Relationship between gamma-glutamyl transferase (GGT) concentrations and lying behaviour in dairy cattle

M Sutherland[a]*, F Huddart[a], G Lowe[a], N Cox[b], E Cuttance[c] and K Schütz[a]

[a]*AgResearch Ltd., Ruakura Research Centre, Hamilton 3216, New Zealand;* [b]*NeilStat Ltd, 9 Ngaere Ave, Hamilton 3210, New Zealand;* [c]*VetEnt, Te Awamutu 3800, New Zealand*

**Corresponding author*. Email: mhairi.sutherland@agresearch.co.nz

Abstract

Facial eczema (FE) in livestock results in liver damage, impaired production and welfare, and is a significant problem in New Zealand. The pathology of this disease has been described in sheep and cattle, however, few studies have investigated the behavioural response to FE and whether behaviour can be used as an indicator of FE. Therefore, the aim of this study was to evaluate lying behaviour of dairy cattle with FE. Six farms located in the North Island of New Zealand with confirmed cases of FE were selected (n=391 lactating cows). Blood samples were collected to analyse gamma-glutamyl transferase (GGT) concentrations and three days of continuous lying behaviour were recorded using accelerometers. There was no correlation between GGT concentrations and either lying times (r^2=0.0008) or lying bout frequency (r^2=0.0008) when all animals were included in the analysis. In addition, lying times (means±SEM: Low: 9±0.1 h/d, High: 9±0.1 h/d; P=0.347) and lying bout frequency (means±SEM: Low: 8±0.2 no. bouts/d, High: 8±0.2 no. bouts/d; P=0.149) did not differ between pair-matched cows with Low (≤ 89 IU/L) or High (≥ 123 IU/L) GGT concentrations. Therefore, it does not appear as though lying behaviour, measured using accelerometers, is associated with GGT levels in dairy cattle.

Keywords: facial eczema; gamma-glutamyl transferase; lying behaviour

Introduction

Facial eczema (FE) or pithomycotoxicosis, a disease that affects ruminants and camelids, is found in all temperate climate zones in the world and commonly occurs in late summer and autumn in the North Island of New Zealand. Animals get FE after eating large numbers of fungal spores produced by *Pithomyces chartarum* which contain the toxin sporidesmin. Ingestion of sporidesmin causes occlusion of the bile ducts and liver damage (Smith & Towers 2002). Photosensitivity then occurs when phylloerythrin, a breakdown product of chlorophyll, is not eliminated from the body but instead accumulates in the blood (Clare 1944). When phylloerythrin reaches high levels, photosensitisation of unpigmented and hairless areas of the skin occurs in response to sunlight (Clare 1944) and these areas can then become reddened, raised and oedematous (Mortimer & Ronaldson 1983). However, most animals affected with FE do not show clinical signs, though they still can have liver damage: for every cow in a herd with skin lesions, up to 10 or more may be affected sub-clinically (Faull 1986). Even though cattle may not show clinical signs of FE they may still be experiencing adverse effects. Serum GGT levels are commonly used to diagnose FE in sheep and cattle, as GGT concentrations have been shown to increase proportionally with sporidesmin-induced liver damage in both species (Towers & Smith 1978; Towers & Stratton 1978).

Facial eczema is generally not fatal, however, it causes liver damage, reduced feed intake, decreased production, and discomfort (Morris et al. 2004). Limited scientific research is available demonstrating the impact of this disease on ruminant behaviour. Anecdotally, once animals become photosensitive from FE, they will often seek shade and may respond to skin irritation by head shaking, skin rubbing and restlessness (Morris et al. 2004). There is little other scientific information regarding the behavioural response of cows with FE and whether animal behaviour can be used as an early indicator of animals with FE. It would, therefore, be of interest to assess how FE affects the behaviour of dairy cattle.

Lying behaviour is an important welfare indicator in cattle (Haley et al. 2000) and well-fed, pastured, lactating dairy cattle spend between 8.3 and 10.1 h/24 h lying down (Kendall et al. 2006; Tucker et al. 2007, 2008; Fisher et al., 2008; Schütz et al. 2013). Health status can affect lying behaviour in dairy cattle; for example, cows performed fewer and longer lying bouts during the three days before clinical diagnosis of metritis (Neave et al. 2018) and a similar pattern was observed in calves before clinical signs of neonatal calf diarrhoea appeared (Lowe et al. 2019). Therefore, lying behaviour could potentially be used as an indicator of FE, possibly even sub-clinical FE, in cattle. Lying behaviour can now be measured relatively non-invasively using accelerometer data loggers that have been validated for measuring lying behaviour of cattle (Ledgerwood et al. 2010). The aim of this study was to evaluate lying behaviour of dairy cattle with FE (e.g., elevated serum GGT concentrations).

Materials and methods

Between March and May 2018 (southern hemisphere autumn) six dairy farms located in the North Island of New Zealand were selected due to cows in those herds having current outbreaks of FE and high GGT concentrations. This study was part of a larger research project designed

to determine both the cost and animal welfare implications of sub-clinical facial eczema in dairy cattle. All procedures involving animals were approved by the Ruakura Animal Ethics Committee (no. 14361) under the New Zealand Animal Welfare Act 1999.

Blood samples were collected from the coccygeal (tail) vein of cattle into tubes that contained no anticoagulant. Blood samples were left to sit at room temperature in an upright position for approximately 30 minutes. Serum samples were analysed for GGT enzyme activity at 37°C, using a Roche colorimetric method and Modular Analytics Model P analyser (Roche Diagnostics, Auckland, NZ).

In total, 413 cows from the six farms were selected based on GGT concentrations, days in milk, milk yield, calving date, age and breed. Cows with GGT concentrations ≥ 123 IU/L were match paired with cows with similar days in milk, milk yield, calving date, age and breed, but with GGT concentrations ≤ 89 IU/L. Due to missing data from some animals it was only possible to include 167 complete matched pairs in the analysis (n = 334 cows).

Once these cows were selected, a HOBO pendant G accelerometer data logger (64k, Onset Computer Corporation, Bourne, MA, USA) was attached to each cow to record lying behaviour continuously for at least three days. The time between blood sampling, selecting animals for inclusion in the trial and attaching the accelerometers to the cows meant that there was a delay between blood sampling and recording of lying behaviour of 14 to 30 days. Following the methodology described by Schütz et al. (2019), the accelerometers were set to record the y- and z-axes continuously at 1-min intervals. The accelerometers were placed in a fabric pouch held in position by Velcro patches and attached on the lateral side of the hind leg above the metatarsophalangeal joint. The data were downloaded using HOBOware Pro software (Onset Corp., Pocasset, MA) and converted to daily summaries of lying behaviour (total lying times and bout information) calculated in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) using a code designed for this purpose (AWP UBC, 2015). Lying behaviour was analysed over three consecutive days (72 h in total) for each cow.

Statistical analysis

The lying data were analysed using a rank transformation, due to the data being clearly inconsistent with the normal distribution. An ANOVA was run in Genstat, version 19 (VSN International, Hemel Hempstead, UK) and the model included the blocking effects of farm and pair (within farm) and treatment effect for GGT category (Low or High).

Results

From the original 413 cows, data were successfully collected from only 391 cows due to accelerometer failure (lost accelerometers or corrupted files). Farm and animal information for these cows is summarised in Table 1.

Table 1 Farm and cow information for the six enrolled dairy farms located in the North Island of New Zealand between March and May 2018.

			Age		Days in milk			GGT concentrations (IU/L)		
Farm	Region	No. cows tested	Mean	Range	Mean	Range	Sampling gap[1]	Mean	SD	Range
1	Taranaki	71	12	(7 - 15)	216	(172 - 237)	14	961	1267.2	(2 - 5278)
2	Waikato	104	11	(8 - 15)	230	(195 - 265)	20	456	710.7	(1 - 3635)
3	Northland	42	11	(7 - 15)	240	(196 - 259)	17	333	440.8	(3 - 2055)
4	Taranaki	39	13	(8 - 15)	218	(170 - 249)	17	285	545.7	(4 - 2809)
5	Bay of Plenty	107	12	(5 - 15)	230	(171 - 289)	30	773	1058.4	(3 - 5158)
6	Waikato	28	13	(6 - 15)	223	(189 - 250)	30	513	786.2	(3 - 2495)

GGT = Gamma-glutamyl transferase

[1]Sampling gap is the number of days between cows being blood sampled and when lying behaviour was recorded.

Table 2 Mean (± SD) gamma-glutamyl transferase (GGT) concentrations, daily lying times and frequency of lying bouts for cows categorised as having Low or High GGT concentrations (n = 167 matched pairs) on six dairy farms in the North Island of New Zealand between March and May 2018.

		GGT concentrations (IU/L)				Lying time (h/24 h)				Lying bouts (no./24 h)			
Farm	Cows (no.)	Low	SD	High	SD	Low	SD	High	SD	Low	SD	High	SD
1	46	37.3	25.01	1783.3	1161.4	9.7	1.47	9.6	1.62	7.8	2.38	7.4	1.81
2	92	19.5	12.90	883.6	736.3	9.7	1.46	9.6	1.66	9.1	3.07	8.7	2.51
3	32	23.8	18.36	675.7	509.7	7.7	1.38	7.8	1.12	7.1	2.16	7.2	2.20
4	38	16.8	11.24	567.2	669.3	9.9	1.69	9.0	1.31	9.1	1.97	8.1	1.72
5	100	22.1	19.09	1509.4	1112.1	9.0	1.75	8.9	1.73	10.3	2.57	10.1	2.70
6	26	22.2	9.38	1056.5	864.6	8.8	2.99	9.1	3.18	6.7	2.00	7.5	2.35

Figure 1 Relationship between daily lying times and gamma-glutamyl transferase (GGT) concentrations in dairy cattle (n = 391) on six dairy farms in the North Island of New Zealand between March and May 2018.

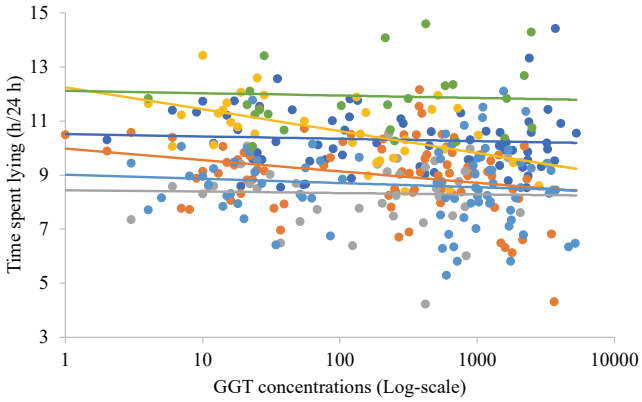
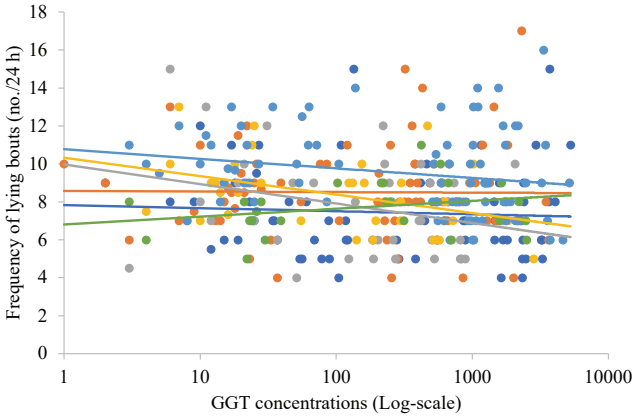


Figure 2 Relationship between number of lying bouts and gamma-glutamyl transferase (GGT) concentrations in dairy cattle (n = 391) on six dairy farms in the North Island of New Zealand between March and May 2018.



Daily lying times and number of lying bouts, averaged over the three data-collection days, are plotted against the GGT concentrations for each animal in figures 1 and 2, respectively. There was no relationship between GGT concentrations and lying times ($r^2 = 0.0008$) or number of lying bouts ($r^2 = 0.0008$) when all 391 cows were included in the analysis.

Due to missing data from some cows, data analysis was based only on 167 matched pairs (n = 334 cows). The range of GGT concentrations for cows categorised as Low and High, across all matched pair cows on the six farms, was 1 – 89 IU/L and 123 – 5278 IU/L, respectively. GGT concentrations, daily lying times and frequency of lying bouts for cows categorised as having Low or High GGT concentrations on the six study farms are presented in Table 2.

Time spent lying, averaged over three days and the six farms, did not differ between the Low and High cows (mean ± SEM: Low: 9 ± 0.1 h/d, High: 9 ± 0.1 h/d; P = 0.347). The frequency of lying bouts, averaged over three days and the six farms, did not differ between the Low and High cows (mean ± SEM: Low: 8 ± 0.2 no. bouts/d, High: 8 ± 0.2 no. bouts/d; P = 0.149).

Discussion

There appeared to be no relationship between GGT concentrations and lying behaviour in dairy cattle; however, two farms did show a slight negative correlation between lying times and GGT concentrations. Due to logistical reasons, lying behaviour was not measured at the same time, or directly after, blood samples were taken to determine GGT concentrations. Depending on the farm, lying behaviour was measured 14 to 30 days after blood samples were taken. It is, therefore, likely that GGT concentrations at the time that lying behaviour was recorded were different than those initially measured. However, previous studies in cattle and sheep suggest that GGT concentrations rise within two weeks after a naturally or experimentally induced sporidesmin challenge and can remain elevated for several weeks after this initial exposure (Blackshaw 1978; Towers & Smith 1978; Towers & Stratton 1978). Therefore, cattle with elevated GGT levels in the current study may still have had elevated GGT concentrations at the time lying behaviour was measured, but concentrations might have changed and not be reflected in the analysis. There is still limited evidence for how GGT levels develop with a natural challenge on-farm and with previous exposure of sporidesmin. It would have been ideal to know the actual GGT concentrations of the cows at the time lying behaviour was recorded. For example, GGT concentrations of 'High' cattle may have declined or GGT concentrations of 'Low' cows may have been in the process of increasing. Without being able to confirm when these animals were exposed to sporidesmin, or how often they were exposed, it is not possible to know what stage of infection or degree of liver damage these animals would have been experiencing, hence, the results in the present study need to be interpreted with caution, and we recommend that more research is undertaken to investigate the relationship between behaviour and FE. It is also worth noting that the cows enrolled in this study, which were selected based on GGT concentrations and their matched pairs, ended up being older animals; the mean age ranged between 11 and 13 years, and we speculate that previous exposure to FE might have affected the livers of these animals ability to produce GGT. Hence, this could have influenced our results, and we recommend that more research investigating the effect of history of liver damage on the excretion of GGT and how this in turn influences animal responses, is needed.

It is unclear why farms 2 and 4 showed a slight negative correlation between lying times and GGT concentrations. The gap between blood sampling and measuring lying behaviour on farms 2 and 4, was 20 and 17 days, respectively. No correlation between lying times and GGT concentrations were observed on farm 1, which only had a sampling gap of 14 days. Therefore, timing

of sampling likely does not explain the slight negative correlations observed on farms 2 and 4. In addition, as mentioned earlier, previous exposure to sporidesmin and possible liver damage may have accounted for the variation in the responses we observed among farms.

The lying times recorded in the present study are, in general, within the range of studies with well-fed lactating dairy cattle in New Zealand (Kendall et al. 2006; Tucker et al. 2007, 2008; Fisher et al. 2008; Schütz et al. 2013) except for one farm in Northland where cows had low lying times (a reduction > 1 h/24 h) compared to the other farms. We did not measure weather conditions at the time of testing, but it was noted that it was very warm at the time of recording of lying times on the Northland farm. It is well-known that cows spend less time lying in warm weather (Tucker et al. 2008; Schütz et al. 2010) and this could be a potential explanation for the lower lying times on the Northland farm.

Lying behaviour in an important welfare indicator in cattle and can be measured relatively non-invasively, and with minor labour input, using accelerometer data loggers. Therefore, we chose to investigate whether lying behaviour could be used to detect FE in cattle in the present study. Since feed intake and milk production is affected by FE (Morris et al. 2004), other behaviours such as grazing would have been beneficial to measure, however, it was not feasible in the present study. Up until recently, it has not been possible to measure grazing activity automatically, though with recent developments in technology, this is now feasible, and we encourage further research investigating the behavioural responses of cows to FE.

In summary, clinical signs of FE are the result of photosensitisation causing unpigmented and hairless areas of skin to become irritated (Mortimer & Ronaldson 1983). Anecdotaly, animals respond to the discomfort and pain caused by photosensitisation by seeking shade, head shaking, skin rubbing and restlessness (Morris et al. 2004). Animals that show these clinical signs of FE, and possibly cows with sub-clinical FE, are likely to experience discomfort from the disease process and possible negative affective states, such as pain and perhaps nausea. We, therefore, recommend further studies investigating behavioural responses of cattle with FE, in particular sub-clinical FE.

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