as young as 2 months can be tested
- Animals only need to be tested once in a lifetime
- Testing is safe and does not interfere with any other diagnostic testing
- Cost benefit analysis show significant savings to producers who identify HIR cows in their herd.

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Figure 1. Combined Estimated Breeding Values for Antibody and Cell-mediated Immune Responses of Holstein Cows in the Canadian Bovine Mastitis Research Network Cohort Herds