Lameness (i.e. structural or functional disorder affecting one or more limbs or the vertebral column that is evident while standing, rising, moving or lying down is considered to be the most severe welfare problem facing both the dairy cow, and the European dairy industry. The disorder causes severe pain and distress for the cow and substantial economic losses. Dairy lameness is primarily caused by diseases or injuries to the hoof, which can be categorised as either affecting the claw horn, or the surrounding skin. Claw horn disorders (CHD; characterised by sole haemorrhages, sole ulcers and white line disease) are non infectious in nature, and result from complex genetic and environmental interactions.Historically, CHD have been thought to arise as a result of inflammation of the laminae within the hoof capsule. However, it has recently become clear that other factors are influential in CHD development. Periparturient changes in hormones are also linked with the onset of CHD as they can cause increased vascular permeability, increasing the risk of edema and ischemia in the hoof. Weakening of connective tissue near the time of calving also causes the pedal bone to drop and compress the corium, further disrupting claw horn formation.

Early and accurate lameness detection means overall prognosis and the welfare of cows is improved. Unfortunately, early detection of lameness and CHD is extremely difficult; cows show little behavioural response to pain until injuries are advanced, and stockpersons find it difficult to detect signs of impaired locomotion. Scoring systems that categorize lameness by degree of locomotory deviation from ‘normal’ lack reliability and sensitivity, and cannot always detect the presence of CHD before the damage is severe. Thus it is unlikely that gait changes alone will provide accurate early detection of lameness caused by CHD, and objective measures to aid lameness diagnosis that can be clinically applied at farm level warrant investigation. Moreover, the prospect of identifying objective biomarkers of inflammatory hoof lesions could help identify animals in need of pain relief and provide appropriate targets for the development and monitoring of novel lameness therapies.

The first step in identification of biomarkers of disease usually begins with exploratory studies to identify characteristics that are unique to tissues from affected and unaffected individuals. Lame cows display several behavioural and physiological responses that are representative of a sickness response, indicating a lameness-associated systemic activation of the immune system.

Peripheral blood mononuclear cells (PBMCs) are immune cells that can be obtained relatively non invasively, and are responsible for surveillance of the body with regard to injury and disease. They display specific expression signatures with regard to several diseases in cattle. Previously, a microarray-based gene expression profiling approach using PBMCs identified a small number of genes that could be part of a signature for lameness in dairy cows. The objective of our first study was to characterise differences in leukocyte profile, cortisol and DHEA response, metabolite profile, and expression of genes associated with lameness between cows with sole ulcers and sound cows. The second study compared these responses in cows with sole haemorrhages ranging from severe to mild/none. The third study was a retrospective longitudinal study, comparing these responses in cows that had severe sole haemorrhaging approximately three months into the lactation with these measures at calving, and with cows that did not develop sole haemorrhages

Study 1

Twelve cows that were clinically lame due to ulceration of the sole (ULCER cows) were identified through weekly locomotion scoring and inspection of lame cows’ feet. Each lame cow was paired with a healthy cow (SOUND) of similar lactation number and stage, breed, body condition score, weight and milk yield. Blood samples were taken from each pair on the same day, then a variety of immune, endocrine and gene expression markers compared. ULCER cows had higher locomotion scores than sound. They also had higher levels of circulating glucose, protein and DHEA and haptoglobin and lower levels of circulating urea and DHEA:cortisol ratio. The had a higher percentage of circulating neutrophils, and a tendency for a lower lymphocyte percentage, which resulted in a higher neutrophil lymphocyte ratio. Several genes that had either previously been associated with lameness, or associated with stress, were also found to be differentially expressed in LAME versus SOUND cows. LAME cows had greater expression of MMP13, IL1-alpha, FAS and CD62L, and tended to have greater expression of IL10, IL1-beta, IL8, GR-alpha and haptoglobin, as well as tending to have lower expression of CSF2 than sound cows.

As well as a targeted gene approach to identifying differences in gene expression, a next-generation sequencing approach was also taken. This was to attempt to identify differences in gene expression accross the bovine genome. We used the Illumina platform, and after analysis of the results using bioinformatic methods, we identified 43 genes that were differentially expressed in ULCER and SOUND cows. Using this methodology we found that only 5 genes were up-regulated in lame cows, and 38 downregulated. We selected 10 of these genes for further investigation using a targeted gene expression approach. We only identified one gene, CXCL13, that was differentially expressed using both methods. In both cases ULCER cows expressed more of this gene.

Study 2

The study subjects consisted of 51 cows. Cows were locomotion and hoof scored at approximately 3 months post partum. They had sole haemorrhages scores ranging from 0 to 91 (higher scores = worse sole damage), but no other hoof pathology, or other health disorder. Blood samples were taken on the same day as hoof and locomotion scoreing were carried out, and analysed for the same potential biomarkers as in study 1.

We found that as sole haemorrhage score increased (worsened) cows had more impaired locomotion. Similar to the results from study 1, cows with more sole damage (higher haemorrhage scores) had higher levels of circulating glucose, urea, haptoglobin, and a higher cortisol:DHEA ratio than cows with healthy hooves. They also had higher levels of circulating cortisol. There was also a tendency for cows with sole damage to have a higher neutrophil:lymphocyte ratio. As regards gene expression, we identified two genes, IL10 and IL1-alpha, that were associated with higher sole haemorrhage scores, similar to the results from study 1 where ULCER cows had higher expression levels of these genes compared with control. In addition, greater expression of CCR5, IL4 (general indicators of stress), CFB and KLRC1 (identified as being expressed more in ULCER than SOUND cows using next generation sequencing) were also associated with higher sole haemorraging.

Study 3

The study subjects consisted of 14 cows. All cows were locomotion scored and had their hooves examined at approximately 2 weeks and 2 months after calving. Six cows had a significant increase in sole haemorrhage score between examinations, and 8 cows had consistently low scores. The biomarkers examined in study 1 were compared between cows and time points, with the exception of differences in gene expression. We found no interactions between the examination and whether or not cows had an increase in sole haemorrhage score, or not. However, overall, cows that did not have an increase in sole haemorrhage score had higher neutrophil, lymphocyte and eosinophil percentages. They also had a higher neutrophil lymphocyte ratio.

Overall interpretation

As expected, cows with claw horn disorders showed a behavioural (locomotary ability) and physiological profile different to healthy cows. The stress hormone, cortisol, was associated with damage to the sole, as was the leukocyte profile (high neutrophil percentage, and low lymphocyte percentage). Morevoer, high circulating glucose levels are indicative of a stress response, as the body mobilises energy from storage to cope with the stress. One cause of stress hyperglycemia could be the presence of high circulating levels of cytokine, in particular IL1. In fact, IL1-alpha was more highly expressed in both ULCER cows, and cows with higher sole haemorrhage scores. We did not find any biomarkers that could be predictive of damage to the claw horn (study 3). With regard to gene expression, we identified several genes that appear to be more highly expressed in cows with claw horn damage than in healthy cows. However, all of the genes that were differentially expressed are associated with general activation of the immune system. Due to the low number of genes that were differentially expressed, we were unable to identify any specific metabolic pathways that were differentially activated as a result of claw damage. Thus, these genes are probably not specifically indicative of claw damage, or thus useful biomarkers of the disorder.

It is possible that even though cows appear to show an overall stress response when claw horn disorders are present, due to the localised nature of damage (the sole of the foot) specific biomarkers of claw horn damage might not be found in circulating white blood cells. Further work should be carried out to determine whether biomarkers could be identified at the site of the damage.