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RESEARCH ARTICLE

# Longitudinal study of the effect of sporidesmin toxicity on lamb production and serum biochemistry in a flock of 46 Romney ewes using a standardised measure of liver damage

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**ABSTRACT**

**Aims:** To evaluate the effect of sporidesmin toxicity on production outcomes and serum biochemistry analytes in mixed age Romney ewes, using a standardised measure of liver damage.

**Methods:** This was a prospective longitudinal study following 46 mixed age Romney ewes from sporidesmin intoxication in April 2019, to slaughter 8 months later. The ewes were blood-sampled up to eight times, with a panel of serum biochemistry tests performed on the final six samples. However, only gamma-glutamyl transferase (GGT) activity was measured in the first two samples collected at the end of sporidesmin intoxication and 2 weeks later. Body condition score, ewe weight and production data were also recorded. Using a standardised liver score, based on histology of liver samples collected at slaughter, ewes were assigned to one of three liver disease categories (LDC); low, middle, and high. These were then used as the outcome or predictor variables for statistical analyses. Finally, two separate decision tree models, using recursive partitioning (RP), were fitted to the biochemistry data and to the GGT data at FE outbreak, to predict ewes in the low LDC.

**Results:** There was no evidence of a difference for the effect of LDC on ewe weight (p = 0.86) with ewes, on average, gaining weight to weaning. Weaning percent, lamb rearing percent and ewe flock efficiency were lower in ewes with high LDC, and scanning-to-weaning lamb loss was significantly higher in sheep with high LDC (p = 0.02). Serum activities of GGT and glutamate dehydrogenase and concentration of globulin were significantly lower in sheep with low LDC than in sheep with middle or high LDC (p < 0.05). However, there was no evidence of a difference for the effect of LDC on other biochemistry variables (p > 0.05). The final RP model for the biochemistry data categorised ewes as low LDC if their GGT was <122 IU/L, 3 months after sporidesmin intoxication, or if their GGT was <514 IU/L, <18 days after sporidesmin intoxication.

**Conclusions and clinical relevance:** Sheep with gross and histological evidence of severe sporidesmin-induced liver damage were able to maintain or gain body weight, suggesting that sporidesmin intoxication alone is not causative of poor body condition. Similarly, many of the serum biochemistry tests were not associated with evidence of liver damage. Lamb production was reduced in ewes with evidence of severe liver damage and the decision tree model showed promise as a basis to select ewes for culling.

**Abbreviations:** BCS: Body condition score; BOH: β-hydroxybutyrate; FE: Facial eczema; GEE: Generalised estimating equation; GGT: Gamma-glutamyl transferase; GLDH: Glutamate dehydrogenase; GLS: Gross liver score; KPI: Key performance indicator; LME: Linear mixed effect; NEFA: Non-esterified fatty acids; RP: Recursive partitioning; TP: Total protein

## Introduction

Facial eczema (FE) is a common, highly seasonal disease of grazing production animals, which has huge financial and welfare implications for farmers and livestock in New Zealand (Boyd 2016; West *et al.* 2017). The disease is usually seen in the mid-to-late summer and autumn and is substantially more prevalent in the North Island than in the South Island. FE is described as a hepatogenous photosensitisation (Smith and Towers 2002) and is caused by the ingestion of sporidesmin toxin, which is produced by the saprophytic fungus *Pithomyces chartarum* (Jordan 2020) which grows on the dead and dying matter at the base of pastures. Rapid fungal growth occurs when weather conditions are warm (minimum temperature >12°C) and humid (close to 100% humidity), with recent light rain (Brook 1963; Townsend 1996). *P. chartarum* is found in all temperate climate zones of the world, with corresponding reports of FE disease (Hore 1960; Marasas *et al.* 1972; Ozmen *et al.* 2008). However, the risk of FE appears much greater in New Zealand owing to a higher proportion of

*P. chartarum* strains producing sporidesmin A toxin (Collin *et al.* 1998; Jordan 2020).

At sporulation, *P. chartarum* spores are eaten by grazing livestock (Brook 1963). The sporidesmin within the spores causes necrotising pericholangitis in the biliary system leading to partial or total obstruction of the medium and large bile ducts, and oedema and necrosis of the bladder lining leading to haematuria (Dodd 1959; Mortimer 1963; Smith and Towers 2002). The leakage of bile, carrying sporidesmin, from the damaged and obstructed bile ducts into surrounding liver tissue may further exacerbate the severity and extent of liver damage (Mortimer 1963).

The clinical signs of "acute FE" result from secondary photosensitisation due to the accumulation in the circulation of phylloerythrin, a natural photo-active product of chlorophyll breakdown in the rumen, normally excreted in the bile (di Menna *et al.* 2009). Clinical signs typically develop 1–3 weeks after ingestion of sporidesmin, depending on both the initial and cumulative toxic challenge (Thornton and Sinclair 1959; di Menna *et al.* 2009). In addition to acute FE, sheep that have been intoxicated with sporidesmin, subsequently develop consistent anatomical and histological changes to the liver and are referred to as having "chronic FE." This is characterised by marked fibrosis and atrophy of the left liver lobes and hypertrophy of the right liver lobe – so-called boxing glove-shaped livers. However, referring to this syndrome as chronic FE is misleading as sheep generally do not display clinical evidence of skin disease in the absence of additional damage to the liver.

While the pathological changes to the liver in chronic FE are well studied and described, there is limited peer-reviewed literature describing the relationship between these changes and subsequent ewe productivity. Previous studies have shown that sporidesmin toxicity can impact flock productivity via an increased proportion of barren (non-pregnant) ewes (Morris *et al.* 1991), a decreased number of ewes with multiple births (Smeaton *et al.* 1985; Jagusch *et al.* 1986; Sheath *et al.* 1987), decreased ewe weights at mating and weaning (Smeaton *et al.* 1985), decreased lamb weights at birth and weaning (Smeaton *et al.* 1985; McMillan *et al.* 1988), decreased ewe survival (McMillan *et al.* 1988) and decreased lamb survival to weaning (McMillan *et al.* 1988). However, in all these studies, the activity of gamma-glutamyl transferase (GGT) in serum was used as a proxy measure for liver damage; the actual extent of liver damage was never quantified using a standardised measure.

Anecdotally, chronic FE is considered causative of weight loss and death in older ewes. Indeed, within the Massey University post-mortem room, sheep in poor body condition and with the characteristic boxing glove-shaped livers are assumed to have lost weight due to impaired liver function (J. Munday personal observation). However, there is currently little robust evidence supporting this assumption. Indeed, the liver is known to have a high regenerative ability and the hypertrophy of the right lobe suggests some compensation for the fibrosis within the left lobe. This suggests there may be less loss of liver function from previous sporidesmin intoxication than currently thought.

Several serum biochemical parameters have been successfully used to monitor the level of acute liver damage following sporidesmin intoxication. Serum GGT, an enzyme with increased activity when there is damage or obstruction of the hepatobiliary system, is the most sensitive known biomarker and highly correlated with liver damage and sporidesmin challenge (Towers and Stratton 1978). Other serum biochemistry parameters show dramatic increases following acute sporidesmin intoxication and include aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and glutamate dehydrogenase (GLDH), which are associated with hepatocyte damage, and bilirubin, alkaline phosphatase, and cholesterol, which are associated with cholestasis (Leaver 1968; Bonnefoi *et al.* 1989; Flåøyen and Smith 1992). No references to the measurement of bile acids (increases may be associated with cholestasis or hepatocellular dysfunction) or β-hydroxybutyric acid (BOH) and non-esterified fatty acids (NEFA) (increases may be associated with energy imbalance) in serum of ewes following sporidesmin intoxication could be found. However, while the biochemical changes that result from acute sporidesmin intoxication are well known, the biochemical changes present and their consistency during chronic FE are less known (Munday *et al.* 2021). Indeed, if biochemical evidence of reduced liver function were detectible in sheep with characteristic pathological changes to the liver, this would be strong evidence of a lasting detrimental effect of sporidesmin toxicity on liver function.

This is a prospective observational study of 46 Romney ewes that suffered an acute FE outbreak. Our purpose is to describe the longer-term impacts over the 8 months following exposure, on ewe production (including ewe live weight and body condition score (BCS), lambing and weaning rates and scanning-to-weaning losses), and overall ewe flock efficiency, and relate these impacts to the level of histopathological liver damage using a standardised measure quantified at necropsy. In addition, changes in ewes' serum biochemical parameters are described for the duration of follow-up. This is a companion paper to Munday *et al.* (2021) which describes the liver pathology of these sheep.

## Materials and methods

In January 2019, a flock of 83, 6-year-old Romney ewes, part of an ongoing study investigating mammary gland defects, were moved from a property in Masterton deemed to be low-risk for FE, to a Massey University dry stock property in Palmerston North, with a known history of FE. On 4 February 2019, each ewe received an intra-ruminal capsule containing zinc (The Time Capsule; New Zealand Agritrade Ltd, Christchurch, NZ) for FE prevention. The ewes were bred to mature Romney rams from 25 March 2019 to 15 April 2019, using ewe synchronisation for the first round of mating.

The FE outbreak was first reported on 26 April 2019, when 2/83 (2.4%) ewes were observed to have clinical signs consistent with FE. Histology following post-mortem examinations of one ewe that died, and two euthanised for ill-health on 29 April 2019, revealed necrosis of the bile ducts, consistent with a diagnosis of acute FE. Examination of the remaining ewes identified mild to moderate swelling and crusting over the ears and face in 15/81 (18.5%) ewes on the 29 April 2019, and these ewes were treated with a single injection of 1 mg/kg meloxicam (Metacam; Boehringer-Ingelheim, Auckland, NZ) S/C. Investigations revealed none of the original 83 ewes had received a second zinc capsule, scheduled for administration on 20 March 2019, 6 weeks after the first.

On 29 April 2019, the remaining 79 ewes were moved to a predominantly plantain pasture with plenty of trees to provide shade from the sun. As *P. chartarum* does not infect plantain, the date of 29 April 2019 was taken as the last day of exposure to sporidesmin toxin.

On 4 June, pregnancy testing (scanning) was undertaken using transabdominal ultrasound. Ewes were identified as barren with no fetus or dead fetuses *in utero* (10/79; 13%), or pregnant with single (25/79; 32%), twin (32/79; 41%) or triplet (12/79; 15%) lambs.

Ram harness crayon information, collected during breeding, was used to allocate ewes into groups according to time of conception; "first cycle" ewes conceived during the first 17 days of breeding while "second cycle" ewes conceived during the subsequent 17 days.

After pregnancy testing, 31/79 (39%) ewes, (10 barren, 12 triplet-bearing, seven with severe udder defects, and two with BCS< 2) were culled, leaving 46/79 (61%) ewes to be followed through pregnancy to slaughter. At the time of the FE outbreak, for the ewes retained in the study, the first cycle fetuses were a mean of 35 days old, compared to 19 days for second cycle fetuses.

The mammary glands of the ewes were palpated pre-lambing (30 July) and categorised normal (both halves normal) or abnormal (lumps and/or hardness in one or both halves).

Throughout the lambing period, the 46 ewes were lambed in a separate group with lambing beats performed by a PhD student and two experienced animal science technicians. Two daily observations were conducted, with each new-born lamb matched to its dam, tagged and its birthweight recorded. Dead lambs were collected, and field necropsies carried out as described by McFarlane (1965), thus establishing likely cause of death. Live lambs that were found starving were fostered if their dams had little or no milk.

### Sampling protocol

The number, type and date of samples taken over the study are shown in Table 1.

### GGT and biochemistry testing

The blood samples were collected by jugular venepuncture into 10 mL serum tubes, left to clot for 30–

**Table 1.** Dates and management period of blood sampling, recording of weight and body condition score (BCS), and udder palpation, of ewes (n = 46) exposed to acute sporidesmin toxicity in late April 2019 in Manawatu (NZ), and followed for 8 months.

| Period | Date[a] | Week[b] | Blood sampled[c] | Weight and BCS recorded | Udders palpated |
|---|---|---|---|---|---|
| | | | Number of ewes | | |
| Pre-mating | 25/03/2019 | −5 | – | 46 | – |
| Outbreak | 29/04/2019 | 0 | 44 (GGT) | – | – |
| Follow up | 16/05/2019 | 2 | 46 (GGT) | – | – |
| Scanning | 4/06/2019 | 5 | – | 46 | – |
| Pre-lambing | 30/07/2019 | 13 | 46 (BP) | – | 46 |
| Early lactation, cycle 1 | 3/09/2019 | 18 | 30 (BP) | – | – |
| Early lactation, cycle 2 | 25/09/2019 | 21 | 16 (BP) | 16 | – |
| Mid-lactation, cycle 1 | 2/10/2019 | 22 | 30 (BP) | 29 | – |
| Mid-lactation, cycle 2 | 18/10/2019 | 25 | 16 (BP) | – | – |
| Late lactation | 18/11/2019 | 29 | 46 (BP) | – | – |
| Weaning | 29/11/2019 | 31 | – | 46 | – |
| Post-weaning | 3/12/2019 | 31 | 45 (BP) | – | – |
| Pre-slaughter | 17/12/2019 | 33 | 46 (BP) | – | – |

[a]Rams were removed on 15 April 2019.
[b]Number of weeks since end of sporidesmin exposure.
[c]Abbreviation in brackets following number of ewes sampled indicates serum parameters measured: BP = serum biochemistry panel and GGT = measurement of gamma-glutamyl transferase activity in serum.

60 minutes, then centrifuged for 10 minutes at 2,000 rpm. Serum was pipetted into microcentrifuge tubes which were transported to SVS Laboratories (Hamilton, NZ) and tested within 24 hours of collection. For the first two blood sample dates, only GGT activity was measured, while for the next six blood sample dates the following panel of biochemistry parameters where measured: concentration of albumin, bilirubin, bile acids, BOH, NEFA, total protein (TP) and urea, and activity of GLDH and GGT. Serum globulin concentrations were estimated by subtracting the concentration of albumin from the concentration of TP. All serum biochemical tests were carried out using a Cobas c501 analyser (Roche Diagnostics International Ltd, Rotkreuz ZG, Switzerland). The reference ranges for all biochemistry variables were provided by SVS Laboratories.

### Liver pathology and development of standardised score

For a full description of ewes' gross pathology and histopathology results see Munday et al. (2021). Briefly, the ewes were slaughtered on 18 December 2019 (33 weeks after sporidesmin exposure ceased) and their livers collected and weighed. A five-point gross liver score (GLS; scored from 0–4), was given to each assessed liver on collection. These GLS were then collapsed down to three GLS categories, none = GLS 0, moderate = GLS 1 and 2, and severe = GLS 3 and 4, owing to little discrimination found between GLS 1 and 2, and GLS 3 and 4 respectively. At the same time, five standardised liver samples were taken from five separate locations on each liver, as described by Munday et al. (2021). For each individual slide from a single ewe, scores were then given for necrosis (0–4), portal inflammation (0–4), biliary hyperplasia (0–6), portal fibrosis (0–6) and the scores for each category summed across the five slides.

Three composite scores were created by combining some or all individual slide scores. Combining all separate slide scores = Total (maximum score of 100), combining the slide scores for portal inflammation, biliary hyperplasia, and portal fibrosis = Bili (maximum score of 80) and combining the slide scores for biliary hyperplasia and portal fibrosis = Bhype_Fib (maximum score of 60).

### Liver disease categories

Histograms of the three composite liver histopathology slide scores, Total, Bili and Bhype_Fib, were plotted. Based on visual inspection of the histograms and results of the Shapiro–Wilk normality tests, the composite score with the distribution that most closely resembled a normal distribution was selected. The chosen composite histopathology slide score was then divided into three liver disease categories

(LDC) low, medium, or high based on the 33rd and 66th percentiles. Thus, each sheep had an outcome liver disease score which could be one of three categories (low, middle, high). The LDC for each ewe was then used as the outcome or predictor variables for all statistical analyses of the production and biochemistry results. The validity of these LDC as a quantitative measure of liver damage was checked against the liver weight and gross liver score data collected post-mortem.

### Weight and body condition score

Ewe live weight and BCS were measured five times during the study (Table 1), with BCS recorded on a 1–5 scale (Jefferies 1961) using 0.5 increments.

### Key performance indicators

Key performance indicators (KPI) for sheep production (see Supplementary Table 1 for definitions) were calculated for the 46 ewes retained in the study.

All procedures performed within this experiment were carried out under the approval of Massey University Animal Ethics Committee, Protocol 19/91.

### Statistics

The following statistical tests were carried out on the data set.

### Effect of LDC on liver weight and gross liver score categories

Analysis of variance was used to compare the mean liver weight across the three LDC. The LSM differences (Lenth 2016) between LDC were then predicted from the ANOVA and tested for significance using Tukey's adjustment method of p-values for multiple post-hoc comparisons. Fisher's exact test was used to test for a significant association between LDC and the GLS recorded post-mortem.

### Udder pathology

The $\chi^2$ test was used to test for an association between udder pathology and LDC, between udder pathology and conception cycle and between udder pathology and whether the ewe weaned a lamb or not.

### Effect of LDC on weight and body condition score

A separate linear mixed effect (LME) model, with a random intercept for ewe, was fitted to the weight and BCS data. LDC, sampling period, cycle conceived (first, second), udder pathology (normal, abnormal) and whether the ewe weaned a lamb (yes, no) were fitted as fixed effects, with variables and biologically plausible interactions retained at p < 0.05 or as a potential confounder using the 10% rule. The fit of

each model was assessed using residual plots and Tukey's adjustment method was used to adjust the p-value for multiple post-hoc comparisons.

### Effect of LDC on key performance indicators

The $\chi^2$ test for trend in proportions was used to test for a linear effect of LDC on the lamb rearing percent and the lamb scanning to weaning loss. The same statistical test was not appropriate for comparing the other measured KPI; weaning percent, lamb rearing percent and ewe flock efficiency, since the numerator is not a dichotomised subset of the denominator for these proportions. So, these data are presented without a statistical test of significance.

### Effect of conception cycle and LDC on lambs born

Fisher's exact test was used to test for a significant association between conception cycle and whether lambs were born (yes, no). The $\chi^2$ test was used to test for a significant association between LDC and conception cycle, and between LDC and whether lambs were born alive (yes, no).

### Effect of LDC, conception cycle and udder pathology on lamb birth weight, lamb weaning weight and weaning probability

Three separate generalised estimating equation (GEE) models were fitted to the production data to model the effect of LDC, the conception cycle (first, second), and udder pathology (normal, abnormal) on lamb birth weight, lamb weaning weight, and lamb weaning probability. A Gaussian link and variance function were used for the birth weight and weaning weight GEE models, and a binomial link and variance function was used for the weaning GEE model (lamb weaned = 1, lamb not weaned = 0). Variables were retained in the model as covariates at p < 0.05 using the Wald statistic or as a potential confounder using the 10% rule. The GEE models allowed for clustering of lambs within ewes, and an exchangeable correlation structure was used as the working correlation structure. The binomial GEE model fits the odds of weaning a lamb, which was converted to the probability of weaning a lamb (odds/(1 + odds)).

### Effect of LDC on biochemistry variables

An LME model, with a random intercept for ewe, was fitted to each of the biochemistry variables, with LDC, udder pathology (normal, abnormal), cycle conceived (first, second), and days from the end of the FE outbreak fitted as fixed effects. Whether a ewe weaned a lamb or not was not included in the model since this was considered a dependant, not independent variable. Days from FE outbreak was also tested as squared and cubic transformations, after standardisation to control multicollinearity. A random slope for days from FE outbreak was also tested in the model with variables and

biologically plausible interactions retained at p < 0.05. The distribution of each biochemistry analyte was first assessed by histograms and a $\log_{10}$ or square root transformation was applied when required to normalise the distribution of the data. The LSM differences for biochemistry outcome variables between LDC (described in Results), adjusted for other variables, were predicted from the fitted model. Plots of those biochemistry variables with a significant association with LDC were made, with fitted regression lines and 95% CI from the LME model for each LDC. Again, Tukey's method was used to adjust the p-value for multiple post-hoc comparisons. The marginal $R^2$ was calculated to show the proportion of variance explained by the fixed effects in each final model. The LME model for GGT included the outbreak and follow-up samples, giving eight sample points instead of six, as for the other biochemistry variables. Correlations between biochemistry variables were estimated with Holm's adjustment for multiple comparisons.

### Effect of LDC on serum GGT activities in outbreak and follow-up samples

An LME model, as described above, was fitted to GGT activity in blood samples collected at the end of sporidesmin exposure (outbreak) and 2 weeks later (follow-up).

### Recursive partitioning to predict low LDC

The LDC groups middle and high were combined into a single category, making LDC binomial "low" or "high" and a decision tree model, using recursive partitioning (RP), was fitted to the biochemistry panel data (first collected 13 weeks after sporidesmin exposure) to predict low *vs.* high LDC using the caret package (Kuhn 2008) in R. A second RP model was also fitted to just the GGT data collected at the outbreak and follow-up samplings, again to predict low *vs.* high LDC. Each RP model was built using 10-fold cross-validation with 10 repeats and pruned by selecting the complexity parameter associated with the highest accuracy. For each iteration of the cross-validation process, the predicted classifications from the model were compared with the actual classifications and the sensitivity and specificity calculated. The mean sensitivity and specificity result across all iterations was used as the estimated sensitivity and specificity and a 95% CI was calculated from the SE for that mean (mean ±1.96 x SE). For the full table of statistical tests used, see Supplementary Table 2.

## Results

### Liver disease

All three composite histopathology slide scores (Total, Bili, Bhype_Fib) passed the Shapiro–Wilk normality test

(p > 0.05). However, after inspecting the histograms, the composite score Bhype_Fib was selected as the liver disease outcome variable and divided into three LDC: low, medium, or high. The livers from the 46 ewes were fairly evenly distributed across the three LDC, with 17 ewes in the low LDC (mean score 10, min 1, max 15), 16 ewes in the middle LDC (mean score 20, min 16, max 24) and 13 ewes in the high LDC (mean score 29, min 25, max 41).

### Effect of LDC on liver weight and gross liver score categories

The mean liver weight was measured post-mortem as 1.28 (95% CI = 1.18–1.38) kg, 1.42 (95% CI = 1.3–1.52) kg and 1.51 (95% CI = 1.39–1.62) kg, for low, middle, and high LDC, respectively. The high LDC livers were 0.225 (95% CI = 0.04–0.41) kg heavier (p = 0.014) than the low LDC livers, with no evidence for a difference between low and middle categories (p = 0.17) or between middle and high categories (p = 0.46), using Tukey's adjustment. There was a significant association between LDC, based on the Bhype_Fib histopathology scores, and the GLS determined post-mortem (Fisher's exact test, p = 0.004), with counts for middle and high LDC increasing with GLS severity (Table 2). The mean Bhype_Fib scores were 3.5 (95% CI = 0–9.84), 15.6 (95% CI = 12.52–18.66) and 23.5 (95% CI = 21.0–26.05), for GLS categories none, moderate and severe, respectively.

### Udder pathology

Overall, 20/46 (43.5%) ewes, when assessed pre-lambing, had abnormal udders. Of these, 7/20 (35%) had a low LDC, 8/20 (40%) a middle and 5/20 (25%) a high LDC. There was no evidence for an association between LDC and udder pathology ($\chi^2$ = 0.45, df = 2, p = 0.8) or between udder pathology and conception cycle ($\chi^2$ = 0.12, df = 1, p = 0.7). For 19/20 ewes with udder pathology there was one abnormal half, while only one ewe had pathology in both halves. At weaning, 11/20 (55%) ewes with abnormal udders and 18/26 (69%) ewes with normal udders weaned at

least one lamb. There was no evidence for an association between udder pathology and weaning a lamb ($\chi^2$ = 0.47, df = 1, p = 0.49).

### Effect of LDC on ewe weight and BCS

Not all 46 ewes were weighed and scored for BCS at early lactation (16/46, 35%) or mid-lactation (29/46, 63%), so only the data for the remaining three periods are presented. The ewes weighed 77.4 (95% CI = 74.7–80.1) kg at pre-mating (5 weeks prior to sporidesmin toxicity), 78.3 (95% CI = 75.6–81.0) kg at scanning (5 weeks after sporidesmin toxicity) and 88.8 (95% CI = 86.1–91.5) kg at weaning (31 weeks after sporidesmin toxicity). They were 11.4 (95% CI = 8.5–14.3) kg heavier at weaning than at premating (p < 0.001) and 10.5 (95% CI = 7.6–13.4) kg heavier at weaning than at scanning (p < 0.001). There was no evidence for a difference in ewe weights between pre-mating and scanning (p = 0.74). The final LME model showed a significant interaction between sampling period and whether the ewe weaned a lamb (p = 0.01), with ewes that didn't wean a lamb being 9.0 (95% CI = 1.1–16.9) kg heavier at weaning (p = 0.02). There was no evidence for a difference in ewe weights for different LDC (p = 0.86), with udder pathology (p = 0.72) or with conception cycle (p = 0.45).

The LME model for BCS showed a significant interaction between sampling period and whether the ewe weaned a lamb or not (p = 0.001), with a ewe not weaning a lamb being 0.8 (95% CI = 0.2–1.4) BCS greater at weaning (p = 0.003). There was no evidence for a difference in BCS with udder pathology (p = 0.97) or conception cycle (p = 0.4). The BCS of ewes with a low LDC was approximately 0.45 BCS units greater than ewes with a higher LDC, but the 95% CI for the difference included zero (−0.035 to 0.94) with a global p-value for the effect of LDC of p = 0.06. Figure 1 shows the fitted values for the LME models for ewe weight and BCS, by sampling period and whether the ewe weaned a lamb or not.

### Lamb cause of death

There were 64 lambs born of which 55 (86%) were alive, eight were stillborn (from ewes with abnormal (n = 4) and normal (n = 4) udders) and one died from trauma. The causes of lamb loss from scanning to weaning, categorised by LDC are shown in Supplementary Table 3.

### Effect of LDC on key performance indicators

The individual counts of ewes and lambs, categorised by LDC at different sampling points in the production cycle, are shown in Supplementary Table 4. Ewes from the middle and high LDC performed poorly at carrying

**Table 2.** Count of ewes (n = 46) exposed to acute sporidesmin toxicity that were assigned, after slaughter 8 months later to each gross liver score category and liver disease category. Gross liver score category is based on the degree of damage detected by gross pathology; liver disease category is based on the degree of biliary hyperplasia and portal fibrosis detected by histology (Munday *et al.* 2021).

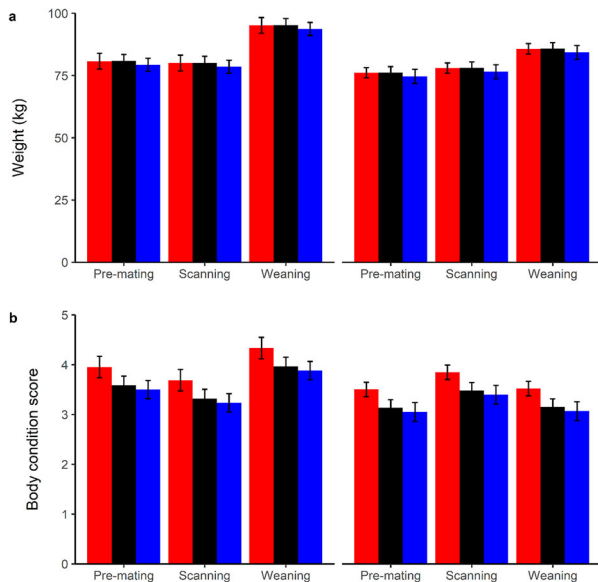| Liver disease category | Gross liver score category | | |
|---|---|---|---|
| | None | Moderate | Severe |
| Low | 4 | 9 | 4 |
| Middle | 0 | 6 | 10 |
| High | 0 | 2 | 11 |

**Figure 1.** Weight (kg; a) and body condition scores (b) of Romney ewes (n = 46) exposed to acute sporidesmin toxicity in late April 2019, recorded at pre-mating (25 March 2019), scanning (4 June 2019) and weaning (29 November 2019) categorised by whether they weaned lambs (right) or not (left). Ewes are grouped by liver disease category (low = red; middle = black; high = blue) based on degree of biliary hyperplasia and portal fibrosis detected by histology (Munday et al. 2021) at slaughter 8 months after exposure to sporidesmin.

lambs to term and rearing lambs to weaning. The KPI for production for these 46 ewes, summarised by LDC, are found in Table 3, and show a significant $\chi^2$ trend in proportions for lamb scanning-to-weaning loss ($\chi^2$ = 5.9, df = 1, p = 0.02), and some evidence for a trend in proportions for lamb rearing percent ($\chi^2$ = 3.7, df = 1, p = 0.06). The table further shows that weaning percent, lamb rearing percent and ewe flock efficiency fell with increasing LDC, and that lamb scanning-to-weaning loss increased with LDC.

### Effect of conception cycle on lambs born
An effect of conception cycle on lambs born was found with 3/16 (19%) ewes conceiving in the second cycle not having any lamb births recorded, compared to 0/30 (0%) ewes conceiving in the first cycle (p = 0.04, Fisher's exact test). These three ewes were from the following LDC: 0/3 low, 2/3 middle, and 1/3 high. There was no evidence for an association between LDC and conception cycle ($\chi^2$ = 1.59, df = 1, p = 0.45) or between LDC and not delivering lambs to term (p = 0.37, Fisher exact test).

### Effect of LDC, conception cycle and udder pathology on lamb birth weight and lamb weaning weight
There were 55 live and 9 dead lambs weighed at birth, the mean birth weight of these lambs was 5.1 (SE 0.15) kg. The GEE model found no evidence for a difference

in birth weights for different LDC (p = 0.72), for different conception cycles (p = 0.71) or for udder pathology (p = 0.88). There were 38 lambs with weaning weights, representing only 38/55 (69%) of birthed-live lambs reared. The mean weaning weight of the first cycle lambs was 35.3 (SE 1.03) kg and for the second cycle lambs was 29.1 (SE 1.29) kg. The GEE found a significant effect of conception cycle on weaning weight, with first cycle lambs being on average 6.9 (95% CI = 3.1–10.8) kg heavier (p < 0.001). There was no evidence for a difference in weaning weight for different LDC (p = 0.41) or for udder pathology (p = 0.61), controlling for the effect of conception cycle.

### Effect of LDC, conception cycle and udder pathology on weaning probability
Adjusting for pre-lambing udder pathology, and conception cycle, the GEE model found that the probability of weaning a lamb was 0.70 (95% CI = 0.51–0.84), 0.49 (95% CI = 0.25–0.72), and 0.28 (95% CI = 0.1–0.58), for low, middle, and high LDC ewes, respectively. The odds of a ewe from the low LDC weaning a lamb were 6.2 (95% CI = 1.05–35.8) times that of a ewe from the high LDC weaning a lamb (p = 0.04). However, there was no evidence for a difference in weaning probability between low and middle LDC (p = 0.37) and between middle and high LDC (p = 0.48).

Adjusting for LDC and pre-lambing udder pathology, the GEE model found that the probability of weaning a lamb for ewes conceiving in the first cycle was 0.67 (95% CI = 0.50–0.80) and in the second cycle was 0.31 (95% CI = 0.13–0.58). The odds of a ewe weaning a lamb conceived in the first cycle were 4.4 (95% CI = 1.2–16.3) times that of a ewe weaning a lamb conceived in the second cycle (p = 0.03).

Adjusting for the effect of LDC and conception cycle the GEE model found no evidence for an effect of udder pathology on weaning probability (p = 0.14).

**Table 3.** Key performance indicators for production stratified by liver disease category for Romney ewes (n = 46) exposed to acute sporidesmin toxicity in late April 2019.

| Key performance indicator (%) | Liver disease category[a] | | |
| --- | --- | --- | --- |
| | Low | Middle | High |
| Scanning percent | 135.3 | 162.5 | 146.2 |
| Lambing percent (alive) | 123.5 | 125.0 | 84.6 |
| Weaning percent | 100 | 87.5 | 53.8 |
| Lamb rearing percent[b] | 80.1 | 70.0 | 50.0 |
| Lamb scanning to weaning loss[c] | 26.9 | 46.2 | 63.2 |
| Ewe flock efficiency | 46.4 | 36.8 | 22.6 |

[a]Based on degree of biliary hyperplasia and portal fibrosis detected by histology (Munday et al. 2021).
[b]$\chi^2$ test for trend in proportions, p = 0.06.
[c]$\chi^2$ test for trend in proportions, p = 0.02.

## Biochemistry results

The mean concentrations or activities for all serum biochemistry parameters by sampling period are shown in Table 4. Activities of the indicators of acute liver disease, GLDH (hepatocyte damage) and GGT (cholestasis) were elevated outside the reference range for the whole sampling period and showed an increase from pre-lambing to early lactation before declining through the subsequent periods. In contrast, concentrations of the indicators associated with energy balance (NEFA and BOH) and most of the indicators of liver function (albumin, globulin, bilirubin, and urea) remained within relevant ovine reference ranges throughout the entire sampling period. The exception to this was the concentration of bile acids, an indicator of liver function and cholestasis, which was elevated outside the reference range for the whole sampling period. Correlations between biochemistry variables from Table 4 showed that GGT was strongly correlated with GLDH ($r = 0.79$, $p < 0.001$) and bile acids ($r = 0.71$, $p < 0.001$), and that bile acids were moderately correlated with GLDH ($r = 0.58$, $p < 0.001$) and globulin ($r = 0.49$, $p < 0.001$). For full table of biochemistry correlation results see Supplementary Table 5.

## Linear mixed effects models for biochemistry data

For only three biochemistry variables did the LME model show a significant effect of LDC, these were GGT, globulin and GLDH.

The final GGT LME model used a square root transformation and showed a significant interaction between LDC and days from FE outbreak ($p = 0.03$) and a significant random effect for days from FE outbreak ($p < 0.001$). A square and cubic transformation of days from FE outbreak also showed a significant effect ($p = 0.001$ and $p < 0.001$ respectively). The back transformed LSM for GGT activity at 19 weeks after sporidesmin toxicity were 284 (95% CI = 173–423) IU/L, 565 (95% CI = 406–751) IU/L and 699 (95% CI = 502–929) IU/L, for low, middle, and high LDC respectively. The LSM for GGT activity for low LDC ewes was significantly lower than that for middle ($p = 0.025$), and high ($p = 0.003$) LDC ewes, whereas there was no evidence for a difference between middle and high LDC ewes ($p = 0.58$). There was no evidence for a difference in GGT by udder pathology ($p = 0.22$), however, there was evidence for a difference in GGT by conception cycle ($p = 0.016$), with the back transformed LS means for GGT activity at 19 weeks after sporidesmin toxicity and controlling for the effect of LDC, being 383 (95% CI = 285–496) IU/L and 632 (95% CI = 461–829) IU/L, for first cycle, and second cycle conceptions respectively, ($p = 0.02$). The residuals were normally distributed, and the marginal $R^2$ of the LME model was 0.4. The fitted results from the LME model show that the GGT results for the first cycle conception ewes were much lower than second cycle conception ewes throughout the sampling periods and for low LDC ewes were much lower than the middle and high LDC ewes throughout the sampling periods (Figure 2).

The final globulin LME model showed no evidence for an interaction between liver disease category and days from FE outbreak ($p = 0.53$); however, a significant interaction was detected between liver disease category and days-squared from FE outbreak ($p = 0.001$). Days from FE outbreak was significant ($p < 0.001$) and a random effect for days was also significant ($p < 0.001$). There was no evidence for a difference in globulin concentration for conception cycle ($p = 0.61$) while the effect of abnormal udder pathology on globulin concentrations spanned the null value (95% CI = −0.33–7.75; $p = 0.08$). The LSM for globulin concentration 25 weeks after sporidesmin toxicity were 44.0 (95% CI = 40.4–47.7) g/L, 50.9 (95% CI = 47.2–54.7) g/L and 55.8 (95% CI = 51.7–60.0) g/L, for the low, middle, and high LDC groups, respectively. The LSM

**Table 4.** Mean (SE) of serum biochemistry analytes for 46 Romney ewes exposed to acute sporidesmin toxicity in late April measured at up to eight sampling periods over an 8-month study period.

| | Sampling period (week[a]) | | | | | | | |
| Analyte | Outbreak (0) | Follow up (2) | Pre-lambing (13) | Early lactation (18–21) | Mid-lactation (22–25) | Late lactation (29) | Post-weaning (31) | Pre-slaughter (33) | Reference range |
|---|---|---|---|---|---|---|---|---|---|
| Albumin (g/L) | | | 32 (0.53) | 32.3 (0.47) | 32.7 (0.48) | 32.8 (0.4) | 32.5 (0.41) | 31.1 (0.41) | 21–41 |
| Bilirubin (µmol/L) | | | 3.7 (0.24) | 3.1 (0.32) | 4.7 (0.5) | 6.2 (0.89) | 3.7 (0.41) | 3.6 (0.62) | 0.0–9.0 |
| Bile acids (µmol/L)[b] | | | 76.1 (11.14) | 113.3 (16.3) | 91.5 (14.1) | 59.3 (10.0) | 63 (12.48) | 66.9 (13.29) | 0.0–25.0 |
| BOH (mmol/L) | | | 0.5 (0.03) | 0.7 (0.07) | 0.5 (0.03) | 0.3 (0.02) | 0.3 (0.01) | 0.4 (0.01) | 0.10–1.50 |
| GLDH (U/L)[b] | | | 472 (65.1) | 551.6 (66.9) | 440.5 (57.98) | 274.3 (40.1) | 209.8 (33.3) | 178.3 (30.35) | 0–20 |
| Globulin (g/L) | | | 53.8 (0.96) | 50.6 (1.3) | 50.2 (1.48) | 49.7 (1.3) | 49.2 (1.16) | 49.6 (1.17) | 34–55 |
| GGT (U/L)[b] | 884.5 (76.9) | 802.1 (67.9) | 486.1 (58.7) | 616 (76.7) | 550.9 (77.5) | 412.6 (57.6) | 357.2 (56.2) | 311.5 (50.9) | 32–70 |
| NEFA (mmol/L) | | | 0.7 (0.05) | 0.8 (0.07) | 0.7 (0.05) | 0.9 (0.05) | 0.2 (0.01) | 0.6 (0.03) | 0.1–0.9 |
| TP (g/L) | | | 85.8 (0.74) | 82.9 (1.03) | 82.9 (1.19) | 82.6 (1.08) | 81.7 (0.94) | 80.7 (0.98) | 56–88 |
| Urea (mmol/L) | | | 8.4 (0.17) | 8.1 (0.23) | 8.5 (0.18) | 6.8 (0.16) | 6.3 (0.14) | 6 (0.14) | 5.1–15.6 |

[a]Number of weeks from the end of the exposure to sporidesmin (29 April 2019).
[b]These analytes were elevated outside the reference range.
BOH = β-hydroxybutyric acid; GLDH = glutamate dehydrogenase; GGT = gamma-glutamyl transferase; NEFA = non-esterified fatty acids; TP = total protein.

globulin concentrations in low LDC ewes were significantly lower than that of the middle LDC (p = 0.03) and high LDC (p < 0.001) ewes, whereas there was no evidence for a difference between middle and high LDC ewes (p = 0.19). The residuals were normally distributed and the marginal $R^2$ of the fitted LME model was 0.23.

The final GLDH LME model used a square root transformation and showed a significant interaction between LDC and days from FE outbreak (p = 0.04) and a significant random effect for days (p < 0.001). A square and cubic transformation of days from FE outbreak also showed a significant effect (p = 0.003 and p = 0.014 respectively). There was no evidence for an effect of udder pathology (p = 0.22) or conception cycle (p = 0.16) on GLDH activities. The back transformed LSM for GLDH at 25 weeks after sporidesmin toxicity were 155 (95% CI = 78–257) IU/L, 384 (95% CI = 253–542) IU/L and 531 (95% CI = 359–736) IU/L, for the low, middle, and high LDC ewes respectively. The LSM for GLDH for the low LDC ewes were significantly less than that of medium LDC (p = 0.02) and high LDC (p = 0.002) ewes whereas there was no evidence for a difference between middle and high LDC (p = 0.42). The residuals were normally distributed and the marginal $R^2$ of the fitted LME model was 0.32. The fitted results from the LME models for globulin and GLDH show that the results for the low LDC are much lower than the middle

and high LDCs throughout the sampling periods (Figure 3).

### Other biochemistry parameters
The respective LME models showed no evidence for an effect of LDC on albumin (p = 0.11), bile acids (p = 0.25), bilirubin (p = 0.43), BOH (p = 0.16), NEFA (p = 0.42) or urea concentrations (p = 0.96). An effect of LDC on TP was found (p = 0.03) but this was likely the effect of LDC on globulin concentrations since there was no evidence of an effect of LDC on the concentration of albumin.

### GGT activity in serum during acute sporidesmin toxicity
The GGT activities recorded at the outbreak and follow-up sampling 17 days later were highly correlated (r = 0.91). The back transformed LSM for GGT activity, averaged over the outbreak and follow-up sampling, were 373 (95% CI = 243–530) IU/L, 981 (95% CI = 756–1,235) IU/L and 1,063 (95% CI = 804–1,358) IU/L, for low, middle, and high LDC respectively. There was no evidence for an effect of udder pathology (p = 0.27) or conception cycle (p = 0.23) on GGT activity, while the 95% CI for the difference in GGT activity between outbreak and follow-up samples was −1 to 120, (p = 0.053). The LSM for GGT activity for low LDC were significantly lower than that of the middle LDC (p < 0.001), and high LDC (p < 0.001), whereas there was no evidence for a difference
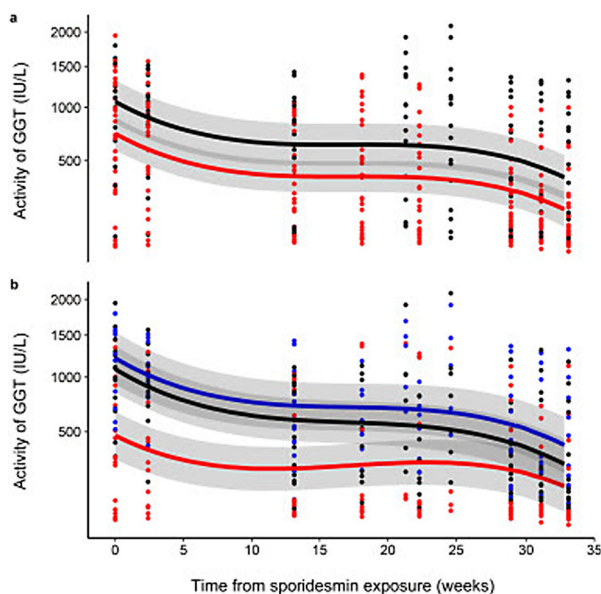


**Figure 2.** Fitted curves with 95% CI (light grey shading) from linear mixed effects model describing the change in gamma-glutamyl transferase (GGT) activity in serum with time, categorised by (a) conception cycle (red = first cycle; black = second cycle) and (b) liver disease category (LDC; red = low LDC, black = middle LDC; blue = high LDC) in ewes (n = 46) exposed to acute sporidesmin toxicity in late April 2019 (time = 0). Note the square root scale of the y-axis.
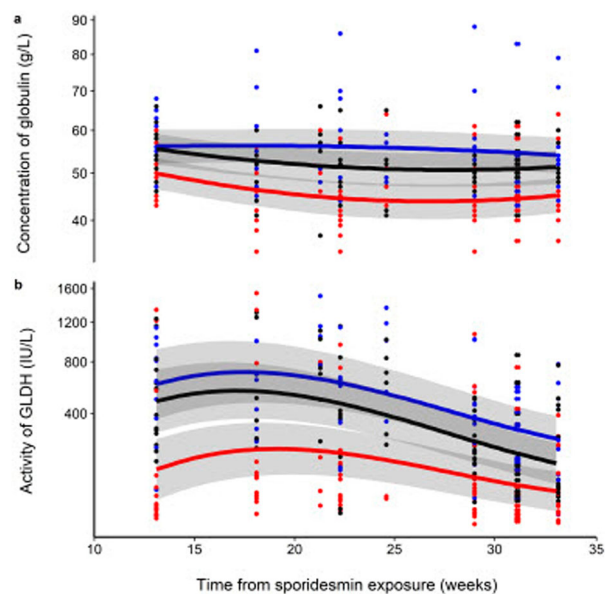
**Figure 3.** Fitted curves with 95% CI (light grey shading) from linear mixed effects models describing the change over time of (a) the concentration of globulin and (b) the activity of glutamate dehydrogenase (GLDH) in serum of ewes (n = 46) exposed to acute sporidesmin toxicity ending in late April 2019 (week 0) categorised by liver disease category (LDC; red = low LDC, black = middle LDC; blue = high LDC). Note the square root scale of the y-axis.

between middle and high LDC (p = 0.89). The residuals were normally distributed and the marginal $R^2$ of the fitted mixed effect model was 0.38.

### Prediction of low LDC

The final decision tree RP model for the biochemistry panel data (collected 13–33 weeks after exposure), after pruning, was very simple, consisting of a single node. The node asked whether the activity of GGT <122 IU/L; answer yes and the sheep was categorised as low LDC, answer no and the sheep was categorised as high LDC. This cut point had a sensitivity of 0.69 (95% CI = 0.61–0.76) and a specificity of 0.90 (95% CI = 0.84–0.95) for predicting low LDC.

The final decision tree RP model for the GGT activities in the outbreak and follow-up samples had a cut point of GGT <514 U/L. This cut point had a sensitivity of 0.70 (95% CI = 0.61–0.80) and a specificity of 0.91 (95% CI = 0.85–0.98) for predicting low LDC.

## Discussion

To the authors' knowledge, this is the most extensive longitudinal observational study of sporidesmin intoxication on lamb production and biochemistry completed in New Zealand to date, with the study ending 8 months after initial intoxication.

The results showed that ewes from the middle and high LDC performed poorly at carrying lambs to term and rearing lambs to weaning. Since the trial ewes had a concurrent high prevalence of udder pathology, some confounding of these results was expected. However, the GEE model showed that, after adjusting for the effect of udder pathology and cycle of conception, ewes from low LDC had a significantly higher probability of weaning a lamb compared to ewes with high LDC. These results are consistent with a previous study in which increasing serum GGT activities were also reported to have a negative effect on reproductive outcomes in sheep (Moore et al. 1990).

The finding of decreasing lamb survival with increasing liver damage assessed by serum GGT activity, also agrees closely with the results of McMillan et al. (1988), who found that lamb survival decreased by 6% for every 100 IU/L increase in GGT activity above the normal in the ewe. Although 20/46 (43.5%) ewes in the presently reported study had some udder pathology, which likely affected overall production performance, for this dataset the effect of liver pathology on lamb survival can be assumed to be independent of these defects since there was no association between LDC and udder pathology found (i.e. ewes with udder pathology were present in similar proportions in each of the LDC groups).

The heaviest livers were from the high LDC and there was a significant association found between the LDC and the gross liver scores given post-mortem. Both these results validate the use of this quantitative measure, based on the combined biliary hyperplasia and portal fibrosis scores at five standardised sample sites on the affected liver, to assess liver damage from sporidesmin toxicity. The use of a standardised scoring system will make it easier to compare results from future ovine FE studies. The heavier, high LDC livers found likely reflect the marked hypertrophy of the medial right lobe seen in the severely affected livers (Munday et al. 2021).

Considering all KPI, it appears that the greatest effect of sporidesmin-induced liver damage is on the ewes' ability to carry and rear lambs from scanning to weaning. Low LDC ewes were 6.2 (95% CI = 1.05–35.8) times more likely to wean a lamb than high LDC ewes (p = 0.04), after adjusting for udder pathology and conception cycle. However, the $\chi^2$ test for trend in proportions found only a weak association between LDC and lamb rearing percent (p = 0.06). This indicates that the rearing ability of the ewes, when the lambs were born alive, was similar irrespective of liver damage. This observation is supported by the finding that there was no difference in weaning weights across the LDC, which would have been expected had the degree of liver pathology negatively impacted milk production and suggests that there is little effect of liver damage due to FE on milk production. This is supported by the results of McMillan et al. (1988), who found no effect of severity of sporidesmin challenge on milk production. If there is no effect on milk production, then this would suggest that the lambs themselves were affected in utero by sporidesmin toxicity at the time of the FE outbreak. This distinction is critical, as it means that even if pregnant ewes develop serious liver disease (i.e. those from the middle and high LDC), their productivity may not be affected during the subsequent lactation. Unfortunately, the only way to determine this is to keep ewes for 2 production years following sporidesmin intoxication, something that was not possible in the current study.

There was a strong effect of conception cycle, on both lamb survival to term and on weaning probability, with ewes conceiving in the first cycle being 4.4 (95% CI = 1.2–16.3) times more likely to wean a lamb (p = 0.03), after adjusting for the effect of LDC and udder pathology. However, this apparent relationship may have been confounded by the significantly higher GGT activities found in the second-cycle ewes compared to first-cycle ewes. This difference in GGT activities may be an artefact introduced into the data by the culling decisions made after scanning on 4 June, when barren ewes and ewes identified with dead or dying lambs were removed. The mean conception date for ewes that conceived in the first cycle was 25 March, and 10 April for the

second cycle ewes. This meant at scanning the first cycle fetuses were an average of 71 days old compared to 55 days for second cycle fetuses. It is possible that ewes were retained from cycle 2, which had they been scanned 16 days later in their pregnancies may have been removed for dead or dying lambs or being barren. By not having the ability to cull these ewes at an equivalent stage in their pregnancy, then ewes with more serious sporidesmin toxicity and higher GGT activities may have been retained from cycle 2, potentially leading to the finding of an association between GGT and conception cycle. Even though it is an artefact of the data, this finding substantially supports our hypothesis of a direct effect of sporidesmin toxicity on lambs *in utero*, which results in either death and expulsion of the fetus or still birth. Sporidesmin has been associated with atrophy of the thymus and hypertrophy of the adrenal glands in sheep with experimental sporidesmin toxicity (Smith and Payne 1991; Smith 2000) which suggests that its toxic effects may not be limited to the cells lining the bile ducts and bladder. Future studies should examine this potential mechanism, via detailed necropsy and histological examination of dead lambs born to ewes with known exposure to sporidesmin intoxication during early pregnancy.

The normal range for New Zealand ewe flock efficiency is 40–70%, with an average of 57%, and the normal range for lamb scanning-to-weaning loss is 15–20%. Only ewes from the low LDC had a normal ewe flock efficiency of 46.4%, whilst no LDC had a normal lamb scanning to weaning loss (Beef + Lamb NZ 2018). If a farmer wanted to retain some ewes affected with FE for the next lambing, it appears that being able to select ewes with a low LDC would limit the negative impact on flock productivity in the current lambing.

No association between LDC and ewe weight or BCS was detected throughout the study. It is important to highlight this result, as anecdotal reports suggest farmers and veterinarians often assume grossly visible liver changes characteristic of previous sporidesmin exposure are the cause of poor body condition. In the present study, even ewes with the most advanced liver shape changes and fibrosis were able to maintain or even gain weight and these animals did not show any changes within serum biochemistry variables, such as albumin, globulin, bilirubin, and urea, to indicate loss of liver function. However, it is important to note that, in the current experiment, there were no unaffected controls, so no observations on the efficiency of ewe weight gain can be made. Additionally, it is very likely these study ewes were on a much greater plane of nutrition compared to ewes in a typical commercial flock. It is, therefore, possible that ewes that have previous sporidesmin-induced liver damage could develop evidence of liver failure if challenged by a lower plane of nutrition. Furthermore, the ewes with more severely affected livers in the present study were less likely to be rearing a lamb and so, as a group, would have lower metabolic demands placed on them than ewes that reared lambs to weaning, which may have contributed to these findings.

The limited effect of sporidesmin intoxication on most serum biochemistry parameters was a surprising result, with almost all measured variables of liver function remaining within their reference ranges. The only abnormality was the increase in the concentration of bile acids; however, this may have been due to cholestasis rather than a reduction in liver function. The lack of evidence for reduced liver function in these ewes may illustrate the remarkable functional reserve of the ovine liver. No references to the use of bile acid measurement in ewes post-sporidesmin intoxication could be found and although elevated throughout the trial, the bile acids measurements were highly correlated with GGT and GLDH measurements, which themselves were highly correlated. This suggests that there is no additional value to measuring bile acids or GLDH over GGT alone.

The LME models for GGT, GLDH and globulin all showed a significant interaction between days since FE and LDC, indicating that the changes over time in GGT, GLDH and globulin are different for each LDC. For all three LME models the GGT, GLDH and globulin measurements were significantly less for the low LDC compared to the middle and high LDC, whereas the middle and high LDC were not significantly different. These results clearly indicate that the changes in GGT, GLDH and globulin associated with low LDC are consistent and different to middle and high LDC over the 8 months from sporidesmin exposure to slaughter. These findings expand the results of Munday *et al.* (2021): not only is GGT a significant marker for liver pathology when measured at FE outbreak, but over the follow-up period the relationship between activity of GGT and severity of liver pathology is consistently maintained through to slaughter.

The results of the RP model for the biochemistry data had a sensitivity of 0.69 and a specificity of 0.90 for predicting low LDC with just a single GGT measurement, 3 or more months after the acute phase of the sporidesmin toxicity. Commercial farmers could use this to assist in prioritising culling decisions following an FE outbreak, as ewes in the high LDC are predicted to have poorer productivity. A GGT activity of 122 IU/L is above the normal reference range for GGT of 32–70 IU/L, which would be expected since these sheep had some evidence of FE damage. The productivity of the ewes from the low LDC was the closest to normal and retaining ewes with this level of liver damage post FE in a flock could be a legitimate strategy, particularly in flocks with a large proportion of FE

affected ewes. If the reproductive outcomes of low LDC ewes could be shown to approach normality in the first season and recover in the second season, then keeping these ewes would minimise the financial losses in the first year. Given that this was a very small data set, we would suggest that a cut point of 122 IU/L is used more as a guideline for making culling decisions until further work can be completed to confirm this finding. However, it is interesting that given the wide range of biochemistry analytes measured, only GGT was retained in the final RP model, again reinforcing the value of this variable for predicting liver damage from sporidesmin toxicity.

The RP model for the outbreak and follow-up GGT data had a similar sensitivity and specificity to that found after 3 months and indicates that in the 2 weeks following an outbreak of sporidesmin toxicity, keeping only those ewes with a GGT < 514 IU/L may offer a method to farmers of selecting those ewes most likely to have low liver damage.

## Conclusions

Assessment of liver damage using a standardised liver score was shown to predict lamb production and some serum biochemistry changes. A simple GGT measurement 3 or more months after an outbreak of clinical FE, using a cut point of 122 IU/L, could be used as a basis to select ewes for culling or retention in the flock. Alternatively, in the 2 weeks following an outbreak of FE, keeping only those ewes with a GGT < 514 IU/L could select for ewes likely to develop low liver damage and limit production losses in the subsequent season.

In the absence of nutritional stress, even ewes in the high LDC group were able to maintain body condition, suggesting FE alone was not associated with poor BCS and that BCS was not a good predictor of sporidesmin-induced liver damage. If the FE outbreak coincides with early pregnancy, then losses of lambs *in utero* and at birth will be increased in severely affected ewes.

## Acknowledgements

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# Longitudinal study of the effect of sporidesmin toxicity on lamb production and serum biochemistry in a flock of 46 Romney ewes using a standardised measure of liver damage

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