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RedHolsteins- Knowledge of genetic variations to breed a more robust dairy cow

Key external stakeholders:

Dairy farmers, cattle breeders, AI companies, suppliers of mastitis treatments and prophylactics

Practical implications for stakeholders:

The main objective of this study was to test the Irish dairy herd for genetic variations in key genes and to determine whether these variations associated with differences in cow performance. The main outcome was that we have identified genetic variations that associate with energy balance and fertility. This information can be used in breeding programs to select for more robust dairy cows.

- Of 24 genetic variations tested, 17 exhibited associations with energy balance traits.
- We identified a variant in the milk protein gene, *lactoferrin* that associated with calving interval.
- We detailed the immune response to the probiotic mastitis therapeutic *Lactococcus lactis*.

Main results:

- 17 genetic variations associated with energy balance in the Irish dairy herd and this knowledge can be used in breeding programs to aid in selection of dairy cows with shorter periods of negative energy balance in early lactation.
- The significance of finding lactoferrin variants that associate with calving interval is important in our efforts to improve dairy cow fertility
- *L. lactis* has been proposed as a probiotic treatment for mastitis. We have proven that, once introduced into the udder, it rapidly stimulates the cow's immune system helping it fight the bacteria that cause mastitis.
- We found no associations between polymorphisms in immune genes with milk somatic cell count. Therefore these polymorphisms should not be used in breeding programmes for improved mastitis resistance in the Irish dairy herd.
- We observed a delayed immune response by the udder when infected with the mastitic bacteria *Streptococcus dysgalactiae* spp. *dysgalactiae*. This will aid in the development of effective therapeutics to treat *S. dysgalactiae* infections, particularly persistent and sub-clinical infections.

Opportunity / Benefit:

- Polymorphisms that associated with energy balance and fertility can be included in breeding programs to select for more robust dairy cows in the Irish dairy herd.
- Immune signals which exhibited a delayed response to the mastitic bacteria *S. dysgalactiae* are targets for mastitis therapeutics.
- Understanding the mode of action of *L. lactis* as a mastitis therapy is important in our efforts to commercialise this probiotic.
- We have developed expertise in intramammary challenge trials. Queries are welcome from companies interested in performing animal trials to test potential mastitis therapeutic products.

Collaborating Institutions:

UCD, UCC, Irish Cattle Breeding Federation

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1. Project background:

The modern dairy cow has been subjected to intense selection for milk production. The onset of lactation imposes a substantial energy demand which leads to a rapid mobilisation of fat stores. This period of negative energy balance differs from cow to cow and breed to breed and has a major impact on the animal's subsequent body condition, fertility and health. Knowledge of genetic variations in energy balance genes may provide a mechanism to select for improved body condition and fertility in breeding programmes without negatively affecting milk production. From a health point of view, mastitis is the most significant production disease and a major source of economic loss on dairy farms worldwide. It is estimated that 30% of dairy cattle are affected at an average cost of €150-€300 per animal due to therapeutic costs, reduced milk yield, milk wastage, penalties for high somatic cell count and involuntary culling. Efforts to reduce mastitis incidence include incorporation of mastitis resistance into modern animal breeding programs, development of non-antibiotic treatments and enhancement of immunity in the dairy cow.

2. Questions addressed by the project:

What genetic variations associate with improved energy balance, fertility and immune response in the Irish dairy herd?
How does the cow's immune system respond to the mastitis bacteria, *S. dysgalactiae*?
Does the probiotic bacteria, *L. lactis*, boost the immune system to help fight against mastitis?

3. The experimental studies:

We quantified the associations between 43 genetic variations (polymorphisms) in 16 genes with performance traits in 848 Holstein-Friesian sires, estimated from performance of up to 43,117 daughter-parity records per sire. The average co-ancestry among the 848 sires was 2.2%. In addition, we quantified associations between genotypes and phenotypes of 246 lactating dairy cows of different breeds (Norwegian Red, Holstein Friesian, Jersey and Montbeliarde) on the same farm.
S. dysgalactiae accounts for up to 16.5% of all clinical mastitis cases worldwide. To understand an *S. dysgalactiae* infection, animal trails were performed where cows in their first lactation were deliberately infected with this mastitic pathogen. Somatic cell counts, bacteriological data and expression of 10 immune genes were monitored, in milk and blood, from time zero to day 14.
We also performed intramammary challenge experiments with the probiotic, *L .lactis*, to understand how it helps the cow fight the bacteria that cause mastitis.

4. Main results:

Of the 24 polymorphisms tested in energy balance genes, 13 exhibited associations with the energetically expensive process of milking. These included a *corticoid releasing hormone* polymorphism, CRH 240, and several *leptin* polymorphisms. Interestingly polymorphisms in energy balance genes that associated with increased milk fat also exhibited associations with heavier, fatter or larger animals. In total, 10 polymorphisms associated with energy storage traits. Associations were also observed between *leptin* polymorphisms and calving difficulty, gestation length and calf perinatal mortality. The lack of an association between the *leptin* variants investigated with calving interval in this large data set would question the potential importance of these *leptin* variants in selection for improved fertility in the Holstein Friesian dairy cow. In contrast, we established a link between a *lactoferrin* variant and the number of days between consecutive calvings in Holstein Friesian dairy cattle. The A to G polymorphism at -190, was associated with shorter calving interval and increased functional survival.
Of the sixteen polymorphisms in seven immune genes genotyped, only *CXCR1-777* tended to associate with milk somatic cell count, albeit only in the on-farm study. The lack of an association between the

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polymorphisms selected and somatic cell count data would question the potential importance of these variants in selection for improved mastitis resistance in the Holstein-Friesian cow.
In animal trials, we demonstrated that the cow's immune reaction to *S. dysgalactiae* infections is unique in terms of timing, immune targets and levels of response. For example, expression of the important immune genes, *TNF-α* and *IL-1β*, peaked at 48 hours post-infusion which is considered a delayed response when compared to the response to other mastitis pathogens. This delayed response probably results from *S. dysgalactiae* ability to evade the immune system by internalising into mammary cells.
The probiotic, *L. lactis*, has potential as a therapeutic or prophylactic treatment for mastitis because of its ability to (a) produce a bacteriocin with broad spectrum antibacterial activity against mastitis-causing bacteria and (b) elicit a rapid and substantial immune response by the udder. *In vitro* studies also indicated that *L. lactis* impeded the ability of *S. dysgalactiae* to internalise into mammary cells.

5. Opportunity/Benefit:

Polymorphisms that associated with energy balance and fertility can be included in breeding programs to select for more robust dairy cows.
The probiotic *L. lactis* is available to commercialise as a mastitis therapeutic. Queries are welcome from companies interested in licensing this product.
We have developed expertise in intramammary challenge trials. Queries are welcome from companies interested in performing animal trials to test potential mastitis therapeutic products.
There is an opportunity to develop an effective mastitis therapeutic by targeting the immune signals which were slow to respond to the bacteria *Streptococcus*.

6. Dissemination:

The exploitation of the (phenotype-genotype) association results in Ireland will be through the Irish Cattle Breeding Federation in Ireland.
Queries are welcome from companies interested in licensing *L. lactis* as a mastitis therapy.
In total, there were 5 peer reviewed scientific publications, 7 presentations at national and international scientific conferences and 2 popular publications.

Main publications:

- Beecher, C., Daly, M., Berry, D.P., Klostermann, K., Flynn, J., Meaney, W., Hill, C., McCarthy, T.V, Ross, R.P. and Giblin, L. (2009) 'Administration of a live culture of *Lactococcus lactis* DPC 3147 into the bovine mammary gland stimulates the local host immune response, particularly IL-1β and IL-8 gene expression' *Journal of Dairy Research* 76: 340 – 348.
- Beecher, C., Daly, M., Childs, S., Berry, D.P., Magee, D.A., McCarthy, T.V. and Giblin, L. (2010) 'Polymorphisms in bovine immune genes and their associations with somatic cell count and milk production in dairy cattle' *BMC Genetics* 11: 99.
- Giblin, L., Butler, S.T., Kearney, B.M., Waters, S.M., Callanan, M.J. and Berry, D,P. (2010) Association of bovine leptin polymorphisms with energy output and energy storage traits in progeny tested Holstein-Friesian dairy cattle sires. *BMC Genetics* 11:73.

Popular publications:

- Beecher, C., Daly, M., Ross, R.P. and Giblin, L. (2007) 'Managing mastitis more effectively.' *T-Research* 2(3): 24-36. http://www.teagasc.ie/publications/2007/22/22_tresearch200708.pdf
- Howard, D., Waters, S., Giblin, L., Berry, D. and Diskin, M. (2009) 'Banking on DNA.' *TResearch* 4(1):18-19. http://www.teagasc.ie/publications/2009/16/16_tresearch200902.pdf

7. Compiled by:

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