Do cows with facial eczema feel pain?

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Abstract

Facial eczema (FE) negatively influences production and welfare. One consequence of FE is reduced feed intake, which could possibly be a consequence of cows experiencing nausea or pain or both. The aim of this study was to evaluate the association of cortisol and acute phase protein (APP) concentrations (indirect measures of pain) with gamma glutamyl transferase (GGT) and bile acid concentrations (indirect measures of liver damage) in dairy cattle. Blood samples were collected from 2,080 lactating dairy cattle from seven farms that were affected by FE and located in the North Island of New Zealand. Blood samples were analysed for GGT, bile acid, cortisol and APP (serum ferroxidase (SF), serum amyloid A and haptoglobin) concentrations. Data were analysed using best subsets linear regression including farm as a factor and various other prediction variables. There was a significant positive relationship between GGT and bile acid ($P<0.001$, Adjusted $R^2=37.3\%$), SF ($P<0.001$, Adjusted $R^2=27.4\%$), and haptoglobin ($P<0.001$, Adjusted $R^2=1.9\%$) concentrations, but not with cortisol. The positive association between GGT concentrations and APP (SF, haptoglobin) may partly reflect the degree of liver damage caused by sporidesmin intoxication, however, it could also suggest that animals with higher GGT levels are experiencing systemic inflammation and potentially pain.

Keywords: acute phase proteins; facial eczema; gamma-glutamyl transferase; inflammation; pain

Introduction

Facial eczema (FE) or pithomycotoxicosis is a disease caused by the ingestion of fungal spores produced by *Pithomyces chartarum* which contain the toxin sporidesmin. Sporidesmin causes occlusion of the bile ducts and liver damage (Smith & Towers 2002) which can then lead to photosensitisation (Clare 1944) in sheep, cattle, deer and goats (Di Menna et al. 2009). Facial eczema is generally not fatal but can negatively affect production and welfare of dairy cattle. One consequence of FE is a reduction in feed intake (Morris et al. 2004), however, the mechanism behind this response is not clear, it may be possible that animals are feeling pain or nausea or both and that this may result in behavioural changes. The pathology of this disease is well described in the literature (Laven et al. 2020; Munday et al. 2020; Fernández et al. 2021); however, we still know little about how animals experience having FE.

An important part of assessing welfare includes measuring affective state, which refers to how the animal is experiencing its life, e.g., free from negative affective states, such as pain or fear, or by experiencing positive affective states, such as pleasure (Fraser et al. 1997; Mellor et al. 2020). Clinical signs of FE, such as skin lesions, are likely to cause pain and discomfort, thereby resulting in negative affective states, however, this has not been well researched. Also, little is known about how cattle may perceive liver damage when clinical signs are not observed.

Facial eczema in ruminants can cause fibrosis which can lead to liver cirrhosis (Di Menna et al. 2009; Laven et al. 2020; Munday et al. 2020; Fernández et al. 2021). Hepatic fibrosis has been shown to develop in sheep after only a single exposure to sporidesmin intoxication (Munday et al. 2020). In humans, pain is commonly experienced by patients with liver cirrhosis (Rogal et al. 2013; Klinge et al. 2018), which may partially be due to systemic inflammation as pro-inflammatory biomarkers (e.g., Interleukin-6 and C-Reactive protein) have been associated with disease severity and pain in patients with cirrhosis (Rogal et al., 2015a; Costa et al. 2021). Therefore, dairy cattle may also experience pain in response to liver damage caused by FE and this could potentially be assessed by measuring pro-inflammatory biomarkers, such as acute phase proteins (APP).

It is difficult to directly assess affective states in animals, therefore, proxy measures are often used instead. Behaviour is commonly used to measure positive and negative affective states in animals, however, this can be time consuming and expensive. Alternatively, biomarkers in the blood can be used as proxy measures of stress and pain. Changes in cortisol concentrations are often used to measure pain and it has been shown to increase in response to painful husbandry procedures in cattle, such as dehorning and castration (Fisher et al. 1997; Ting et al. 2003; Sutherland et al. 2013). Moreover, APP can be used as a diagnostic tool in cattle (Eckersall 2000). The acute phase response refers to a wide range of neuroendocrinal, physiological and metabolic changes that occur in animals in response to infection, inflammation or trauma (Eckersall 2000). Part of this response involves the release of APP, such as haptoglobin, into the circulation. In cattle, APP have been shown to become elevated in response to acute painful procedures such as castration (Fisher et al. 1997; Ting et al. 2003; Ballou et al. 2013) and acute and chronic inflammatory conditions (Horadagoda et al. 1999). Therefore, in the present study cortisol and APP (serum ferroxidase, serum amyloid A and haptoglobin) were used as proxy measures of pain. The aim of this study was to evaluate the association of cortisol and APP concentrations

with indirect measures of liver damage (gamma glutamyl transferase (GGT) and bile acids concentrations) in dairy cattle selected from herds with evidence of FE.

Materials and methods

Between March and April 2019 (Southern Hemisphere autumn) seven commercial dairy farms located in the North Island of New Zealand were selected based on these herds having evidence of FE. The methodology for identifying herds for the study is described by Cuttance et al. (2021). All procedures involving animals were approved by the Ruakura Animal Ethics Committee under the New Zealand Animal Welfare Act 1999.

Herd selection

This study was part of a larger research project designed to determine both the financial cost and animal welfare implications of sub-clinical facial eczema in dairy cattle. Briefly, a notice was sent to members of the Dairy Cattle Veterinarians branch of the New Zealand Veterinary Association and Livestock Improvement Corporation (LIC) asking whether they knew of any farmers who had farms where clinical or sub-clinical FE had been identified during the current season and who would be interested in being involved in a FE study. From this identification procedure, seven herds were identified as being suitable for enrolment in our study. These herds were selected based on at least one cow in the herd having clinical FE or the presence of serum GGT activities >300 IU/L (Cuttance et al. 2021). Blood samples were taken from all cows in these seven herds and then a herd test was conducted by LIC between -12 and 15 days in relation to blood sampling (mean -0.3 days). Herd-test data included milk solids, milk volume and somatic cell counts. Blood samples were taken from 2,080 cows in total. General information about the animals was also collected and used in the analysis, including calving date, age and breed.

Blood sampling

Blood samples were collected from the coccygeal (tail) vein of cattle into 10mL vacutainers (BD Diagnosis, Auckland NZ) that contained no anticoagulant. All blood samples (except for two missing samples) were analysed by Gribbles Veterinary pathology laboratory for GGT concentrations and a sub-set of samples were further analysed for bile acid, cortisol, serum ferroxidase (SF), serum amyloid A (SAA) and haptoglobin concentrations. The sub-set of samples was selected based on GGT concentrations (a representative spread of GGT concentrations), age, breed and farm. Number of blood samples analysed for each blood parameter are summarised in Table 1 and below.

Statistical analysis

Data were analysed using Genstat, version 19 (VSN International, Hemel Hempstead, UK) using best subsets linear regression including farm as a factor and various other prediction variables. Predictor variables included in the analysis were: log cortisol, log haptoglobin, log SF, log bile acids, calving date, milk total (kg), milk solids (kg), age and farm. The relationships among GGT (log transformed) and the range of explanatory variables measured were examined using linear regression. In addition, best subsets regression was used to determine the combination of variables that best correlated with GGT. The optimal prediction for the relationship between GGT and the other variables was calculated based on Mallows Cp, a criterion for selecting among alternative subset regressions (Thompson 1978). The regression model only used data where all variables were present for an individual animal.

The number of samples per variable used in the model are: GGT (n = 2078), bile acid (n = 851), cortisol (n = 806), SF (n = 851), haptoglobin (n = 852), total milk volume (n = 1931), total milk solids (n = 1929) and somatic cell counts (n = 1792).

Serum amyloid A concentrations were not statistically analysed as all animals tested had levels below the detectable limit (5 µg/mL), except for one animal that measured 8 µg/mL.

Results

Number of blood samples analysed and mean and range concentration for each blood parameter are summarised in Table 1. Herd testing was conducted on 1,931 cows. Milk production data are summarised in Table 2.

A linear regression model was fitted to log (GGT) against a range of explanatory variables (e.g., blood parameter, cow parity, calving date). When one variable at a time was fitted to the model, there were significant (linear) positive relationships between log GGT and log bile acids ($P<0.001$, Adjusted R^2 = 37.3%; Figure 1), farm ($P<0.001$, Adjusted R^2 = 29.1%), log SF ($P<0.001$, Adjusted R^2 = 27.4%; Figure 2), milk solids ($P<0.001$, Adjusted R^2 = 19.0%), milk total ($P<0.001$, Adjusted R^2 = 14.9%), log haptoglobin ($P<0.001$, Adjusted R^2 = 1.9%) and calving date ($P=0.002$, Adjusted R^2 = 1.2%). The strongest relationship was between GGT and bile acid and the weakest was between GGT and calving date.

When two or more variables were fitted to the model, the optimal prediction (based on Mallows Cp) for the relationship between GGT (log) concentrations and the other variables involved six variables, which all contributed significantly: farm (factor), calving date, haptoglobin (log), SF (log), bile acids (log) and milk solids (kg). The adjusted R^2 for this model is 65.2% and the Mellows Cp is 12.9 with 12 df.

Discussion

In the present study, GGT was used as a proxy for liver damage caused by FE (ingestion of sporidesmin in spores of *P. chartarum*). Gamma glutamyl transferase is commonly used as an indirect measure of FE as several studies (in sheep and cattle) have shown a positive relationship between GGT concentrations and liver damage associated with sub-clinical and clinical FE (Blackshaw 1978; Towers

Table 1 Gamma glutamyl transferase (GGT), bile acid, cortisol, serum ferroxidase (SF), and haptoglobin concentrations of cows from seven dairy farms located in the North Island of New Zealand.

Farm		Age	GGT (IU/L)	Bile acids (µmol/L)	Cortisol (nmol/L)	SF (IU/L)	Haptoglobin (mg/dL)
1	Number	222	272	153	152	153	153
	Mean	4.6	1539.6	98.9	73.6	21.2	47.0
	Range	2.0 - 12.0	2.0 - 6337.0	13.2 - 784.0	8.1 - 226.0	10.4 - 37.8	28.0 - 273.2
2	Number	261	262	58	59	58	59
	Mean	4.4	763.9	135.1	64.4	23.2	40.3
	Range	2.0 - 11.0	3.0 - 6456.0	26.1 - 712.0	7.4 - 173.0	13.6 - 41.9	20.7 - 128.2
3	Number	216	216	93	94	93	93
	Mean	4.9	342.7	87.5	66.6	17.6	43.6
	Range	1.9 - 9.1	2.0 - 4704.0	8.5 - 718.0	6.6 - 156.0	5.9 - 31.2	22.9 - 127.2
4	Number	539	540	72	72	72	72
	Mean	4.6	88.8	62.8	58.3	15.8	46.2
	Range	1.8 - 14.9	2.0 - 5233.0	14.7 - 171.0	10.6 - 147.0	5.6 - 24.0	28.5 - 83.3
5	Number	226	225	118	118	118	118
	Mean	4.4	1196.0	126.1	83.7	17.5	51.0
	Range	2.0 - 10.0	2.0 - 6406.0	8.4 - 858.0	14.2 - 448.0	7.1 - 34.5	22.3 - 196.0
6	Number	398	399	296	250	296	296
	Mean	4.7	270.0	60.7	39.4	16.1	33.0
	Range	2.0 - 11.0	2.0 - 4032.0	7.4 - 662.0	5.1 - 138.0	3.6 - 37.8	3.2 - 300.7
7	Number	108	164	61	61	61	61
	Mean	5.4	1794.5	225.5	45.7	20.6	50.1
	Range	2.0 - 12.0	2.0 - 5300.0	29.0 - 699.0	9.7 - 132.0	12.8 - 33.0	0.0 - 184.7
All farms	Number	1970	2078	851	806	851	852
	Mean	4.7	856.5	113.8	61.7	18.9	44.5
	Range	1.8 - 14.9	2.0 - 6456.0	7.4 -858.0	5.1 - 448.0	3.6 - 41.9	0.0 - 300.7

Table 2 Selected herd-test data from seven dairy farms located in the North Island of New Zealand.

Farm		Age	Total milk volume (kg)*	Total milk solids (kg)	Somatic cell counts (kcells/mL)
1	Number	222	222	221	222
	Mean	4.6	5.0	0.5	231.5
	Range	2.0 - 12.0	0.2 - 12.1	0.0 - 1.3	0.0 - 2232.0
2	Number	261	259	259	132
	Mean	4.4	6.0	0.6	152.6
	Range	2.0 - 11.0	0.9 - 13.4	0.1 - 1.4	1.0 - 1524.0
3	Number	216	186	186	186
	Mean	4.9	11.0	1.2	191.8
	Range	1.9 - 9.1	4.9 - 20.7	0.6 - 2.3	0.0 - 9999.0
4	Number	539	536	536	535
	Mean	4.6	9.5	1.0	209.6
	Range	1.8 - 14.9	3.1 - 16.6	0.3 - 1.7	12.0 - 15182.0
5	Number	226	223	223	212
	Mean	4.4	9.3	1.0	224.0
	Range	2.0 - 10.0	0.4 - 17.6	0.1 - 1.8	11.0 - 7352.0
6	Number	398	398	397	398
	Mean	4.7	7.8	1.0	137.6
	Range	2.0 - 11.0	2.5 - 15.9	0.4 - 1.6	0.0 - 3440.0
7	Number	108	107	107	107
	Mean	5.4	7.3	0.6	228.0
	Range	2.0 - 12.0	1.3 - 12.4	0.1 - 1.1	0.0 - 1791.0
All farms	Number	1970	1931	1929	1792
	Mean	4.7	8.0	0.8	196.4
	Range	1.8 - 12.0	0.2 - 20.7	0.0 - 2.3	0.0 - 15182.0

*Some of these values are low as some of the animals were being milked once a day.

Figure 1 Linear relationships between log gamma-glutamyl transferase (GGT) and log bile acids concentrations in cattle (n = 851) on seven dairy farms in the North Island of New Zealand between March and April 2019. Different-coloured dots signify different farms

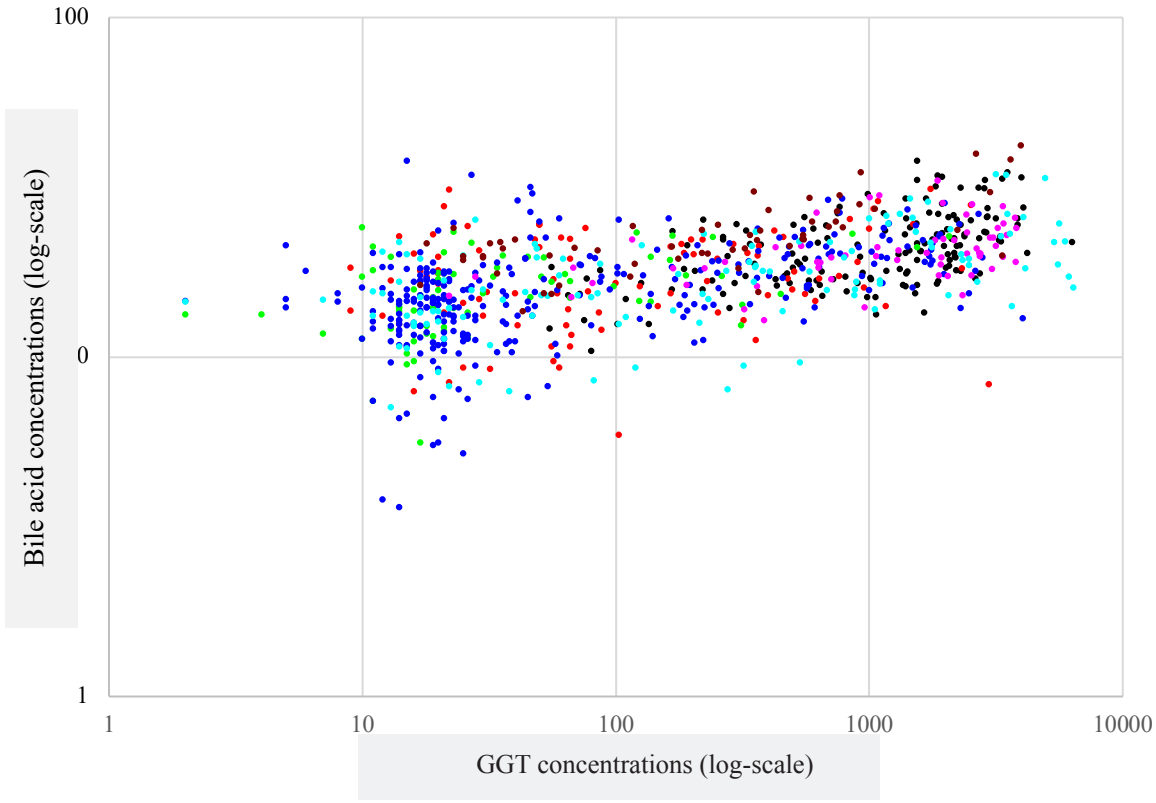
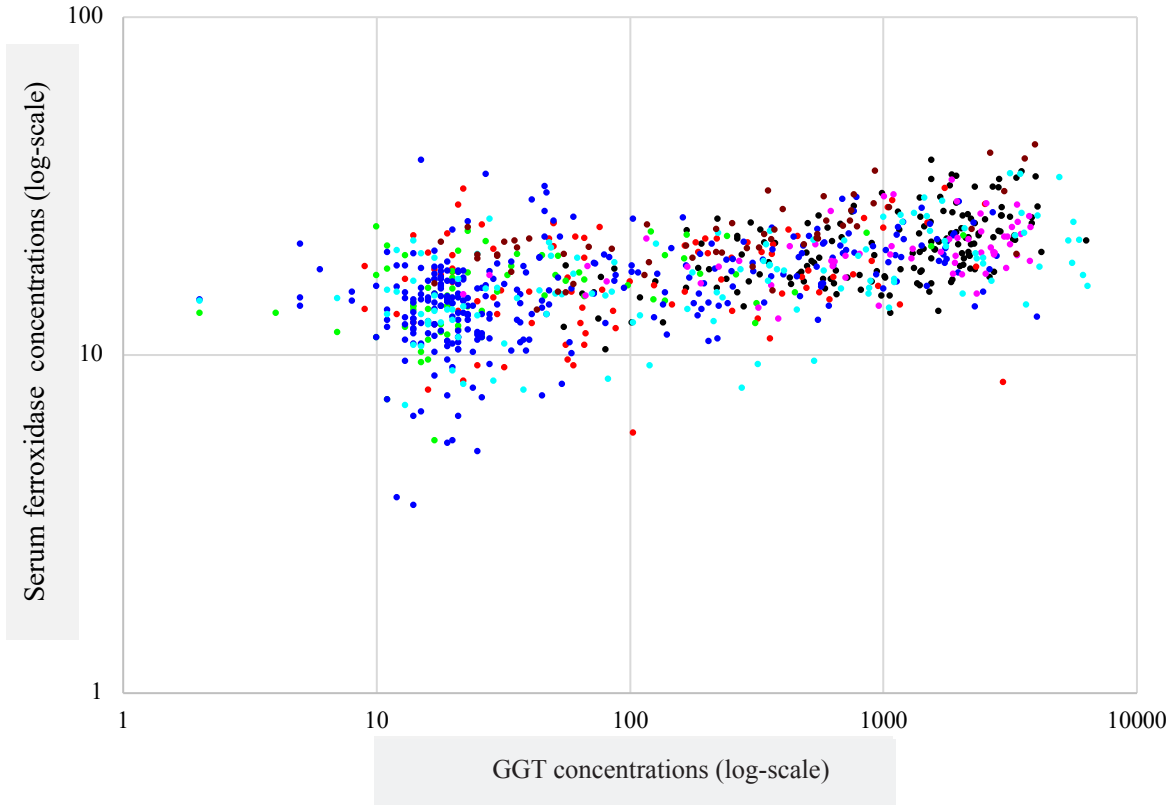


Figure 2 Linear relationships between log gamma-glutamyl transferase (GGT) and log serum ferroxidase concentrations in cattle (n = 851) on seven dairy farms in the North Island of New Zealand between March and April 2019. Different-coloured dots signify different farms.



& Smith 1978; Towers & Stratton 1978). However, elevated GGT concentrations in the autumn could also have been caused by ingestion of *Brassica spp*., muscle damage, ragwort toxicity, fasciolosis, lipidosis or kidney damage (Blackshaw 1978; Cuttance et al. 2021). Cuttance et al. (2021) considered these possible alternative causes of liver damage in relation to the farms and animals enrolled in their study (and ours); given the time of year, region, lack of *Brassica spp*. being fed and low levels of liver fluke exposure they concluded that the liver damage (elevated GGT concentrations) seen in these herds was most likely due to ingestion of sporidesmin. Histopathology of the liver would be required for definitive diagnosis of FE, but this type of sample collection is invasive and is not practical on a large sample size.

Our results suggest that there is a positive relationship between GGT concentrations and the acute phase proteins haptoglobin and SF; however, SAA concentrations were below detectable levels for the majority of animals tested. The acute phase response is a systemic response to infection, inflammation or trauma and results in the increased production of acute phase proteins by the liver (Eckersall 2000). Haptoglobin, SAA and SF (also called ceruloplasmin) are APP that have been used in bovine medicine diagnostics. Haptoglobin and SAA have been shown to be elevated in response to a range of inflammatory conditions in cattle (Horadagoda et al. 1999) and ceruloplasmin concentrations have been shown to increase in response to conditions such as mastitis (Conner et al. 1986) and endometriosis (Kaya et al. 2016). Moreover, haptoglobin has been shown to increase in response to painful husbandry procedures, such as castration (e.g., Fisher et al. 1997; Ting et al. 2003; Ballou et al. 2013). Interestingly, providing animals with pain relief (a local anaesthetic and analgesic) prior to castration prevented the increase in haptoglobin concentrations that were observed in animals not provided with pain relief (Ballou et al. 2013). Therefore, it appears as though APP concentrations can increase in response to pain as well as disease in cattle, suggesting that animals with higher GGT levels may be experiencing systemic inflammation and potentially pain. It is unclear, why SAA was not elevated in response to FE. This could possibly be explained due to the short half-life of SAA and the timing of the initial liver damage in relation to when animals were sampled. Therefore, more research is needed to understand the relationship between FE and the APP response in cattle, including the relationship between the degree of liver damage and the APP response.

In humans, pain is commonly experienced by patients with liver disease (Rogal et al. 2013; Klinge et al. 2018) and pain has been reported in up to 80% of patients with cirrhosis caused by hepatitis C virus infection, non-alcoholic steatohepatitis or alcohol (Rogal et al. 2015a). Abdominal pain is reported by the majority of patients with liver disease (Klinge et al. 2018), however, a large proportion of patients also report widespread pain (Rogal et al. 2015a,b). Several factors are likely related to abdominal pain in patients with cirrhosis including ascites, hepatic capsular distension, and splenomegaly (Rogal et al. 2015a; Klinge et al. 2018). However, widespread pain may be due to systemic inflammation, as cirrhosis is a proinflammatory state and an increase in pro-inflammatory cytokines (e.g., IL-6: Interleukin-6) and acute phase proteins (e.g., CRP: C-Reactive Protein) have been associated with liver damage in humans (Rogal et al. 2015a). Moreover, IL-6 and CRP have been associated with the presence of abdominal pain and the severity of pain related to cirrhosis (Rogal et al. 2015a). These findings have led Rogal et al. (2015a) to suggest that increases in systemic inflammation may contribute to the prevalence of pain in patients with cirrhosis. Like CRP, Haptoglobin, SAA and SF are produced by the liver in response to pro-inflammatory cytokines (Eckersall 2000). Therefore, if increased IL-6 and CRP are a predictor of pain experienced by people with liver damage due to cirrhosis, then Haptoglobin and SF could potentially be a predictor of pain experienced by dairy cattle due to liver damage. Though, some histological features are similar between sporidesmin-induced liver damage in ruminants (e.g. sheep) and cirrhosis in humans, there are significant differences in the pathogenesis and clinical effects of these diseases (Munday et al. 2020). Therefore, more research is needed to investigate whether liver damage associated with FE causes pain in dairy cattle. If liver damage caused by FE results in animals experiencing chronic pain, then this has greater welfare implications for the disease than currently considered.

Cortisol concentration ranged from 5.1 to 448 nmol/L (mean 60 nmol/L) across the seven farms used in the present study; however, there was no relationship between cortisol and GGT concentrations. Cortisol concentrations are elevated in response to acute stress and pain in cattle (Fisher et al. 1997; Ting et al. 2003; Sutherland et al. 2013) and were elevated after the onset of photosensitisation in sporidesmin-dosed sheep (Smith & Payne 1991). Therefore, we predicted that any discomfort/pain caused by FE may result in elevated cortisol concentrations. However, it is difficult to interpret the stress response of an animal from a discrete sample as many factors can influence an animal's cortisol response, including handling, physical activity and the time of day. Therefore, the very large range in cortisol concentrations within and across farms in the present study could be due to multiple factors and may have hidden any potential effect of FE on the cortisol response in these animals. Alternatively, cortisol (measured in serum) may be a more sensitive measure of acute stress (e.g., castration, photosensitisation), but a less sensitive indicator of chronic stress caused by disease, such as liver damage. In sheep, cortisol concentrations in sporidesmin-dosed hoggets tended to increase more when photosensitisation developed rather than the onset of liver injury (Smith & Payne 1991). In the present study, cattle showing clinical signs of FE were not identified, so it is not possible to establish from this research if cattle with high cortisol concentrations had clinical FE.

In the present study there was a positive relationship between bile acid and GGT concentrations. In sheep dosed with sporidesmin, bile acid concentrations rose to very high levels during the biliary obstruction phase of the disease (Peters & Mortimer 1970). A positive correlation between bile acids and GGT activity in cattle has also been found, which the authors concluded could be considered a 'downstream' consequence of sporidesmin induced liver injury (Morris et al., 2004). Therefore, the positive relationship between GGT and bile acid concentrations in the present study further supports that GGT was associated with liver damage.

To increase our understanding of the impact of FE on the welfare of cattle, future studies should evaluate biomarkers of pain and disease obtained simultaneously, include other biomarkers of pain (e.g., substance-P) and increase the number of animals sampled. Moreover, it would be advantageous to confirm that animals with elevated GGT concentrations have FE and the degree of liver damage, however, currently the methodology to confirm liver damage in cattle is very invasive and not practical on a large sample size.

In summary, few studies evaluating the effects of FE on animals have focused on assessing the welfare of these animals beyond their health status. Further research focused on assessing affective state in animals with FE would help our understanding of the disease and its implications to animal welfare.

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