Executive summary

1. Introduction

Long-term problems of cattle performance were observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. These problems presented intermittently during the early 1990s, but were reported to have progressively worsened since 1997. There is some anecdotal evidence (from a private veterinary practitioner) that the problem may have presented as early as 1979, when cattle of small stature were observed. The problem principally presents as stunted growth and ill-thrift in growing cattle, although poor body condition and reduced milk yield in adult cattle were also observed. Marked stunting was generally first noted in calves after the first few weeks of life, with calves gaining as little as ~0.18 kg/day during the first year of life. Animals appeared normally-proportioned, but of small stature. The problem affected both home-born and introduced young animals. Affected animals appeared to improve (in condition, but not stature) if moved away from the index farm. In addition, substantial fluctuations in average daily gain (ADG) of cohorts of growing stock have been reported. These fluctuations are not consistent year-to-year, nor are they associated with periods of insufficient nutrition. These problems may be geographically clustered, noting that poor animal performance has been reported on at least one neighbouring farm.

The index farm is located in hilly topography on the edge of the Castlecomer plateau, approximately 1 km from Castlecomer in Co. Kilkenny. The Castlecomer area is located near the centre of the Leinster coalfield (which covers an area of approximately 250 km^2) and has a long history of anthracite coal mining, from the 1600s to 1969. The local Deerpark mine, located approximately 3 km north of Castlecomer, was the largest opencast coalmine in Ireland, producing nearly 100,000 tons of coal annually at its peak in the 1940s. In this area, the soils are generally considered unsuitable for cultivation or intensive grassland production unless properly managed, due to poor to very poor natural drainage. The index farm (total area 67 ha; in 2008: 207 cattle) is divided into three separate fragments, including two that are separated by a roadway. It was run as a dairy farm for many years, and as a mixed dairy/suckler (beef cattle) operation since the early 1990s. Stock water has consistently been sourced from a disused on-farm shale quarry, except for three years from 1999 when an on-farm well was used. A programme of grassland improvement was conducted during the 1990s.

It is well established that adverse health events frequently cluster, either in time and/or space, regardless of whether the cause is infectious or not. A broad range of methods can be used to investigate these events, including those drawn from clinical, laboratory-based and epidemiological disciplines. Epidemiological methods are generally well-suited to the investigation of event clustering, as they can provide an understanding of the patterns of presentation, and an insight into clues that might potentially be associated with the source and/or cause of the problem. Results from epidemiological studies can prove helpful in guiding in-depth laboratory studies. These methods are well suited to investigation of rare diseases, to situations where exposure leads to a highly specific presentation and can be useful as a tool to determine the potential disease risk from a defined exposure. With care, epidemiological approaches can also be used to investigate single-site clusters.

Approach adopted

The problem of cattle performance on this farm has been investigated intensively by several organisations, including the Department of Agriculture, Fisheries and Food (DAFF), Teagasc and the Environmental Protection Agency (EPA), and considerable data have been generated, particularly for growing cattle. In these studies, a broad range of methodologies has been used, both epidemiological and otherwise. A detailed review of these data was conducted, from an epidemiological perspective, to identify patterns of presentation that might provide clues as to the cause(s) of the problem. Key findings were distilled, then considered in the context of opinion from national and international experts and based on information in the general international literature. Then, a number of general working hypotheses were developed, including:

- hypotheses specific to the index farm (such as management, nutrition, genetics and health), as well as
- hypotheses not necessarily specific to the index farm, including essential element imbalances and environmental pollution.

Five separate studies have been completed over a three year period (*Studies 1 to 5* in the supporting documentation), from late 2006, to investigate the validity of these general working hypotheses. These studies were conducted primarily on the index farm, although two other farms (in counties Kilkenny and Meath) were also used for comparison. In support of this work, reviews were conducted of the international scientific literature and detailed data analyses were undertaken.

In the *Overview* (found in the supporting documentation), a critical epidemiological review is presented of all available evidence relevant to this problem, including the results of these five studies and the earlier work. The *Overview* was conducted to determine whether any or all of the above-mentioned working hypotheses could be plausibly associated with the observed problems of animal performance on the index farm. Each hypothesis was assessed by considering all available sources of data, taking account of data validity and precision, and using the international benchmark (the Bradford-Hill criteria, as outlined in the *Overview*, p5) when assessing evidence for causation during decision-making.

Study 1. **Winter feeding trial, January to May 2007**

The objective of this study was to identify, if possible, any underlying mechanisms of poor performance in growing animals on the farm, thereby providing possible clues for the ongoing problems that were, or still are, being observed.

Some 46 cattle were enrolled in the study from the index farm and a further 31 control cattle were sourced from a farm in County Meath. These cattle were divided into five separate treatment groups, each being supplied with defined feed and water combinations. The index farm cattle had lower initial weights than the control cattle but were matched in all other respects. Three of these groups were based on the index farm and two were resident on a control farm located approximately five km away. Throughout the study, the animals were weighed, bled and assessed at regular intervals. An extensive battery of laboratory testing was undertaken to assess factors that could potentially be linked to poor growth, including nutrition (energy status, an assessment of elemental status), disease and immunity (serology, serum biochemistry, general immune status) and endocrine parameters associated with growth. Analysis of feed and water was also conducted. The animals were vaccinated, and treated for internal parasites, at the start of the study.

All cattle performed well throughout the study period, thereby limiting the ability of this study to identify any causes of the ongoing intermittent poor performance problems. During January to May 2007, the index farm and control cattle achieved ADGs of 0.66 and 1.00 kg/day, respectively. Among the many parameters assessed, few significant differences were identified within and between groups. Mineral deficiencies/imbalances had been identified in the past, and were comprehensively assessed and described in this study. At the start and

throughout the study, the cattle from the index farm were selenium (Se) deficient. Selenium also decreased among the control cattle, reaching the threshold for deficiency concentrations towards the end of the study. Results from *Study 1* indicated that Se deficiency may be a contributor to the ongoing performance problems on the farm. IGF-1, a principal mediator of growth hormone in animals, was markedly lower in the index sourced cattle at the start of the trial, though the concentrations increased in both groups throughout the period. Cadmium (Cd) was measured in cattle towards the end of the study, the significance of which is discussed later.

Study 2. **An evaluation of underlying performance mechanisms in an experimental group of animals kept on the index farm from January 2007 to April 2008**

Following the outcome of *Study 1*, it was decided to keep ten of the above-mentioned 31 outsourced Aberdeen Angus/Friesian cross cattle on the index farm for a further extended period of observation. During the first study, these 10 animals had been held on the index farm. The objective of *Study 2* was to determine whether periods of poor weight gain or weight loss, if observed, were associated with changes in haematological parameters, essential element status, or exposure to heavy metals.

The programme of weighing and sampling was essentially as previously described, albeit at a lower frequency.

Weight gains were intermittent during the period of observation: very good ADGs were observed during the initial housing period (January to May 2007; 0.96 kg/day), very poor during summer grazing (May to October 2007, 0.13 kg/day) and good during the subsequent housing period (October 2007 to April 2008, between 0.55 and 1.2 kg/day). During the summer grazing period, the cattle experienced a coincident reduction in weight gain, which is suggestive of a single insult over a defined period of time. At this time, there was no overt evidence of disease in these cattle. Adequate ADGs during summer 2007 have been reported elsewhere in Ireland. Selenium concentrations declined during the study period and were considered to be deficient from October 2007 to January 2008. However, the concentrations improved on winter feeding and returned to normal by February 2008. Copper (Cu) concentrations declined after cattle were introduced onto the index farm, and were considered deficient from May 2007 until the end of the study. In this study, essential element deficiencies were again identified as potential contributors to the ongoing performance

problems on the index farm. A single Cd peak was measured in cattle during this study, the significance of which is discussed later.

Study 3. **An assessment of responsiveness of calves from the index farm with known essential element deficiencies to selenium supplementation, May 2007 to April 2008**

Having identified Se deficiency as a potential contributor to poor performance on the index farm, *Study 3* was conducted to evaluate the efficacy of different forms of Se supplementation in the maintenance of both Se status and animal performance, and to compare the performance of Se supplemented animals with unsupplemented animals moved to an unaffected farm.

This study was conducted over a one-year period from May 2007 to April 2008. At the start of this study, 42 calves from the index farm were randomly allocated to one of four treatment groups. Three groups were kept on the index farm: two groups were treated with different commercially available Se supplementation products, and the third group (the on-farm controls) received a sterile water placebo. The fourth group (the off-farm controls), which also received the same placebo, were moved after six weeks to an unaffected farm in County Meath for four months (from June to October 2007) and then returned to the index farm. Blood samples and animal weights were collected from all calves on a fortnightly basis for the first six months and thereafter at monthly intervals. Additional work was conducted on several calves at the end of the study, including radiological and post-mortem examinations.

All animals had normal Se concentrations at the start of the study, which is typical for recently born animals even in areas of Se deficiency. Among the on-farm control calves, Se declined rapidly; these animals were considered Se deficient from June 2007. Among the other two on-farm treatment groups, only one of the two products (barium selenate) succeeded in maintaining the Se status above concentrations indicative of deficiency. Increased Se was observed among the calves that were moved from the index farm, though this was subsequently reversed when the animals returned home. This observation confirmed the deficient Se status of the index farm, as found in *Study 1*. The largest difference in ADG between treatment groups was observed between the three on-farm groups and the off-farm controls. Following an initial period of adjustment, after this group had been moved, the ADG among calves in this group was almost twice that of all three groups of calves that remained on the index farm. Selenium supplementation had a positive impact on blood Se status, but

was not sufficient to overcome the shortfall in animal performance, suggesting that Se deficiency may be only a component of a more complex aetiology. In support of this view, marginal to deficient concentrations of Cu and iodine (I) were also observed in all groups. The presence of radiodense growth retardation lines is consistent with poor calf growth, but does not provide any definitive insight into the cause of the problem.

Two patterns of Cd excess were observed in the *Study 3* animals: a large Cd peak (similar to the *Study 2* Cd peak) and background Cd exposure. The significance of these findings is raised later, and considered in detail in the *Overview*.

Study 4. **A survey of essential elements and heavy metals in soil and herbage in a small area encompassing the index farm, April and September 2007**

Given that Se, Cu and other essential elements were identified as deficient or marginally deficient in *Studies 1 to 3*, a comprehensive survey of essential elements and heavy metals was undertaken of the soils and herbage on and surrounding the index farm.

The survey was carried out over two time periods, April 2007 and September 2007, in a 2.5 sq km grid incorporating the index farm at its centre. One hundred and six sampling points were used in April 2007, and 46 of these sampling points were repeated in September 2007. Soil and herbage samples were collected: in total, 152 soil samples were analysed for 18 variables, and 140 herbage samples were analysed for some 16 variables

Low soil pH and high soil liming requirements were identified throughout the sampling area, including the index farm. Soil pH greatly influences nutrient uptake by plants from soil, and subsequently nutrient availability in grazing animals. Calcium (Ca), Cu, Se and zinc (Zn) concentrations were low in soil and herbage in the sampling area and on the index farm. These conditions are not dissimilar to those found on farms in other mineral deficient areas of Ireland. In a small number (4%, 4/97) of sampling points (near to but not on the index farm) in April 2007, fluoride (F) concentrations exceeded the maximum tolerable level for cattle (40 mg/kg). No measurement of Cd was undertaken in herbage samples. In soil samples, Cd was below the level of detection for the test used (<0.1 mg/kg) in over 90% of samples tested, and those where Cd was reported, the maximum Cd concentration was 0.72 mg/kg, which is within the typical range for Irish soils (0.1 to 1 mg/kg). Mineral imbalances (in particular Ca,

Cu, Se and Zn) in pastures caused by low soil mineral status, exacerbated by low soil pH, could lead to significant mineral deficiencies in cattle, thus impeding optimal performance.

Study 5. **Kidney cadmium concentrations in cattle from the index farm, 2003-2005 and 2009**

Study 5 was prompted by the Cd excess observed in *Studies 1 to 3*. In order to assess Cd exposure on the index farm, concentrations of four toxic metals were measured, including Cd, in livers and kidneys collected from index farm cattle. Of all animal tissues, liver and kidney have the greatest potential to bioaccumulate a range of toxic metals, including arsenic (As), Cd, lead (Pb) and mercury (Hg).

In 2009, heavy metal analyses was conducted on livers and kidneys collected from nine cull cows that had spent between 5 and 13 years on the index farm. Additional kidney Cd results were obtained from the Department of Agriculture, Fisheries and Food for 14 mixed-age animals submitted for post-mortem during 2003-2005.

In all nine cull cows, the concentrations of liver and kidney As, Pb and Hg were within the normal range for animal health purposes. In one kidney, the Pb concentration exceeded 0.5 mg/kg (the maximum concentration allowed for human consumption). When all 23 animals (from 2003-2005 and 2009) are considered, 8 (37.8%) animals had kidney Cd concentrations exceeding 1.5 mg/kg, the upper limit of the normal range for animal health. Kidney Cd concentrations in animals were associated with both increasing age and time spent on the index farm. Based on current knowledge, exposure at this level is not associated with adverse effects in terms of animal health or performance.

Overview. **An epidemiological evaluation of all relevant data gathered to date and an assessment of hypotheses that could plausibly be associated with poor animal performance on the index farm**

In recent years, there have been a number of studies conducted to investigate the performance problems among cattle on the index farm. The aim of this final study was to critically evaluate all of the relevant data, and to assess hypotheses that could plausibly be associated with the problems of poor animal performance on the index farm.

In total, 12 (including nine major) government-commissioned studies (including the five described above) and three other studies were reviewed. These range in date from 2004 to 2009, and in scope from large government-commissioned studies to short reports of on-farm observations. An additional seven supporting analyses and reviews were undertaken, including detailed air dispersion modelling and a review of all available literature on Cd in cattle, and these are presented in appendices accompanying the *Overview.* The clinical presentation was as described by the herdowner. Growing animals are very small in both size and stature, but well-proportioned apart from a slightly oversized head. Animals generally appear in good health with a good appetite, apart from stunted growth and a rough hairy coat. Within the locality, farmers believe that the problem is localised, predominantly affecting cattle on the index farm. It has been difficult to rigorously test this hypothesis, as only limited objective data on growing cattle performance are available from other farms in this locality. Nonetheless, this view is supported by the qualitative data that are available.

For some years prior to 2007, a number of animal health problems associated with infectious conditions were identified on the index farm, including acute and chronic pneumonia, infection with *Salmonella dublin,* and high calf mortality rates. Since 2007, however, there has been an observed improvement in the health of animals on the index farm, and few animal health concerns were observed during intensive experimental and observational work (the above-mentioned *Studies 1 to 5*) conducted from 2007 to 2009. Animal health concerns do not, on their own, provide an adequate explanation for the observed poor performance currently reported on the index farm, for a number of reasons. Episodes of poor performance have continued since 2006, regardless of changes in disease management and biosecurity procedures, and despite the observed reduction in clinical morbidity. In addition, and at odds with what might be expected with a single infectious disease or parasitic condition, the presenting problem is intermittent, without regard to either season or defined management events such as housing or grazing. Periods of poor performance have also been interspersed between periods where animal performance has been entirely satisfactory. They have also coincided with times when preventive health measures have been at their most-robust. General aspects of farm management, including preventive animal health, are not dissimilar to those of other farms in Ireland. We have also observed a number of essential element deficiencies, at concentrations sufficient to adversely affect both growth and production in cattle. We have no evidence in support of other hypotheses, including management, genetics or nutrition, currently contributing to poor animal performance on the index farm.

Deficiencies in several essential elements appear to be contributing to, but on their own do not provide an adequate explanation for, poor animal performance on the index farm. A range of element deficiencies in both soil (Cu, Se) and herbage (Ca, Cu, Se, Zn) have been identified, as well as soil conditions (low pH, high liming requirements) known to adversely affect the availability of essential elements. Several elements in soil and herbage (Ca, Cu, Se, Zn) are at concentrations insufficient to meet animal requirements, particularly of dairy cows. Further, cattle on the index farm were Se deficient, based on *ad hoc* monitoring and during formal longitudinal studies on growing animals. The lack of improved performance in animals on the index farm in the presence of Se supplementation is consistent with an aetiology more complex than Se deficiency alone.

In several studies, two patterns of Cd excess were observed, including *acute Cd excess* (based on a single Cd peak observed during August 2007 in *Study 2,* and in July and August 2007 in *Study 3*) and *background Cd exposur*e (in *Studies 1 and 3*). These results gave rise to a further investigation (*Study 5*), a detailed review of the scientific literature (in appendix 7 accompanying the *Overview*) and consultations with international experts. On balance, after considering both the concentrations detected and the pattern of detection, the large Cd peak (suggestive of an acute Cd excess) appears to be biologically implausible, and therefore is most likely to be invalid. This issue is discussed in detail in the *Overview*. Background Cd exposure has been corroborated from several studies, including *Study 5.* The measured kidney Cd concentrations are not of toxicological significance, and therefore would not be expected to affect animal health. Certainly, based on current knowledge there is no evidence that Cd exposure at this level could contribute to the stunting and poor growth rates that were observed on the index farm. Nonetheless, they are much higher than those reported in a very recent pan-European study. The Cd source on the index farm has not been determined, and additional testing may be warranted. Although there is evidence of intermittent F exposure within this locality, it is at concentrations that are unlikely to affect animal health.

There has been ongoing concern about a possible role of the local brick factory in the observed animal performance problems. This factory was established in the late 1960s and is located to the northwest of the index farm. As part of this review, we conducted detailed air dispersion modeling of gaseous emissions from this factory. This modeling was conducted using local emission, meteorological and terrain data. Results from the model do suggest that the index farm consistently experiences some of the highest predicted gaseous (SO_2) exposure of any farm in the immediate locality with all its land situated in the direct environs of this

factory. However, several neighbouring farms, with no reported evidence of poor animal performance, experience similar levels of annual gaseous (SO_2) exposure. In this work, SO_2 was used as a crude proxy for gases emitted from this facility. The factory has not operated since December 2008. Based on the modelling results and all other relevant data, there is no current evidence to associate the factory with the specific problems that have been investigated on the index farm.

Conclusion

A number of element deficiencies have been identified in soil, herbage and cattle on the index farm, including Ca, Cu, Se, Zn and, to a lesser extent, I. Similar concentrations have been measured on other farms in the area, where problems of poor animal performance have not been reported. Observed soil conditions (low pH, high liming requirements) are known to adversely affect the availability of essential elements. The condition is not responsive to Se supplementation, consistent with an aetiology more complex than Se alone. A number of animal health concerns were identified on the index farm prior to 2007, with adverse effects on animal performance. Since 2007, however, the potential for such effects has been substantially reduced, following the introduction of robust preventive measures and as a consequence of intensive veterinary supervision by members of the project team and the farmer's own private veterinary practitioners. There is evidence of background Cd exposure among cattle on the index farm, based on an assessment of Cd kidney in 23 animals during 2003-2005 and 2009. However, these concentrations are not of toxicological significance based on current knowledge, and would not be expected to adversely affect animal health. Fluorine is present intermittently, but not at concentrations considered detrimental to animal health.

This investigation has sought to address two related questions about this case: the possible cause(s) of the problem and reason(s) why this problem appears to cluster, both in time and space. It is likely that this is a multifactorial condition. A number of factors have been described that undoubtedly have some influence on the performance of cattle on the index farm. However, no comprehensive understanding has yet been established to fully explain the epidemiological presentation of cases, in particular the localisation of cases to the index farm and the temporal distribution of periods when negative performance was recorded. Further work to clarify the spatial extent of the problem, and to provide baseline information about Cd concentrations in Irish cattle at slaughter, may be warranted.