Project number: 5582
Funding source: DAFF (06RDTMFRC437)
Date: September, 2011
Project dates: Nov 2006 – Mar 2010

Releasing the potential of bovine lactoferrin

Key external stakeholders:

Dairy farmers, cattle breeders, AI companies, dairy food companies

Practical implications for stakeholders:

Lactoferrin is a bioactive protein found in milk. It has anti-microbial, anti-cancer, anti-viral and anti-oxidative properties. Dr Linda Giblin and her colleagues in University College Dublin have identified cows that naturally produce more of this protein. They have also shown that different lactoferrin proteins have different levels and types of bioactivity.

Main results:

- We quantified the associations between genetic variations in lactoferrin with performance traits in 848 Holstein-Friesian sires and found associations with calving interval, improved survival and lower milk somatic cell score. Selecting for these variants in breeding programmes will benefit the health and fertility of the national dairy herd.
- We identified a lactoferrin genotype in cows which produced higher levels of Lactoferrin protein in milk.
- We also studied the naturally occurring variation in the Lactoferrin protein. 6 novel Lactoferrin proteins were purified from milk of genotyped cows and dried in powder form. Several were found to have enhanced anti-microbial activities against bacterial pathogens *Listeria innocua, Escherchia coli* and *Streptococcus dysgalactiae* compared to current commercially produced bovine Lactoferrin preparations.

Opportunity / Benefit:

The project provides genetic information to cattle breeders to select cows programmed to produce higher levels of lactoferrin in milk. These cows are more fertile, have improved milk quality and survive longer in the dairy herd than their herdmates.
Milk with increased Lactoferrin content or increased Lactoferrin bioactivity is an ideal raw material for producing Lactoferrin ingredients for the food supplement, infant formula and food safety markets.

Collaborating Institutions:

UCD, Irish Cattle Breeding Federation

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1. Project background:

Lactoferrin plays an important role in the innate immune system, with well-characterized anti-bacterial and anti-viral properties. It is found in bodily secretions and is purified, commercially, from bovine milk. It is considered to be a highly safe food additive. Applications for this protein include infant formula, skim milk, yogurt, chewing gum and nutritional supplements. Lactoferrin spray applied to beef carcasses inhibits bacterial growth. Lactoferrin health supplements boast of anti-ageing properties, anti-cancer properties and promotion of good bacteria in the gut. 90metric tonnes of bovine Lactoferrin are produced per year worldwide selling at a price of $300US per kilogram. It takes 3 litres of milk to produce approximately 60mg of purified Lactoferrin. This project aims to make bovine Lactoferrin production more cost effective by identifying milk with high Lactoferrin concentration and/or increased Lactoferrin bioactivity. This should have knock-on benefits to the food supplement, functional food and meat carcass industry. Also at the farm level, increasing Lactoferrin levels or bioactivity in milk may be of health benefit to the dairy cow.

2. Questions addressed by the project:

- Do variations exist in the bovine *lactoferrin* gene?
- If so, do these variations alter levels of Lactoferrin in milk?
- Do Lactoferrin protein variants have altered bioactivity?
- Are these variations linked to health, fertility or milk production in the Irish dairy herd?
- Is *lactoferrin* gene expression and Lactoferrin protein levels increased in the udder during a mastitis episode?

3. The experimental studies:

One of the principle objectives of this project is to mine different cattle breeds for Lactoferrin variants which effect levels of expression and/or protein activity. As such DNA was extracted from (a) blood samples from 70 unrelated cows from 5 commercial breeds and (b) semen samples from 8 Kerry bulls (traditional native breed). Once amplified, the *lactoferrin* promoter was sequenced and 44 variations were identified. Bioinformatic and haplotype analysis were performed on the sequencing data. Promoter transcription efficiencies of haplotypes were assessed using luciferase reporter assays in mammary epithelial and endometrial cell lines. As *lactoferrin* gene expression is both constitutive and inducible, 3 variations located within important transcriptional motifs were genotyped in 814 Holstein Friesian sires representing the Irish dairy herd. Associations between genotypes and performance traits were quantified using weighted mixed models with genotyped individuals included as a random effect, and average expected relationships among individuals accounted for through a numerator relationship matrix.

The coding region of the *lactoferrin* gene was sequenced from RNA isolated from fresh blood samples of the 70 selected dairy cows. A method was established in-house to purify Lactoferrin protein from the milk of cows carrying *lactoferrin* gene variations that resulted in amino acid substitutions. Anti-bacterial, Anti-inflammatory, anti-proliferative, heparin and anti-biofilm assays were developed in-house to test the bioactivity of the purified Lactoferrin protein variants.

To investigate the role of Lactoferrin in the bovine innate immune system, *lactoferrin* gene expression and Lactoferrin protein levels were determined *in vivo* during a deliberate challenge with the udder pathogen, *Streptococcus dysgalactiae*.

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4. Main results:

- 44 variations were identified in 2.2kb of the *lactoferrin* regulatory region. In silico analysis of sequencing data revealed two major haplotypes (H1a and H2a) that differed at 10 sites where constitutive and inducible transcription factors may bind. The basal promoter transcriptional activity of H1a was 1.44-fold higher than that of H2a in mammary epithelial cells. More importantly, cows with the H1a haplotype had increased Lactoferrin protein concentration in milk at various time points over the lactation curves, compared to herdmates with the H2a haplotype. The discovery of cows with increased Lactoferrin levels in milk identifies designer dairy herds for Lactoferrin production.
- Phenotype–genotype associations revealed that the C to T polymorphism at −586bp in the *lactoferrin* promoter, which distorts a putative activating protein 2 binding site, was associated with a shorter calving interval and higher somatic cell score in the Irish dairy herd. The G to A polymorphism at −190bp, located in a putative selective promoter factor 1 transcription binding site, was associated with a longer calving interval and decreased functional survival. A third polymorphism (A to C) at position −28bp, found within the noncanonical TATA box, had a tendency to associate with functional survival. As there was no affect on milk production, the G allele at -190bp could be used in breeding programmes to select for improved fertility in the Irish herd.
- 27 Lactoferrin protein variants were identified from sequencing data. 6 Lactoferrin protein variants were purified from milk of genotyped cows and dried in powder form. Using the bioassays developed in-house, these novel Lactoferrin powders were compared to current commercially produced bovine Lactoferrin preparations. Several had enhanced anti-microbial activities against bacterial pathogens *Listeria innocua, Escherchia coli* and *Streptococcus dysgalactiae* and improved anti-biofilm activity. Identification of Lactoferrin variants with increased bioactivity will have a knock-on benefit to the food supplement, infant formula and meat carcass industry.
- As Lactoferrin is known for it ability to boost the immune system the researchers wanted to establish if high levels in milk aided the animal fight a mastitis infection. In deliberate challenge experiments with a mastitis pathogen, the researchers found significant cow to cow variation and more work is need for a definitive result.

5. Opportunity/Benefit:

- This project provides genetic information to cattle breeders to select cows with improved fertility, milk quality and survival in the herd.
- Milk with increased Lactoferrin content or increased Lactoferrin bioactivity is an ideal raw material for producing Lactoferrin ingredients for the food supplement, infant formula and food safety markets. Queries are welcome from interested companies.
- The exploitation of the phenotype-genotype association results will be through the Irish Cattle Breeding Federation in Ireland. Selecting for the Lactoferrin variants in breeding programmes will benefit the health and fertility of the national dairy herd and thereby contribute to productivity in the industry.

6. Dissemination:

Project results have been disseminated through the Food Institutional Research Measure Relay website www.relay.ie.

Main publications:

Bahar, B., O'Halloran, F., Callanan, M.J. McParland, S., Giblin, L. and Sweeney, T. (2011) 'Bovine lactoferrin (*LTF*) gene promoter haplotypes have different basal transcriptional activities.' *Animal Genetics* 42:270-279.

O'Halloran, F., Berry, D. P, Bahar, B., Howard, D. J., Sweeney, T., and Giblin, L. (2010) 'Polymorphisms in the Bovine Lactoferrin Promoter are Associated with Reproductive Performance and Somatic Cell Count.' *J Dairy Sci.* 93 (3):1253-9.

O'Halloran, F., Bahar, B., Buckley, F., O'Sullivan, O., Sweeney, T. and Giblin, L. (2009) 'Characterisation of single nucleotide polymorphisms identified in the bovine lactoferrin gene sequences across a range of dairy cow breeds.' *Biochimie* 91: 68-75.

Popular publications:

Beecher, C., Daly, M., Ross, R.P. and Giblin, L. (2007) 'Managing mastitis more effectively.' *T-Research* 2(3): 24-36.

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Giblin, L. (2007) 'Extracting and concentrating Lactoferrin'. *Relay Report* 12 Feb.

Giblin, L. (2009) 'Cows with the right lactoferrin gene can save farmers money' *Relay Report* 29th Oct.

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