Final report

South Gippsland Autumn Setback Syndrome

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Prepared by: Leah Tyrell and John Larsen
The Mackinnon Project, The University of Melbourne

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Meat & Livestock Australia Limited

PO Box 1961

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Abstract

A syndrome of unknown aetiology, characterised by lost production and chronic ill-thrift, has been recognised in yearling and possibly two year old cattle in the south Gippsland area of Victoria during autumn. A number of producers have reported losses, however the range of clinical signs observed means it may be overlooked or confused with other conditions, such as facial eczema. An observational study with monthly sampling was undertaken over a five month period on three farms in south-east Gippsland. The syndrome was observed in early winter on one of the three farms, with 7% of steers affected by diarrhoea, photosensitisation and weight loss. A proportion of these were confirmed to have severe liver damage. At this time, there was an increase in mould counts on pastures that the steers were grazing. It is suspected that a mycotoxin produced by an as yet unidentified fungus is causing the syndrome, although this has not been confirmed. However, as a result of this study of the syndrome, ways of investigating it and its impact on production are now better understood. There have been anecdotal reports of clinical signs similar to these seen in other high winter rainfall areas, such as King Island and parts of Tasmania. Further work should include observations on farms in these areas as well as south Gippsland.

Executive summary

An as yet uncharacterised syndrome causing weight loss, liver damage and photosensitisation in beef cattle has been described in several high winter rainfall regions of Australia, particularly south Gippsland. The range of clinical signs include weight loss, severe diarrhoea, decreased immunity resulting in secondary bacterial infections, such as Salmonella and Yersinia, and liver damage accompanied by elevated liver enzymes and often severe photosensitisation.

A previous clinical investigation on a farm in south-east Gippsland had found no specific cause, but suggested that mycotoxins produced by fungi growing on the pasture may be a possible cause. The range and ill-defined nature of the clinical signs means the prevalence, occurrence and impact of the syndrome is difficult to determine. Consequently, this study aimed to better describe the lost production and identify a causative agent associated with the syndrome.

A mob of 200-500 cattle was monitored on each of three beef producing farms in south-east Gippsland. Monthly blood samples, bodyweights, faecal worm and fluke egg counts, pasture quality and quantity assessments and pasture mould counts were used to describe and quantify the impact of the syndrome. On one of the three farms, 7% of steers had severe diarrhoea and were losing an average of 400 grams/day in June. This was despite the remainder of the mob gaining an average of 150 grams/day over the same period. However, unless producers are constantly weighing their stock, these changes in bodyweight will be difficult to detect. Of the steers losing weight, 22% were exhibiting the classical features of the syndrome, namely photosensitisation and elevated liver enzymes (glutamate dehydrogenase; 'GLDH'). Furthermore, liver biopsy confirmed there was severe liver damage present in the affected cattle.

Before the onset of these clinical signs, pasture mould counts began to increase in April on all three farms. The first indication of the syndrome was early signs of photosensitisation in some cattle. The affected farm had a mixture of Herefords and Angus, but only the Herefords had photosensitisation (not unexpected because skin without pigmentation is more sensitive). Nevertheless, both Angus and Herefords were losing weight and had severe diarrhoea.

The predominant breed on the remaining two farms was Angus, and so no signs of photosensitisation were noted. Cattle were not weighed in June, because this was later than when the syndrome was previously seen, and so the planned monthly observations stopped in May. Thus, although mould counts did increase on the unaffected farms in April and May, it is not known if the syndrome was occurring because more detailed investigations, such as blood sampling and liver biopsies, were not able to be undertaken.

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1. Background

A syndrome characterised by losses in production has been recognised in yearling and possibly two year old cattle in the south Gippsland area for several years. The syndrome appears to occur from late summer and autumn, usually with a sudden onset. Clinical symptoms include abdominal upset with diarrhoea and discomfort, varying degrees of photosensitisation and immunodeficiency (decreased immunity), with some animals succumbing to secondary diseases. Affected cattle have elevated concentrations of liver enzymes, strongly suggestive of liver damage. However, this damage is not consistent with that of facial eczema, a disease caused by the fungus Pithomyces chartarum that commonly occurs on irrigated pastures around Maffra in central Gippsland, and parts of the high rainfall country in west Gippsland. Because of the liver damage, it is suspected that South Gippsland Autumn Setback Syndrome ('SGASS') could be caused by a mycotoxin produced by an unidentified fungus on the pastures.

Our initial investigations found that the syndrome has probably occurred every autumn for at least the past three years. This was reinforced in discussions with local veterinarians, who suspected that a problem with poor growth rates of weaner cattle had been occurring for some time, but had not investigated this in any detail. This region has a consistently high rainfall, and so there is a high probability that this poor performance occurs, at least to some degree, annually. It is also highly likely that the syndrome occurs in other temperate high rainfall regions of south-eastern Australia, such as King Island and other parts of Tasmania.

An initial investigation was performed on one affected farm (Farm 2), primarily to rule out worms as the main cause of illthrift and poor growth of cattle. This investigation found that, in a mob of 500 yearlings, 25% lost weight, 10-15% later showed signs of photosensitisation and 5% succumbed to a secondary disease, such as Salmonellosis, Yersiniosis, pneumonia or Coccidiosis. About 1% of the affected cattle died, and 3-4% never recovered, having poor growth rates and chronic ill-thrift. Preliminary investigations indicated that liver enzymes were elevated, indicating a form of liver damage, although not consistent with facial eczema. In addition, an investigation on a neighbouring property revealed that 600 kg bullocks grazing 2.5 t/ha of high quality perennial pasture were performing below expectations. Cattle that were losing weight in the mob were targeted for blood testing and results revealed that they had elevated liver enzymes with a similar clinical pathology pattern to the neighbouring property, Farm 2

2. Objectives

The objective was to quantify the impact of this syndrome, which causes yearling cattle to grow less than what is expected from reasonable quality pastures over the late summer/autumn by:

- Assessing cattle growth rates by monitoring live weight monthly.
- Comparing the growth rates to growth rates modelled on Grazfeed™ and Grassgro™.
- Collecting blood samples to assess liver damage by monitoring liver enzymes monthly .
- Monitoring trace element status at the start and after six months to exclude trace element deficiencies as a cause of illthrift.
- Monitoring blood pepsinogen levels and worm egg counts monthly to exclude worms as a cause of illthrift.
- Describing the pathology of the disease through necropsy of yearling cattle.
- Assessing composition, availability and digestibility of pastures.
- Analysing pastures for mycotoxins, including ryegrass and other endophytes and fungal spores, to identify if the causative agent is a fungus.

The project aimed to improve the production of beef per hectare by:

- Increasing awareness of the syndrome amongst producers and advisers in the affected areas, and
- Providing an insight into how the syndrome may be managed (this would require another study to evaluate any control measures that were suggested).

3. Methodology

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research needs to be done under the auspices of a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

3.1 Farms

The location of the three farms are displayed in Figure 1.

Farm 1 is located 20 km south of Leongatha, in south-eastern Victoria, and has predominantly coastal sandy loam soils. The rainfall is winter-spring dominant, averaging 930 mm, and the growing season extends from April to January. The farm consists of 350 ha of improved pastures, predominantly ryegrass (*Lolium perenne*) and white- and sub-clover (*Trifolium subterraneum*), with pigeon grass (*Setaria incrassata*), annual fog grass (Holcus annuus) and flatweed.

Farm 2 is located 30 km south of Leongatha, on low dunes with coastal sandy loam soils and some heavier clay loams. The rainfall is winter-spring dominant, averaging 900mm, and the growing season extends from April to January. The 430 ha of pastures are ryegrass (Lolium perenne) and sub-clover (*Trifolium subterraneum*) with a mix of fescue (variety unknown) annual fog grass (*Holcus annuus*) and kikuyu (*Pennisetum clandestinum*).

Farm 3 is near Yanakie, 30 km south of Foster in south-eastern Victoria, with black to very dark grey clays or clay loam soils. The annual rainfall is 950 mm, which falls predominately in winter-spring. The growing season extends from March to January. The 1570 ha of improved pastures are predominantly ryegrass (*Lolium perenne*) and sub-clover (*Trifolium subterraneum*).

Figure 1: Map of south-east Victoria showing the relative locations of each farm

3.2 Animals

On Farm 1, a self-replacing Angus herd of 550-660 cows are joined to Angus bulls, half for a spring-calving and half for an autumn-calving. The steers are kept until they are 450-500 kg. Some heifers are either sold at the same weight or joined and sold pregnancy tested in-calf (PTIC). The remaining heifers are kept as replacements.

Farm 2 has 1100 weaner cattle. The weaners are purchased or transferred onto the property in December/January, then kept through to the following December/January when they are sold at about 580-590 kg.

Farm 3 has a self-replacing Angus herd with 300 cows, plus 3500 purchased and transferred trade cattle and weaners/yearlings. Trade cattle are sold once they reach market specifications of approximately 500 kg.

3.3 Management of animals in the study

On Farm 1 only heifers were used. These were born over a 10 to 12 week period in late February/March 2011. At weaning in late December, calves were drenched with Vetmec® (abamectin), vaccinated for a second time with 5 in 1 and Piligard® (pinkeye vaccine), and supplemented with short-acting Selenium and Copper. Throughout the study the heifers were rotated to a different paddock every one to two weeks. They were also supplemented with one bale of hay and silage every second day in the month leading up to joining in May 2012.

On Farms 2 and 3 only steers were used. Those on Farm 2 were born in western Victoria over a 10 to 12 week period, starting in March 2011. They were moved to south-east Gippsland at weaning in late-December, when they were drenched with Vetmec® and given their second 5 in 1 vaccination. During the study the steers were rotated to a different paddock every one to two weeks, with two to

three different paddocks included in the rotation. In November and May each year, the pastures are sprayed with Copper, Selenium and Cobalt.

On Farm 3 the steers were born during spring in western Victoria and transferred to southeast Gippsland at weaning in mid-January. At weaning the steers were drenched with Vetmec® and given their second 5 in 1 vaccine. The steers were set stocked in one paddock for the duration of the study.

3.4 Measurements

A summary of the measurements collected at each visit on each farm are displayed in Table 1. Blood, faeces, pasture and bodyweights were collected each month.

Visit	Drench[B]	Selenium	Liver enzymes	Liver biopsies[E]	Trace elements	Serum pepsinogen	WEC indiv.	WEC bulk	Fluke egg count	Pasture assess.	Mould and yeast	Sporides-min
Jan	2,3		✓		✓	✓	✓			✓	✓	
Feb		1,3	✓		2		3	1,2	3	✓	✓	2,3
Mar	2,3		✓			2,3	2	1,3		✓	✓	✓
Apr	2,3		✓	2	1	1	2	1,3		✓	✓	2
May	3	2	✓		2,3			✓	✓	✓	✓	
Jun	2		2	2		2	2		2	2	2	2

Table 1: A summary of the measurements and samples collected at each visit.
✓ denotes that measurement or samples were collected on all three farms whereas a number denotes which farm was sampled if not all three were done

4. Results and discussion

4.1 Bloods

Blood samples were collected and analysed each month for concentration of liver specific enzymes, such as gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH). Important trace elements (copper, selenium & vitamin B12 to assess cobalt nutrition) and pepsinogen were also monitored, although this did not occur every month (refer to Table 1). The results are summarised below.

4.1.1 Liver enzymes

Increases in serum GGT are a result of increased production and release of the enzyme from hepatobiliary tissue. Serum GGT is used as a reliable indicator of cholestatic disease, such as damage from sporidesmin toxicity in facial eczema. Concentrations become elevated two to three weeks after exposure to the sporidesmin toxin and can remain elevated for two to three months. A tenfold increase in serum GGT is not uncommon after exposure whereas a concentration less than 100 IU/L is regarded as normal.

Serum GLDH is a sensitive marker of hepatocellular damage. A tenfold increase or more indicates acute liver damage, however this increase is only short-lived and serum GLDH may not be elevated in cattle with chronic liver damage. The reference range for serum GLDH is 0 to 30 IU/L. However, in this study we were using a value less than 100 IU/L as normal. A value between 30 and 100 IU/L indicated very mild liver damage, or release of GLDH from other organs.

There were only mild elevations in serum GGT and GLDH in cattle on all three farms from January until the end of May. The minimum concentration ranged from 0 to 3 IU/L and 5 to 9 IU/L for GGT and GLDH, respectively, and the maximum concentrations ranged from 24 to 68 IU/L and 69 to 88 IU/L, respectively. In this study, serum GLDH was used as an indicator, along with decreased weight gain, for when the syndrome was occurring. There was no significant increase in the serum GLDH. Therefore, it was concluded that the syndrome was not present up until the end of May on all three farms.

4.1.2 Trace elements

Blood samples were collected to assess the major trace elements – selenium, copper and cobalt. Red blood cell concentrations of glutathione peroxidase, serum copper and serum vitamin B12 were used to determine the concentrations of selenium, copper and cobalt, respectively.

On Farm 1, weaners are routinely supplemented with selenium and copper at weaning time in December. Although the heifers in this study had been treated with a selenium supplement, blood tests collected in January indicated severe selenium deficiency. Serum copper and vitamin B12 were adequate. The heifers were supplemented with Selovin LA™, a slow-release selenium injection, in February, and concentrations of glutathione peroxidase were adequate by March.

The concentration of glutathione peroxidase and copper in weaners on Farm 2 indicated mild selenium and copper deficiency in January. Vitamin B12 was adequate. No treatments were given, with the steers re-tested the following month because a foliar spray (Tech-Flo basic copper, mixed with N'FOL Cobalt and Selenium) had been applied to pastures in November, and weaners had only arrived on the property in December. Both glutathione peroxidase and copper had increased by February but they were both marginally deficient. Due to the increase in both these trace elements, supplementation was not given on Farm 2. A second foliar spray was applied in May. Prior to this application, blood tests indicated that the yearlings were still marginally deficient.

The weaners on Farm 3, which arrived from western-Victoria in January, had marginally low concentrations of both glutathione peroxidase and copper. Similar to Farm 2, they were retested in February to check if the concentrations had changed. Glutathione peroxidase decreased, but copper increased. Selovin LA™ was given in March, and by May the concentrations of both selenium and copper were adequate.

4.2 Faeces

4.2.1 Worm egg counts

Faecal samples were collected and worm egg counts (WEC) conducted at each visit on all farms. The average WEC for the 30 animals in the study are displayed in Figure 2

The drench program on Farm 1 was vastly different from that on Farms 2 and 3. Heifers on Farm 1 received a drench at weaning in December, but were not drenched again until the end of autumn, which was the normal practice for this farm. The average WEC slowly increased over the duration of the study, reaching 60 e.p.g. at the end of the study in May.

On Farms 2 and 3, the steers were drenched at intervals ranging from 5 to 15 weeks with abamectin. The average WEC on Farm 2 was quite variable. For example, the steers were drenched on the 25th of January, and by the 21st of February the average WEC was 55 e.p.g. The time taken for ingested larvae to develop to egg producing adults is 21 days. Therefore, for the WEC to increase to 55 e.p.g.

over 28 days, (25th Jan to 21st Feb), the pasture contamination and pick up must have been considerable, and/or the drench did not effectively reduce the WEC to 0 e.p.g. A pre- and post-drench WEC in June indicated that there was resistance to abamectin on Farm 2, with a reduction of 89%. Seasonal conditions over the summer on this farm meant that there was green grass for the entire summer and autumn, whereas in a typical year, the pastures would generally dry out. This probably reduced larval mortality over the summer, thus increasing larval populations the following autumn and winter.

On Farm 3, the WEC began to increase after the first drench in January, decreased after the second drench in March, but then significantly increased after the third drench in April. The significant increase in WEC following the April drench probably indicates there were high populations of infective larvae once conditions favoured their development. The continual deposition of eggs onto the pastures which were set-stocked, as well as the contamination from the previous spring meant that during April when moisture and ideal temperatures were present, larval availability increased, corresponding with an increase in WEC in May.

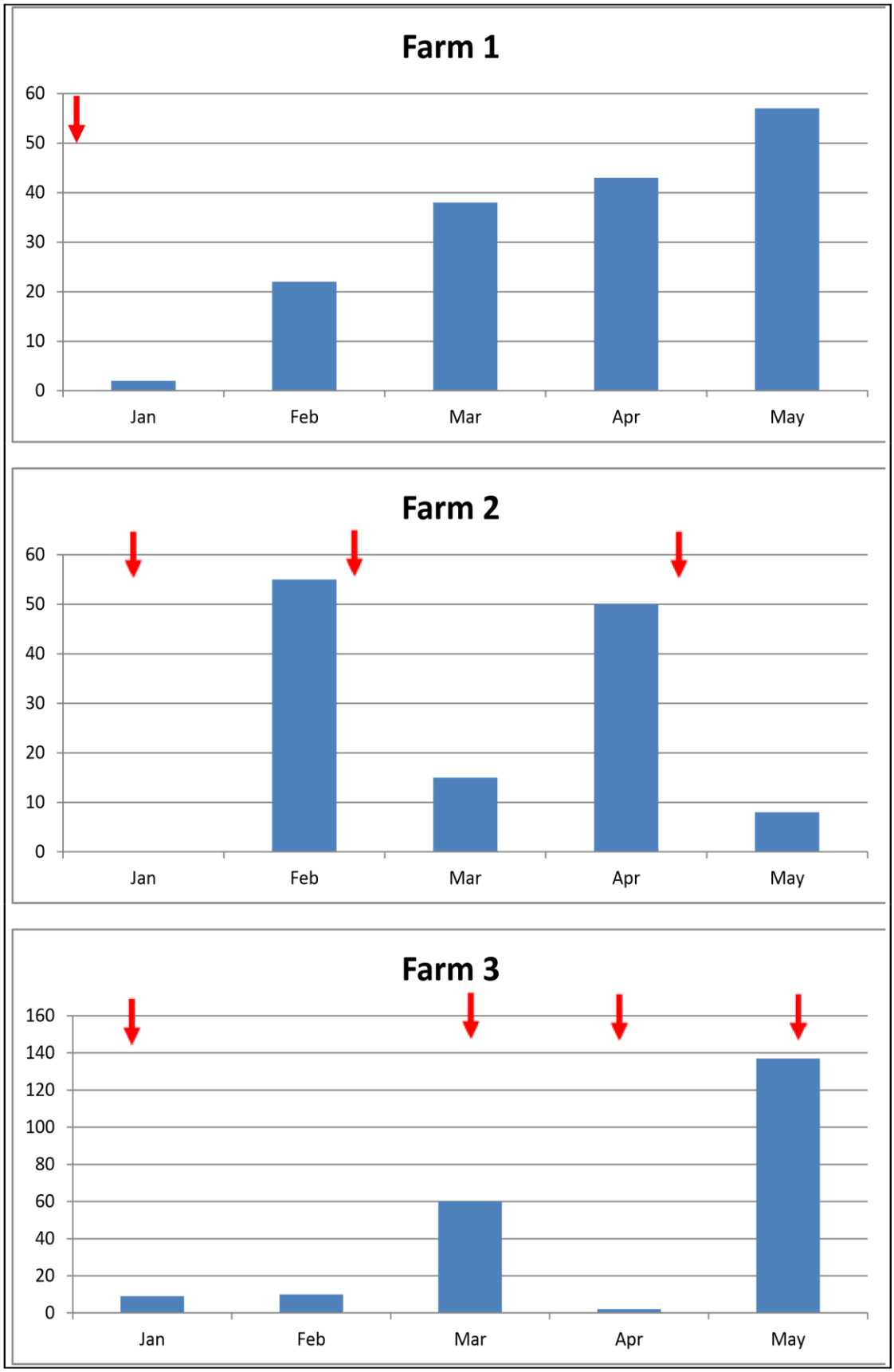

Figure 2: The average worm egg counts (e.p.g.) of the weaners on Farms 1, 2 and 3 (red arrows indicate when the steers were treated with an anthelmintic)

4.2.2 Fluke egg counts

Liver fluke can cause significant liver damage and so it was important to exclude this as a possible cause of the syndrome. Bulk fluke egg counts were conducted on all farms after the May visit and all were zero.

4.3 Pastures

4.3.1 Quality and Quantity

The results for the pasture assessments are displayed in Figure 3. Assessment of dead dry matter did not begin until the 3rd visit (March). However, photos taken of the paddocks indicated that there were varying amounts of dead dry matter present on all farms from January to March.

The heifers on Farm 1 were grazed on similar paddocks from January to March. The pasture species were uniform across the three paddocks – ryegrass, clover and pigeon grass. The quantity and quality in these paddocks did not vary greatly during this period. Despite the pasture always having green feed present, it remained of moderate quality, with about 8 megajoules of metabolisable energy per kilogram of dry matter (MJME/kg DM) during the first three months. During April and May, heifers were grazed on a different part of the farm with predominantly ryegrass and clover pastures. There was less green dry matter available but the quality was greater and there was no dead dry matter present. At the start of June, the heifers were due to be joined but were slightly below mating weight, and so the farmer began supplementary feeding at the start of May. Ninety heifers received two round bales every second day, one of pasture hay, the other silage.

The steers on Farm 2 were rotationally grazed between five paddocks of 40 ha each, with a 7 day rotation interval at the time of this investigation. The quantity of green dry matter remained between 1000 and 1500 kg/DM/ha, with reducing amounts of dead dry matter, from January to May. However, there was still a lot of dead litter present due to large amounts of carryover feed from the previous spring. Interestingly, the quality of the pasture remained fairly constant from January to May, probably due to the continual growth of green feed throughout the summer and early autumn. At no point was there a marked autumn break with a sudden increase in the amount of fresh, highly digestible green feed available.

On Farm 3, the steers were set stocked but there was a large amount of carryover dead feed from the previous spring. Similar to Farm 2, the quality remained constant throughout the study due to the continued presence of green feed throughout the summer and early autumn.

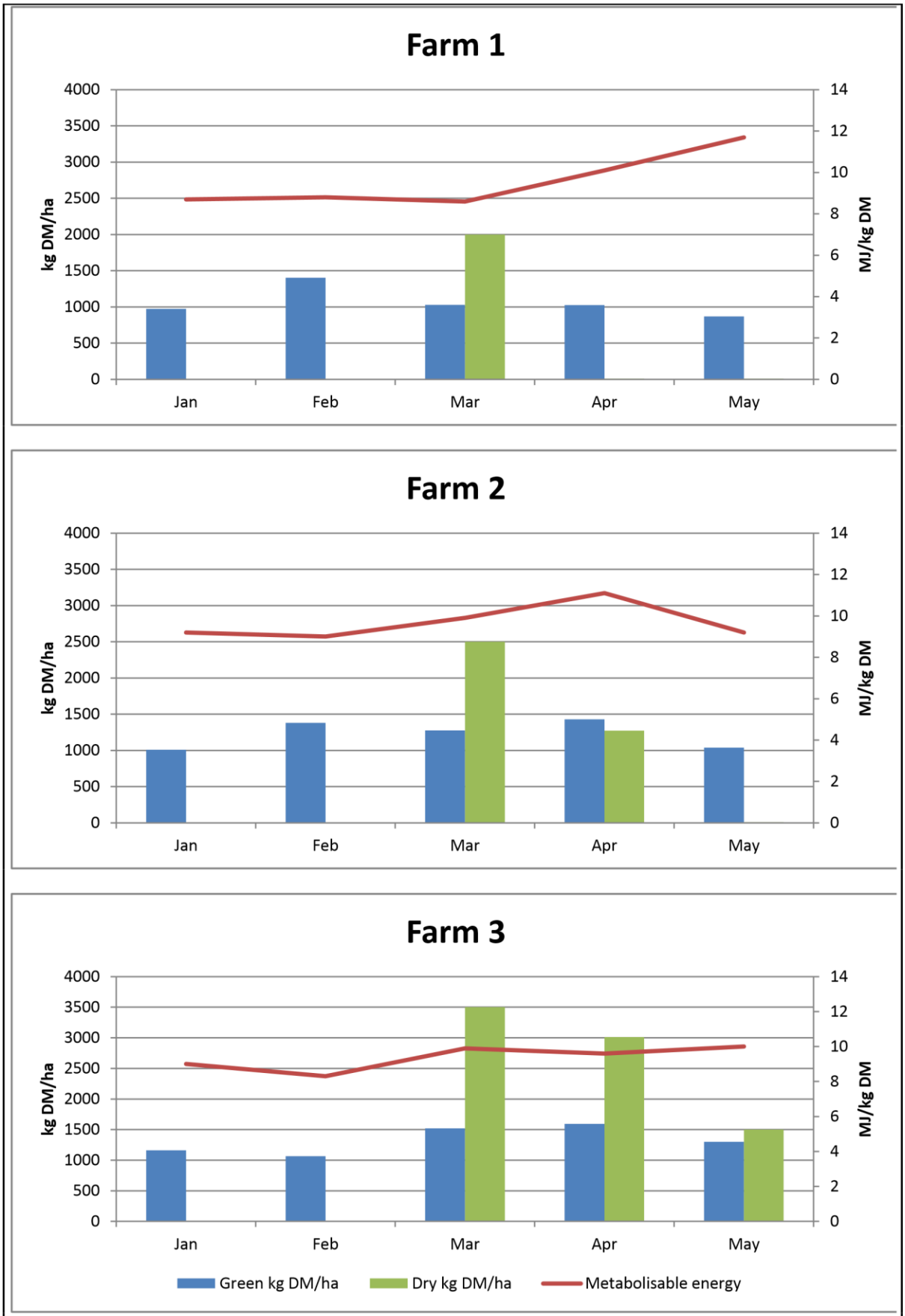

Figure 3: The average quantity of green and dead dry matter and the quality of pasture at each visit on Farms 1, 2 and 3.

4.3.2 Fungal (mould) assessment

Pasture samples were collected and submitted for mould counts at monthly intervals. The results are displayed in Figure 4.

The pattern of change in mould counts was similar on all three farms. Counts were very high in January, then reduced tenfold in February and remained low in March and April. In May mould counts began to increase on all three farms, which corresponded with the expression of the syndrome on Farm 2 in June. The counts in January were markedly greater than in May, but no signs of liver damage or photosensitisation were seen on any of the three farms. One explanation could be the duration of exposure to mycotoxins. For example, on Farms 2 and 3, the cattle were only introduced on to the farm in December/January, compared to weaners on Farm 1, which were born on this property. Another explanation could be presence of different fungi producing a variety of mycotoxins at different times of the year. Thus, there may be high counts of moulds which do not exert toxic effects on stock in January, whereas in June, the moulds present could be very toxic even when present in relatively low numbers.

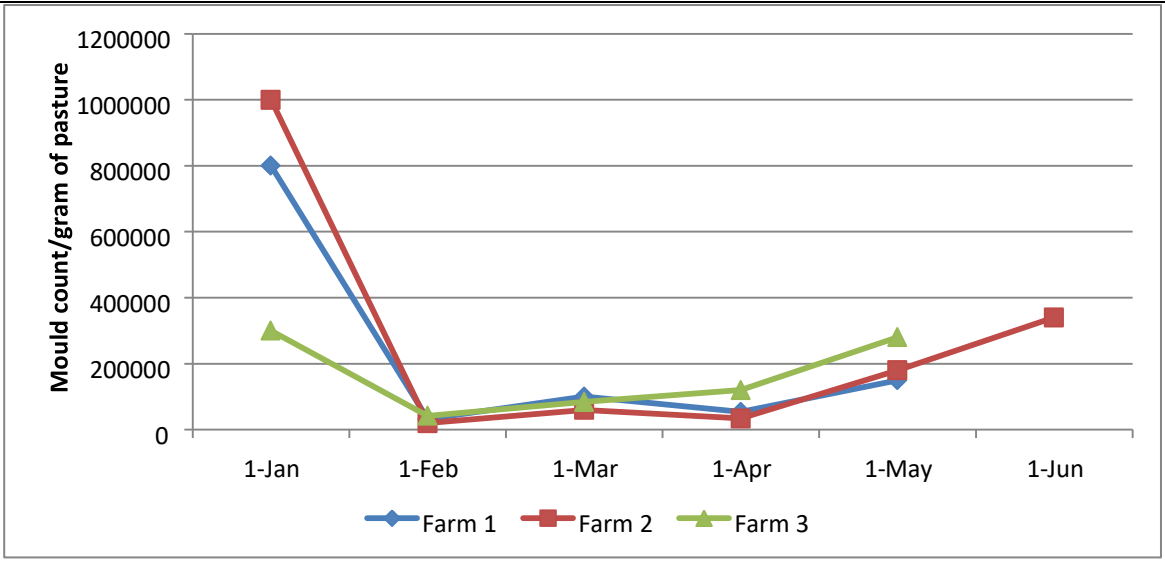

Figure 4: The mould counts from the pastures on Farms 1, 2 and 3

Figure 5 shows the average rainfall, maximum temperature and the mould counts fitted to a polynomial function. Interestingly, the increase in mould counts in May corresponded to increased rainfall on all three farms. Ideal conditions for fungal growth are typically warm, humid conditions. In May, increased rainfall meant that more moisture was present, although the temperature was decreasing. However, these conditions might support different species of fungi compared to those in January, when the mould counts were very high.

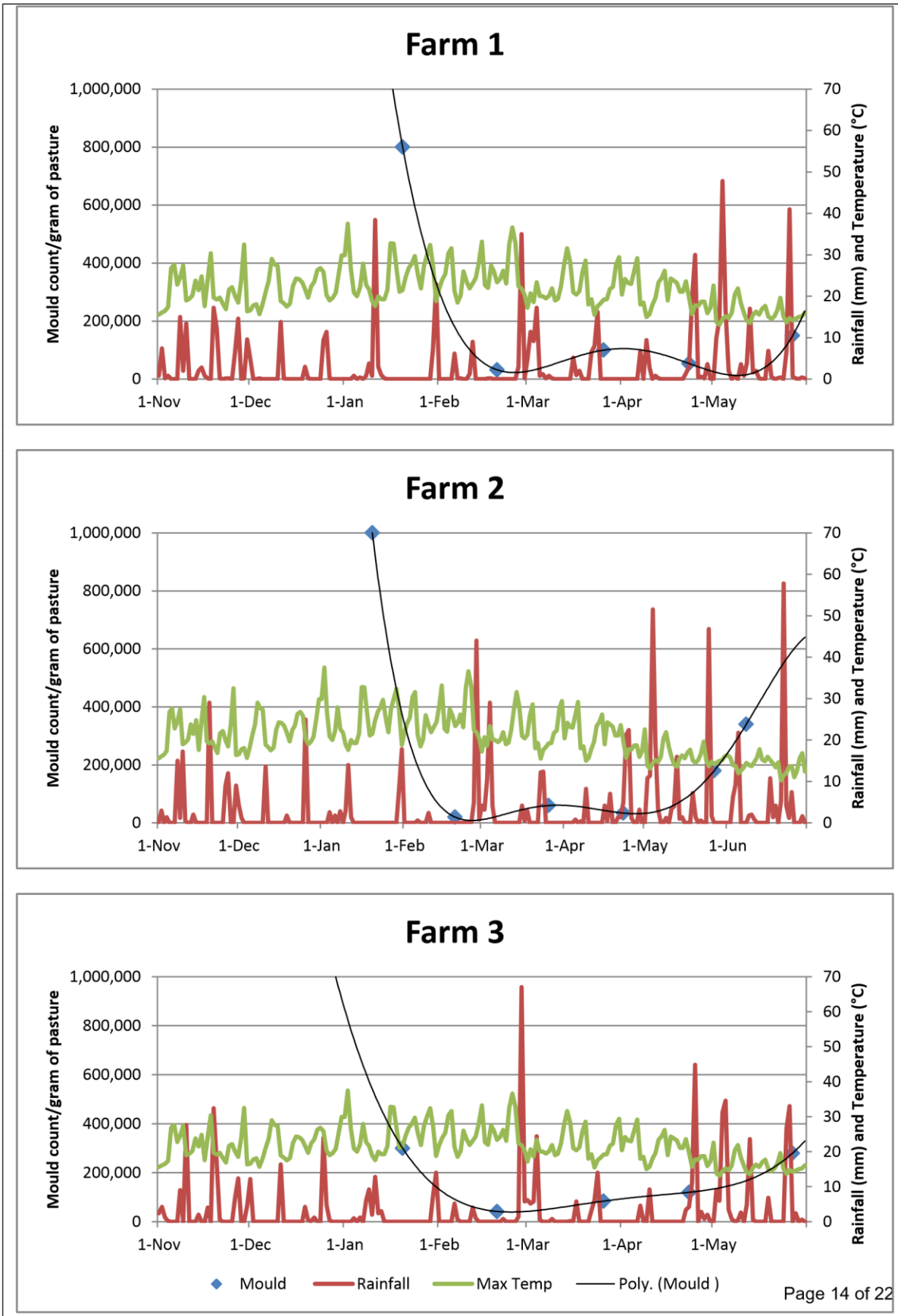

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Figure 5: The average maximum temperature, rainfall and mould count on Farms 1, 2 and 3

4.4 Bodyweights

The average bodyweights of the weaners at each visit, along with the average weight modelled on the decision support tool GrassGro™, are displayed in Figure 6. One of the criteria used to assess if the syndrome was occurring was daily weight gain, and how much this differed from that modelled by GrassGro. Over the five month period, the average weights closely matched the estimates produced by GrassGro. Consequently, with no evidence of liver damage (elevated liver enzymes) or decreased growth rates we are confident that the syndrome was not present from January to May.

On Farm 1, the bodyweight of the heifers closely matched that modelled by GrassGro from January until March, then there was a slight variation between the observed and predicted values from March to May. From March to April, the heifers gained more weight, whereas from April to May the heifers gained less weight than predicted by the model. The failure to gain weight between April and May is of interest. There was only 870 kgDM/ha of green pasture available, however the quality of pastures was very high, 11.7 MJ/kg (refer to Figure 3). In addition, the heifers were receiving supplementary feed in the form of silage and pasture hay. A possible explanation for the failure to gain weight may be high worm burdens. Figure 2 shows that the worm egg count (WEC) was slowly increasing from January to May, reaching 60 e.p.g. by the end of May, and worm egg counts as low as 50 e.p.g. can be associated with decreased weight gains in yearling cattle (Rolls and Webb Ware 2011).

On Farm 2 the average weight gain after February was very similar, but varied slightly from that predicted by GrassGro. Between January and February, the steers did not gain as much weight as expected. This meant that their average bodyweight after January continued to be about 25 to 30 kg lower than that modelled on GrassGro. Once again, endoparasites may explain the difference observed between January and February, with the average WEC increasing from 0 e.p.g. in January to 55 e.p.g. in February. However, the average WEC increased from 15 to 50 e.p.g. between March and April, without any differences between the observed and predicted weight gain, and so there may be another explanation of this difference.

The steers on Farm 3 were transported for six hours after being weaned on another farm in January, so this may explain the difference in their weight compared to that predicted by GrassGro in February. Subsequently, the steers gained more weight than modelled on GrassGro between February and April, but they did not gain as much as expected between April and May. The WEC increased significantly between April and May (Figure 2), from 0 to 140 e.p.g. At this time the steers were still gaining an average of 400 grams/day, but it is likely that the increased worm burden reduced their growth and contributed to the discrepancy between the actual bodyweight and GrassGro predictions during April and May.

Overall, the bodyweights of the steers were very close to that predicted by the GrassGro model. There were minor differences, but these are explained by the worm burdens encountered, or reduced pasture growth and seasonal effects.

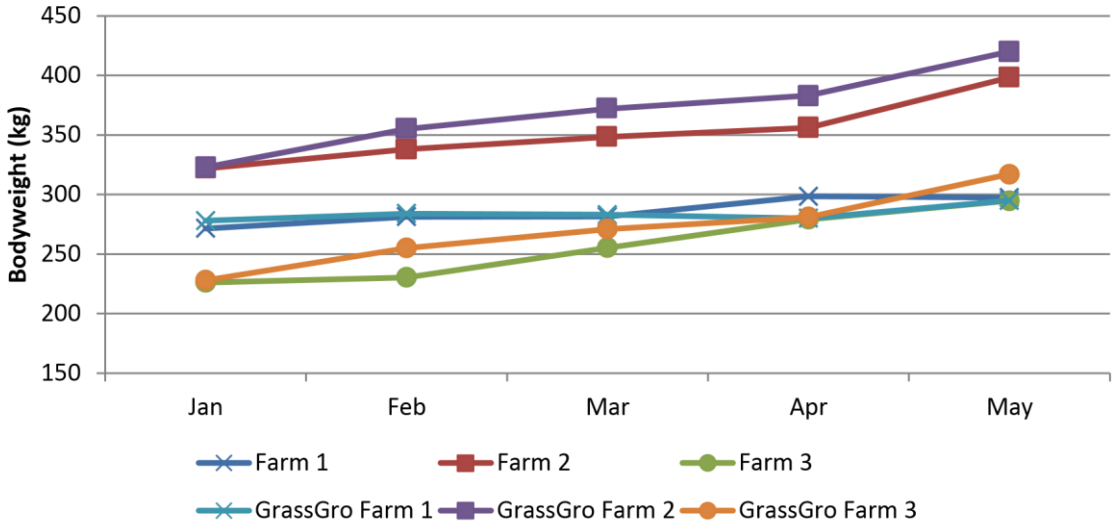

Figure 6: The average bodyweight of steers on all three farms compared to those predicted by the GrassGro™ model

5. Results on farm 2 in June

At the start of June, the farmer detected that the steers were losing weight, a proportion had severe diarrhoea and some had early signs of photosensitisation. On the 8th of June, 35 animals were drafted from the main mob of 471 steers because they were displaying the above signs, but unfortunately these were not from the previously monitored mob. Blood samples were collected to measure liver enzymes, faeces collected for WEC, fluke egg counts and bacterial culture, and pasture samples collected for mould and sporidesmin spore counts. Eleven out of the 35 steers sampled had mild to moderate signs of photosensitisation, and the results from this visit are summarised below.

5.1 Liver enzymes

Blood samples were collected from all 35 steers. Eight of the 35 steers had significantly elevated GLDH (>650 up to 1300 IU/L) and two had moderately elevated concentrations (<600 IU/L). Five of the ten steers with an elevated GLDH also had mildly elevated GGT (>35 but <150 IU/L). All other parameters were within normal limits.

5.2 Faeces

5.2.1 Bacterial culture

Faeces from the steers with severe diarrhoea were cultured, but were negative for the bacteria of interest, Yersinia spp. and Salmonella spp., both of which can cause diarrhoea and weight loss in young cattle. In previous years, steers affected with the syndrome appear to have a decreased immunity and had succumbed to bacterial infections such as Salmonellosis and Yersiniosis, but this was not seen during this study.

5.2.2 Worm and fluke egg counts

The WEC of the 35 steers ranged from 0 to 570 e.p.g. and averaged 62 e.p.g., with no fluke eggs detected. This WEC is quite high and may have contributed to the weight loss and diarrhoea,

however worms do not explain the photosensitisation and increased liver enzymes described below in section 5.5.

5.3 Bodyweight

The average bodyweight of the steers in the mob in which the affected steers were identified, and that modelled by GrassGro, are shown in Figure 7. The observed bodyweight of the steers was very similar to that modelled from January through until 18 April. However, between 18 April and 6 June the bodyweight of the steers was much lower than predicted, and the steers did not start gaining weight until August.

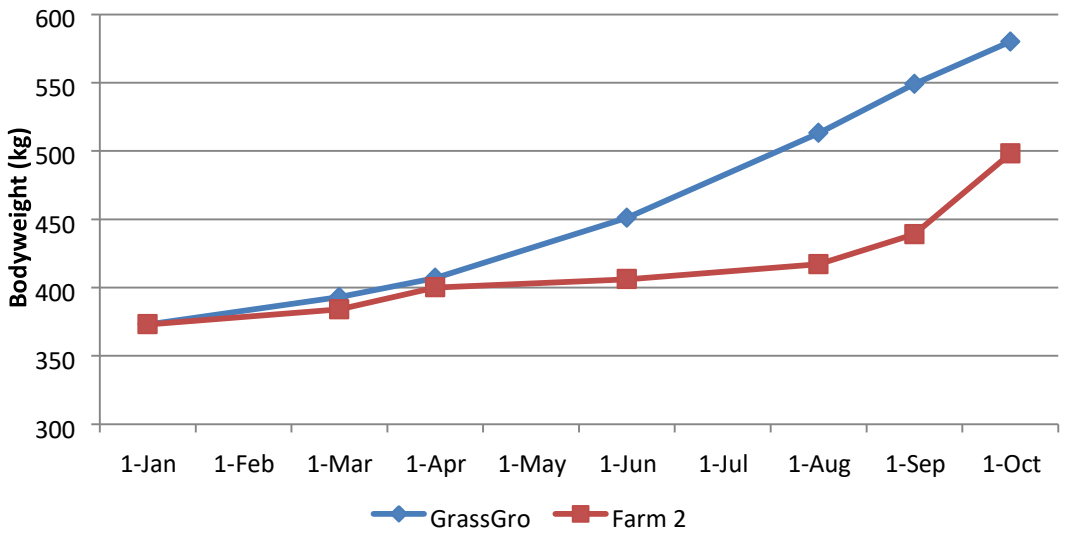

Figure 7: The average bodyweight of the affected mob compared to the expected bodyweights modelled on GrassGro

The bodyweights of the affected steers compared to the remainder of the mob are shown in Figure 8. The affected steers lost nearly 20 kg between 18 April and June 6 before they started to gain weight again in Jul-Aug.

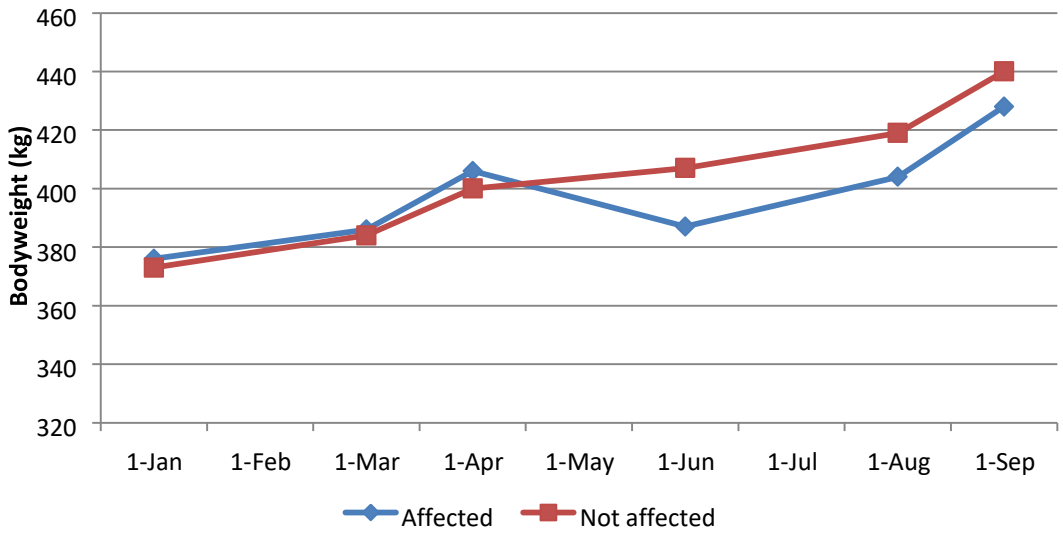

Figure 8: The average bodyweight of steers which displayed signs of the syndrome compared to the remainder of the mob

5.4 Pasture

When the farmer reported that the steers were showing signs of the syndrome, they were grazing a paddock with an old established mixed species pasture consisting of mainly perennial ryegrass, fescue, sub-clover and fog grass. There was approximately 1000 kgDM/ha when the paddock was assessed on 8 June. Mould counts on this paddock were elevated (340,000 counts per gram of pasture; Figure 4), and the sporidesmin count was zero spores per gram of pasture.

After the 35 steers were drafted off, the main mob was drenched with abamectin injectable and moved onto a new pasture sown in 2011. This paddock consisted of mainly perennial ryegrass and clover and had about 1150 kgDM/ha when the steers entered the paddock. The mould count was much lower, 74,000 counts per gram of pasture, and the sporidesmin spore count was also zero spores per gram of pasture.

5.4.1 Mycotoxin analysis

Pasture samples from Farm 2 were sent to Romer Labs in Singapore for mycotoxin analysis using high performance liquid chromatography (HPLC). Samples collected in March, when mould counts were low, and June, when animals were showing signs of the syndrome, were sent to try and identify differences between the concentrations of various mycotoxins. Unfortunately, only a small amount of each pasture sample was available and so only ergot analysis was conducted. The results are summarised in Table 2.

The pasture sample from paddock 1 (where the steers showed signs of the syndrome) had elevated concentrations for 6 of the 10 ergots tested, compared to 2 out of 10 for the March sample and 3 out of 10 for the sample from paddock 2. A previous study examined the effect of feed contaminated with ergot on growth of pigs (Mainka et al. 2005). That study found that concentrations of ergot broadly similar to those detected in the present study caused a significant reduction in bodyweight gain and significant increases in GLDH, although the increased concentrations of GLDH were not above the reference range for pigs and did not correspond with any pathological changes in the liver. These results appear consistent with the present study with

cattle, although the less spectacular rise in GLDH may be because pigs are less susceptible to the hepatotoxic effects of ergots. Overall, these preliminary results underline the need for more detailed investigations to identify any association between mycotoxins and the occurrence of this syndrome.

Table 2: Results from ergot analysis (high performance liquid chromatography) of pasture samples from Farm 2.

Month		March	June	
			Paddock 1[A]	Paddock 2
Mould count (count/gram of pasture		60,000	340,000	74,000
Parametre (µg/kgDM)*	Ergocornine	<10	<10	<10
	Ergocorninine	33	56	29
	Ergocristine	<10	<10	<10
	Ergocrisitinine	<10	25	<10
	Ergocryptine	<10	<10	<10
	Ergocryptinine	<10	48	<10
	Ergometrine	<10	<10	129
	Ergosine	218	349	91
	Ergotamine	<10	42	<10
	Ergotaminine	<10	56	<10

*< "value" means not detected
[A] Paddock which cattle showed signs of the syndrome were grazing

5.5 Pathological findings

On the 19th of June, 11 days after the initial blood samples were collected, liver biopsies and blood samples were collected from eight steers which had elevated GLDH. At this time there was less diarrhoea and steers had started to gain weight. The steers which had mild to moderate signs of photosensitisation on the 8th of June were now severely affected, including the mucosa on the nose and around the eyes, and skin with no pigmentation was sloughing. A steer was bought back to the Werribee Veterinary Clinical Centre for necropsy and the results from the biopsies, blood samples and necropsy are summarised below.

The most remarkable histological findings from all the biopsies was the marked atrophy in the liver parenchyma, which appeared as narrowing of the portal tracts and the smaller than normal size of hepatocytes. There was also a varying degree of cholangitis and inflammatory infiltration of the small bile ducts. The hepatocytes were not only small, but had diffusely granular cytoplasms, with large nuclei and increased number of mitotic figures. The granularity of the cytoplasm is due to an increased number of mitochondria.

Grossly the liver of the necropsied steer was shrunken. The histopathology of the liver indicated diffuse and severe atrophy, chronic cholangitis and pericholangitis. Hepatocytes also had finely granular cytoplasms, some with disrupted membrane, vacuolated cytoplasm and large and variably sized nuclei with condensed chromatin.

The changes observed in the liver are suggestive of an acute hepatotoxic insult, with expert pathologists indicating that the changes were similar to liver damage seen in humans with a drug (a hepatotoxin) overdose.

In conjunction with the changes to the liver, histopathology of the abomasum indicated severe and chronic Ostertagiasis. There was the occasional cross section of Ostertagia ostertagi, diffuse infiltration of inflammatory cells and hyperplasia of the abomasal mucus neck cells. A total worm count on this steer found about 22,000 worms, mainly O.ostertagi, with the majority being larval and immature stages. This steer had been drenched on the 8th of June, but the severe damage to the abomasum and recent drench history is strongly suggestive of a high larval challenge from pastures on this farm.

The liver enzymes of the biopsied steers and the necropsied steer showed that GLDH had returned to normal limits 11 days later. The half-life of GLDH is short, with concentrations of this enzyme being elevated soon after an acute insult to the liver but decreasing soon after.

The liver has an important role in the metabolism and biotransformation of exogenous substances. Therefore, it is exposed to a large volume and variety of substances which are absorbed into the blood stream, most commonly from the digestive tract. Toxins known to cause liver damage include mycotoxins (e.g. aflatoxin, phomopsin which causes lupinosis, sporidesmin and trichothecenes), various phytotoxins, many which are unidentified, chemicals (e.g. anthelmintics such as the macrocyclic lactones) and cyanobacteria (bluegreen algae).

A number of the above heptotoxins can be ruled out as a cause of this episode of liver damage and weight loss in steers grazing improved pastures on Farm 2.

Aflatoxins are metabolites which are produced by fungi which grow on poorly stored feed. The steers in this study had no access to stored feeds.

Phomopsin is produced by a fungus which grows on dead lupin plants or seeds. Again, the steers did not have access to lupin plants or seeds.

Sporidesmin (facial eczema) was initially high on the list of differential diagnoses, but the clinical presentation is not consistent with facial eczema. The sporidesmin spore count was zero when the liver enzymes of the affected steers were elevated. Typically, ruminants which have been exposed to sporidesmin do not show clinical signs until about ten days after ingesting the toxin, at which time the number of sporidesmin spores on the pasture may have already decreased. However, the results from the blood test and the liver pathology indicated that sporidesmin was not the cause, as GGT concentrations usually remain significantly elevated for up to three months after ingestion. In facial eczema, GLDH is not usually elevated and the pathology of the liver would indicate primarily bile damage, rather than hepatocellular damage, which was evident in the liver biopsies and the necropsied steer.

The possible involvement of phytotoxins cannot be completely ruled out. The paddocks which steers grazed were monitored at each visit, when twenty pasture samples were collected and an assessment of pasture species was made. While collecting this information, the presence of toxic plants was noted, with none identified in the paddock the affected mob was grazing. However, the paddock was very large and so it was difficult to comprehensively assess the entire paddock. One pasture species which is thought to be associated with hepatotoxicity is Rat's-tail fescue (Vulpia spp.), but this was not identified and the farmer and a local adviser have not seen this plant in south-east Gippsland.

No blue-green algae were detected on the water source.

A recent paper has described hepatic damage, particularly mitochondrial damage, in rats following the administration of the macrocyclic lactone anthelmintic abamectin (Castanha Zanoli et al. 2012).

Rodents have a lower threshold for abamectin toxicity than ruminants (Fisher and Mrozik 1992), but the cellular damage described in the liver biopsies and necropsied steer was considered indicative of mitochondrial damage and the cattle on Farm 2 and 3 had been treated with abamectin at 4 to 6 weekly intervals (refer to Figure 2). This was done because the farmers considered that the steers would lose weight and appear in poor health if they were not treated regularly. What is not clear, is the relationship of the molar in vitro concentrations of abamectin seen to cause mitochondrial damage, with the oral dosages causing in vivo toxicity in rats Abamectin is considered to have a wide therapeutic margin and there was no evidence of gross overdosage of the observed cattle, although the treatment frequency was deemed to be too high.

Further investigations were conducted on the ten steers with elevated GLDH on the 8th of June. These steers were blood tested and liver enzymes analysed on the 3rd of August (Table 3). They were treated with abamectin at this time, and blood tested again ten days later. The GLDH concentration was within normal limits in nine of the ten steers tested on the 3rd of August. However, ten days after abamectin treatment, GLDH was elevated in eight of the ten steers, and significantly so in three of these eight animals. This indicates that acute liver damage had occurred between the two blood tests. Pasture spore and sporidesmin counts were low at the time of the second blood test. A potential acute hepatotoxin for this event was abamectin. However, this is only a tentative conclusion, as these steers previously had severe liver damage and there were no control animals included in this very small study.

Aspartate aminotransferase (AST) is also released from damaged hepatocytes, although this increase is generally after the GLDH concentrations have increased and are then returning to normal. All the steers, which had an elevated GLDH also had elevated concentrations of AST. The half-life of GLDH is as short as 14 hours in cattle, and so any increase in GLDH may have been missed as the animals were blood sampled at a 10 day interval. However, because AST increases after a rise in GLDH, the increased AST may indicate that elevated GLDH occurred in these animals, but the peak fell between the first and second blood sample.

Table 3: The change in the concentration of GLDH and AST between the 3rd of August (pre) and 13th of August (post)

Steer ID	Pre-GLDH*	Post-GLDH	Change (↓↑)	Pre-AST*	Post-AST	Change (↓↑)
1	25	56	↑	112	124	↑
2	28	67	↑	115	183	↑
3	66	753	↑	112	423	↑
4	13	163	↑	94	254	↑
5	22	61	↑	114	139	↑
6	48	93	↑	108	125	↑
7	266	30	↓	372	126	↓
8	40	20	↓	152	133	↓
9	69	607	↑	167	511	↑
10	26	76	↑	130	188	↑

*GLDH and AST measured in U/L

6. Extension

Feedback about the results found in this study was given to producers in the Gippsland area through a presentation at a farmer meeting. Also, to extend the results and awareness of the syndrome to a wider audience, a presentation was given to agricultural advisors servicing the beef industry in Victoria.

7. Conclusion

The results from this study continue to implicate a hepatotoxin as a contributing cause to the impaired liver function seen in beef cattle suffering decreased weight gain and photosensitisation in the high winter rainfall region of south Gippsland. However, neither the exact nature of the toxin, nor the fungi that may be producing it, have been identified. It is suspected that a mycotoxin produced by an unidentified fungus is the primary cause of this syndrome. Based on recently published results on the effect of abamectin on rat liver mitochondria, it could be asked if the liver damage may have been exacerbated by frequent administrations of abamectin. In regions that have a high risk of excessive fungal growth on pasture, which includes much of the high winter rainfall regions of southern Australia, compromised liver function and poor growth rates may be occurring but not be recognised by most beef producers. If the liver has been damaged, there is a risk of causing more severe damage and increased production loss by the frequent administration of products, such as abamectin, that rely on the liver for their metabolism. Angus cattle are less likely to show obvious signs of photosensitisation, and so the effects of the syndrome may not be observed in these herds unless producers are weighing cattle on a monthly basis. The benefits from more frequent weighing will not only be the early detection of weight loss or sub-optimal growth rates, but also monitor how cattle are progressing to meet the specifications of the target market.

8. Bibliography

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