Photosensitivity in Cattle Grazing *Brassica* Crops

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Abstract

Fast-growing *Brassica* forage crops, comprising turnip, rape, rutabaga, and kale varieties or interspecies crosses, are important in the provision of high-quality, easily digestible animal feed in many countries. The feeding of *Brassica* is associated with a number of potential problems, including photosensitization. This photosensitivity ranges from mild to severe. This article reports data on the implicated *Brassica* cultivars, as well as clinical observations, serum chemistry findings, skin biopsy and liver biopsy histopathology, gross necropsy and histopathological observations of spontaneous cases of *Brassica* (in particular turnip) photosensitivity in dairy cattle, and treatment and prevention strategies. In cattle, *Brassica* photosensitization is associated with increased activities of γ-glutamyl transferase and glutamate dehydrogenase, and raised phytoporphyrin (phylloerythrin) concentrations in serum. Thus, it is classified as a hepatogenous, or secondary, photosensitization. Histopathological lesions in the skin and liver of affected animals and bile duct changes, distinctly different from those seen in facial eczema (sporidesmin toxicosis), are described for the first time. In contrast to the situation with many cases of chronic facial eczema, the biliary and fibrotic changes appear to regenerate and not become relentlessly progressive. The toxin(s) responsible for the hepato- and cholangiotoxicity in cattle grazing *Brassica* is unknown. On the basis of a brief review of the literature on *Brassica* secondary compounds, and work done in rats, it appears possible that toxicity may be caused by degradation products of glucosinolates, in particular the nitrile or isothiocyanate derivatives.

Keywords: *Brassica* forage, glucosinolate, photosensitization

Introduction

It is well known that yearling and adult cattle grazing *Brassica* crops occasionally develop bloat, ruminal stasis, constipation or diarrhea, acute pulmonary edema and emphysema (fog fever), goiter, hemolytic anemia, jaundice, nitrate poisoning, poor growth rates, reproductive failure, blindness, polioencephalomalacia, or enterotoxemia (Cote 1944, Nicol and Barry 1980, Forss and Barry 1983, Wikse et al. 1987). However, in an Australian survey of disease signs in dairy cattle associated with the consumption of *Brassica* forage crops, photosensitization was by far the most prevalent (Morton and Campbell 1997). Fast-growing forage crops, comprising turnip *(Brassica rapa* ssp. *rapa*; syn. *B. campestris),* rape *(B. napus* ssp. *biennis),* swede (rutabaga) *(B. napus* ssp. *napobrassica),* and kale (*B. oleracea* ssp. *acephala*) varieties and interspecies hybrids, fill an important niche in the provision of high-quality, easily digestible feed during dry months of the year in many countries worldwide. On the North Island of New Zealand, *Brassica* spp. are considered “safe” crops during late summer and autumn, when facial eczema risk is high (Nicol and Barry 1980). Daily access by dairy cattle to such crops is normally restricted according to time and/or intake per cow (such as with break feeding). Animals should be introduced gradually, starting at about 2 kg dry matter/cow per day for a few days and then increased to 5 kg dry matter/cow per day once the cows are accustomed to the crop. Other feeds, especially those that are fiber rich, such as hay, are generally offered after cows have eaten *Brassica* plants (Morton and Campbell 1997). Provided that access is well managed and the crop is of good quality, associated disease incidents are normally rare, with small numbers of cattle affected. It is likely, however, that disease problems are underreported and that there is "substantial potential for selective reporting of signs to veterinarians" (Morton and Campbell 1997).

Throughout New Zealand, from Kaitaia to Gore, sporadic outbreaks of photosensitivity in dairy cows grazing turnips or "forage *Brassica*" (= interspecies hybrids, usually rape x kale or turnip x kale) occur during summer and autumn (January to April) each year. Such outbreaks may involve 1 or 2 animals or 10% or more of the herd. Sometimes animals are only mildly affected. Cases can also be very severe, and some animals may die or need to be euthanized. Serum samples of affected animals generally show markedly raised activities of γ-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH), and sometimes abnormally high bilirubin concentrations, indicating that the photosensitivity is hepatogenous (Anonymous 2008, 2011). On the North Island, both veterinarians and farmers have difficulty distinguishing *Brassica* photosensitivity from sporidesmin toxicosis (facial eczema), and it is likely that many cases are misdiagnosed.

Turnip and forage *Brassica* crops are not the only *Brassica* plants associated with liver damage in cattle. There is a report from Southland of cows, grazing chou moellier (marrow-stem kale) (*B. oleracea* ssp. *acephala* var. *medullosa*) and swedes during spring, that developed acute hepatotoxicity, with markedly raised GGT and GDH activities, followed by recumbency and death within days (Anonymous 2009).

The photosensitization in cattle seems to differ from that seen in young sheep grazing *Brassica*. "Rape scald," the disease in sheep, appears to be a primary photosensitivity (Cunningham et al. 1942, Clare 1955, Connor 1977, Vermunt et al. 1993, Westwood and Nichol 2009). Anecdotal observations by seed merchants, farming organizations, and veterinarians of risk factors include application of nitrogenous fertilizers, overconsumption of turnips, feeding crops that are low yielding or "drought stressed" to cattle, and intake by lambs of immature rape—that is, before the leaves "ripen" to a purplish, reddish, or bronze color. (Vermunt et al. 1993, Morton and Campbell 1997, Westwood and Nichol 2009).

Despite the wealth of information on the phytochemistry of *Brassica*, the nature of the hepatotoxic agent(s) in cases of *Brassica* photosensitivity in cattle is still unknown. In addition, the liver lesions in such cases have not yet been characterized. In this article, we record information on the implicated *Brassica* cultivars, as well as clinical observations, serum chemistry findings, skin biopsy and liver biopsy histopathology, and gross necropsy and histopathological observations of natural cases of *Brassica* (in particular turnip) photosensitivity in dairy cattle. We report for the first time lesions of the bile ducts that would appear to be important in the pathogenesis of the photosensitivity and that are distinctively different than those seen in sporidesmin toxicosis. We also briefly review the existing data on *Brassica* secondary compounds and the possibility that one or more nitrile or isothiocyanate derivatives of glucosinolates, toxic to laboratory rats, may be responsible for the liver lesions in affected cattle.

Materials and Methods

The information collected and collated for clinical cases included the following: the *Brassica* cultivar involved; *Pithomyces chartarum* spore counts (Chapman and di Menna 1982) performed on the *Brassica* crop, neighboring pasture, and feces of affected animals; clinical signs in affected cattle; hematology (n=5); serum chemistry (n=121); urinalysis (n=7); skin biopsy histology (n=1); liver biopsy histology (n=12 cows with varying degrees of acute clinical photosensitivity and n=5 healthy cohorts); gross necropsy and histopathological findings (n=5); and treatment and prevention strategies over a 5-year period (January 2008 to March 2012). The most prevalent breeds implicated were Friesian and Friesian crosses. Most were black and white, but some were predominantly black with only small nonpigmented patches, such as portions of the udder and teats. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Venous blood samples were collected into Vacutainers (BD Vacutainer, Franklin Lakes, NJ, USA) that contained either K_3EDTA (glass; for hematology) or a clot activator (silicone-coated plastic; for serum separation). Full hematology was performed on selected samples. Serum was processed for GGT and GDH activities and total bilirubin concentrations using autoanalyzers located in commercial veterinary diagnostic laboratories countrywide. Phytoporphyrin (phylloerythrin) concentrations in serum were measured using the method of Campbell et al. (2010).

Following surgical preparation and infiltration of local anesthetic, two 15 x 10 mm elliptical skin biopsies were aseptically excised from a severely photosensitized cow using a scalpel. One biopsy was taken from affected, stiff and hard, nonpigmented (white), haired skin on one side of the dorsal thorax and the other from normal black skin on the other side. The biopsy wounds were closed with interrupted sutures. Routine liver core biopsies were performed under local anesthesia through the 11th intercostal space on the right side (West 1981) of 17 cows. Skin and liver biopsies were placed immediately in 10% buffered formalin for histopathology. In all of the biopsied animals, the photosensitivity lesions in the skin took some time to heal, and all cows eventually returned to clinical normality.

Necropsy examinations were performed within 1 hour of death or euthanasia (captive bolt and exsanguination). Samples for histopathology were fixed in 10% buffered formalin, processed routinely, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E).

Results

Brassica *cultivars associated with photosensitivity*

The implicated cultivar was established in 26 of 36 turnip-photosensitivity outbreaks that were investigated. Twenty of the 26 comprised the Barkant cultivar; Green Globe and White Star were each implicated in 2; and Rival, Marco, Envy, and Winfred (turnip x kale) were each associated with a single outbreak. Five of the 7 forage *Brassica* outbreaks investigated were associated with Titan (rape x kale), and 1 was the Greenland cultivar.

Pithomyces *spore counts*

No spores were found in debris collected from *Brassica* crops (n=8), nor in the feces of affected cattle (n=2). Most neighboring pastures (n=6) in North Island outbreaks had counts of 0, while one had 5000 and one 10,000 spores/g of grass. Counts of 100,000 are regarded as dangerous (Chapman and di Menna 1982).

Clinical presentation

A feature frequently noted was that animals may have had access to a *Brassica* crop for only 3 or 4 days before clinical photosensitivity manifested. In the early stages, affected cattle would seek shade, become agitated, and kick at any attempts to examine or palpate the teats or udder. In some animals, skin lesions were confined to the teats, udder, and escutcheon. The teats became red and raw and oozed serum, such that the cow could not be milked and had to be dried off. Later the skin of the teats would harden, crack, and slough. In more severe cases, however, skin lesions were more extensive, and large areas of hairless or nonpigmented skin would become stiff, leathery, and wrinkled in the early stages (figure 1), eventually cracking (figure 2) and sloughing in irregular dessicated or hairy sheets a week or more later. Occasionally, animals became conspicuously jaundiced. Subcutaneous edema would develop, especially in the lower limbs. Three acutely affected cows (Cows 1, 2, and 3) were “down,” weak, reluctant to move, and dehydrated, and they were euthanized. Cow 4 died after becoming moribund with jaundice and severe photosensitivity. Cow 5, which had been photosensitive for 9 days, had to be euthanized a day after it went “down” (sternal recumbency with its head drawn to its flank).

Hematology, clinical chemistry, and urinalysis

In the early stages of photosensitivity, all hematology parameters were within the normal ranges. About a week later, however, a leukocytosis, comprising a neutrophilia with a left shift, as well as raised fibrinogen, were evident. Red cells showed a

Figure 1. Close-up of the acutely photosensitized cow that was subjected to the skin biopsy procedures, showing normal, smooth, pigmented (black) skin; and roughened, slightly wrinkled, stiff and boardlike, nonpigmented (white) skin, closely resembling the respective biopsy sites. Serum GGT and GDH activities and phytoporphyrin concentration at the time were 813 U/L, 482 U/L, and 0.38 µM, respectively.

Table 1. Activities of the liver enzymes γ-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH), total concentration of bilirubin, and concentration of phytoporphyrin (phylloerythrin) in the serum of cattle with clinical photosensitivity associated with the consumption of turnip (*n*=101), forage *Brassica* (interspecies turnip x kale or rape x kale) (*n*=19), and swede (*n*=1) crops.

	GGT[a] (U/L at 37°C) (*n*=121)	GDH[b] (U/L at 37°C) (*n*=119)	Bilirubin[c] (µM) (*n*=109)	Phytoporphyrin[d] (µM) (*n*=69)
Mean	832	490	18	0.72
Maximum	4,018	2,281	99	3.6
Minimum	77	15	1	0.1

[a]Normal range 0-36 U/L.
[b]Normal range 8-41 U/L.
[c]Normal range 0-13 µM.
[d]Clinically normal control animals (*n*=17): range 0–0.14 (mean 0.06) µM.

Figure 2. Left thigh, groin, udder, and teat of a subacutely photosensitive cow, showing prominent wrinkles, fissures, and early peeling of deadened nonpigmented (white) skin, as well as localized alopecia and dried crusts, and a raw and scab-covered teat. Serum GGT and GDH activities at the time were 365 U/L and 36 U/L, respectively.

mild poikilocytosis, but Heinz bodies were absent. The activities of GGT and GDH and the concentrations of bilirubin and phytoporphyrin in clinically photosensitive cattle are given in table 1. Marked elevations (>500 U/L) of either GGT and/or GDH enzyme activities were frequently encountered. The serum calcium of Cows 4 and 5 that were down prior to euthanasia were 1.64 and 1.75 mmol/L (normal range 2.0 to 2.6 mmol/L), respectively. Urine samples varied from a pale yellow to orange to greenish-brown to dark brown and turbid. At the time of the liver biopsy procedure, one of the acutely affected cows had hemoglobinuria, and its serum was red-tinged. The pH of the urine samples obtained ranged between 6 and 9, and the specific gravity was between 1024 and 1032.

Skin and liver biopsy histopathology

The biopsy of black skin was normal (figure 3), apart from a few eosinophils near capillaries in the superficial dermis. The affected skin showed diffuse necrosis of the entire epidermis and superficial dermis to the depth of the apocrine sweat glands, while sebaceous glands still appeared viable (figure 4). There were thromboses in dermal vessels and sheets of fibrin in the hypodermis.

Liver biopsies from the clinically normal cohort cows were unremarkable. Three of the 12 biopsies from photosensitive animals had extensive, sometimes bridging, periportal fibrosis and bile duct hyperplasia, typical of that seen in chronic sporidesmin toxicity. Prominent peribiliary edema with loose concentric rings of fibrosis, possibly of significance with respect to the history of *Brassica* consumption and photosensitivity, were also noted in these cases. This characteristic peribiliary lesion (figure 5) was present in a cow with acute turnip photosensitivity that had recovered from turnip photosensitivity a year previously. Lesions in the remaining affected cows varied from mild hepatocellular swelling, mild portal fibrosis, and mild bile duct hyperplasia, with occasional hepatocytes containing fatty vacuoles, to more pronounced cellular swelling and anisokaryosis with clumps of hepatocytes showing fatty change, as well as moderate peribiliary edema, associated concentric fibrosis, and bile duct hyperplasia. A striking feature in many livers comprised circumscribed areas of increased eosinophilia of periportal hepatocytes, with smaller, more darkly stained nuclei. These were in stark contrast to adjoining hepatocytes that had paler, hydropic cytoplasm nd vesicular nuclei (figure 6). In some cases, darker staining hepatocytes were intermingled with ”normal” ones. Occasional small inflammatory foci were seen in the parenchyma in some biopsies.

Gross necropsy findings

Affected leathery skin was hard and boardlike when cut. Corresponding subcutaneous tissues, particularly of the ventrum and distal limbs, were frequently bright yellow and contained excessive watery fluid (figure 7). Areas in the groin and medial thigh were hyperemic and covered with hard, dried crusts in places. The subcutaneous tissue of the brisket was often thickened and doughy due to edema (figure 8). The muzzle and nostril skin were often reddened and eroded, and the ventral midline of the tongue was fissured, brownish, and hardened. Mucous membranes varied from normal to pale yellow.

The livers of 4 of the 5 cows that were necropsied were diffusely enlarged, pale brown to bronze, and the edges of the lobes were rounded (figure 9). In Cow 4, the ventral (left) lobe was about a third smaller and much firmer than normal. Gallbladders contained normal dark-green bile. In the liver of Cow 2, dozens of irregularly shaped, often coalescing, dark reddish-brown 10- to 20-mm foci were present on the diaphragmatic surface near the caudal vena cava. In all 5 cows, the rumen wall and contents, comprising grass and turnip leaf digesta, looked normal. In Cow 4, the omasal laminae had scattered red flecks (congested capillaries). In Cow 5, the omasum appeared very large, and most of the laminae had small (10-mm diameter), sometimes coalescing to larger (50-mm diameter), irregular brown infarcts surrounded by pink-rim reaction zones. The abomasum of this cow

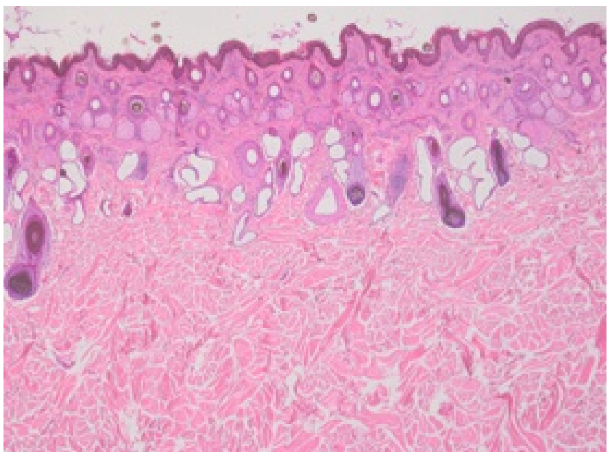

Figure 3. Photomicrograph of a biopsy of normal pigmented (black) skin from the cow in figure 1. H&E 4X.

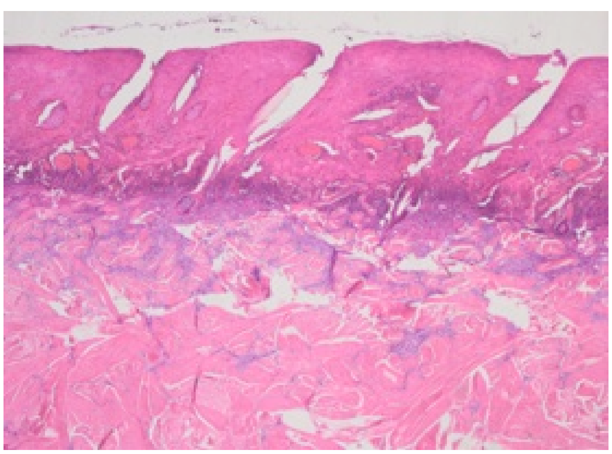

Figure 4. Photomicrograph of a biopsy of affected nonpigmented (white) skin from the cow in figure 1, showing diffuse necrosis of the epidermis and superficial dermis to the depth of the apocrine sweat glands and hair follicle bulbs, while sebaceous glands are still recognizable. Involved blood vessels were thrombosed and necrotic. Serum GGT and GDH activities and phytoporphyrin concentration at the time were 813 U/L, 482 U/L, and 0.38 µM, respectively. H&E 4X.

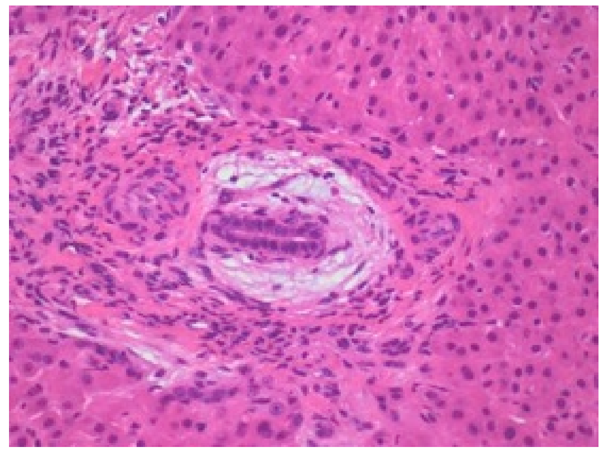

Figure 5. Photomicrograph of a liver biopsy from an acutely photosensitized cow, showing characteristic peribiliary oedema and mild loose concentric fibrosis. This cow had recovered from turnip photosensitivity a year prior. The serum GGT and GDH activities at the time were 867 U/L and 515 U/L, respectively. H&E 40X.

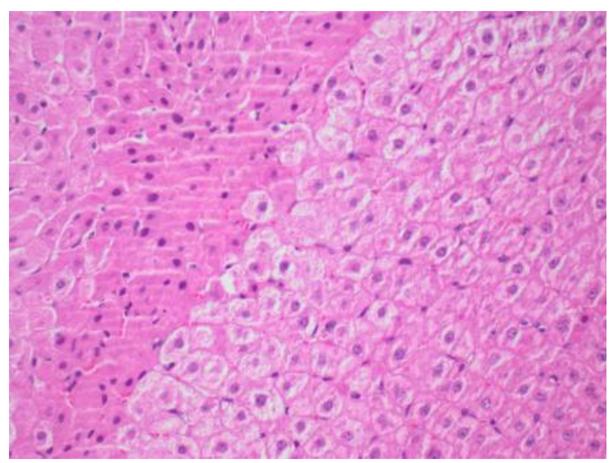

Figure 6. Photomicrograph of a liver biopsy from an acutely photosensitized cow, showing marked delineation in staining intensity between more eosinophilic hepatocytes with small, dark nuclei (top left) and cells with more vesicular cytoplasm and normal nuclei (right and bottom). Serum GGT and GDH activities and phytoporphyrin concentration at the time were 429 U/L, 465 U/L, and 0.64 µM, respectively. H&E 40X.

Figure 7. Longitudinal skin incision of a foreleg of Cow 2 showing prominent jaundice and excessive subcutaneous fluid. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 277 U/L, 1260 U/L, 30 µM, and 0.39 µM, respectively.

Figure 8. Close-up of incised brisket of Cow 2 showing markedly thickened, yellow, subcutaneous edema. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 277 U/L, 1260 U/L, 30 µM, and 0.39 µM, respectively.

had 3 small (10-mm diameter) discrete ulcers that did not appear to have bled. In Cow 2, the abomasum had numerous deep, hemorrhagic, linear (up to 80 mm long) and punctate (10-mm diameter) ulcers, and it contained malodorous dark-red/brown watery fluid admixed with gravel and small stones. The colonic contents of this cow were dark and tarry.

Histopathology

Affected skin revealed full-thickness coagulative necrosis of the epidermis and superficial dermis with occasional hair follicles and sebaceous glands still

Figure 9. Diffusely enlarged and pale-brown liver from Cow 2. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 277 U/L, 1260 U/L, 30 µM, and 0.39 µM, respectively.

identifiable. The epidermis and superficial dermis had a “cooked” appearance, like that of a severe thermal burn. Beneath the necrosis and caught up in it was a thick band of inflammatory cells with occasional thromboses (capillaries mainly, but sometimes also veins and/or arteries) and fibrinoid necrosis of vascular walls. Some dermal blood vessels were surrounded by inflammatory cells comprising macrophages, lymphocytes, and occasional neutrophils and eosinophils. Masses of fibrin were embedded in the deep dermis and between collagen bundles, and fibrin thrombi were visible in some lymphatics. The latter were severely dilated and were sometimes also associated with foci of inflammation. On the edges of the lesions, fibrinocellular crusts overlay the epidermis. The skin of the muzzle, nostrils, and eyelids was similarly affected, but worse in places with extensive ulceration and bacterial colonization of the exposed surface. The dermis and hypodermis of the udder also had moderate numbers of scattered neutrophils. Nonpigmented teats showed necrosis of dermal and epidermal papillae and capillary thrombosis. Even pigmented teat skin showed necrosis of dermal papillae.

In a portion of nonpigmented skin from Cow 4, there were a number of suprabasilar clefts (separation between the stratum basale and the stratum spinosum, resulting in intraepidermal vesicles) that contained pinkish-grey fluid and loose acanthocytes as singles or rafts (figure 10). The deep subcutaneous tissues were markedly edematous, with prominent fibrin exudation (even where the overlying epidermis was pigmented). In a pigmented (black) ear, the dermis of the dorsal surface had

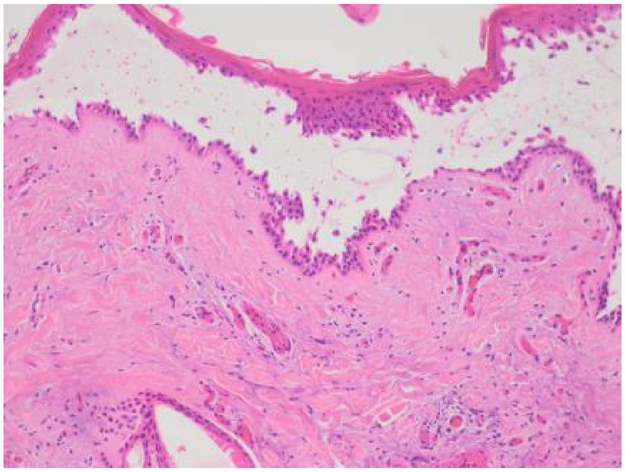

Figure 10. Photomicrograph of a section of nonpigmented skin from Cow 4 showing a suprabasilar intraepidermal cleft containing free-floating acanthocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 636 U/L, 720 U/L, 45 µM, and 1.61 µM, respectively. H&E 20X.

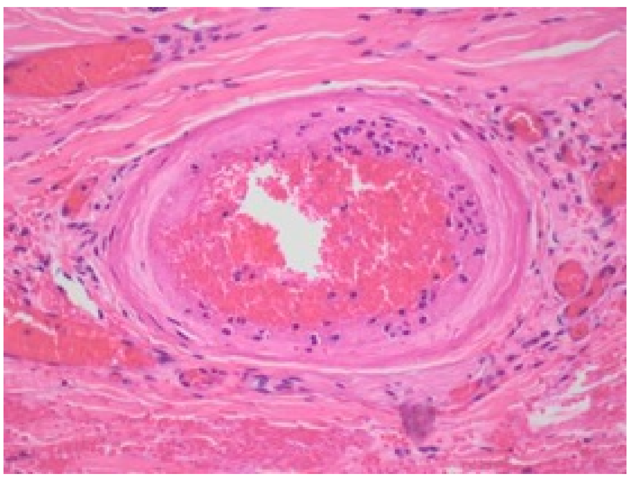

Figure 11. Photomicrograph of the dorsal dermis of a pigmented (black) ear of Cow 4 showing fibrinoid necrosis of the wall of a medium-sized blood vessel. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 636 U/L, 720 U/L, 45 µM, and 1.61 µM, respectively. H&E 40X.

some vessels with fibrinoid necrosis (figure 11), scattered thrombosed capillaries, and extensive hemorrhage adjacent to the cartilage.

The ventral midline of the tip of the tongue showed localized parakeratotic hyperkeratosis, with associated necrosis of the mucosal epithelium overlying prominent papilliform proprial capillaries and the infiltration of neutrophils.

In Cow 4, with the grossly smaller ventral liver lobe, lesions consistent with chronic sporidesmin exposure (i.e., severe bile ductule hyperplasia and periportal to bridging fibrosis) (figure 12) were present. In Cows 1, 2, and 3, liver lesions were far

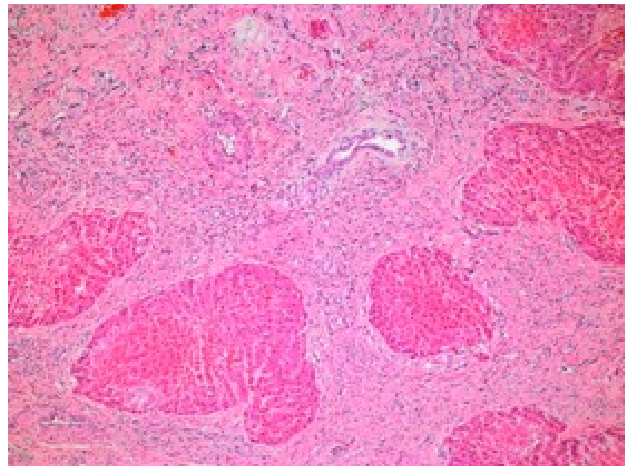

Figure 12. Photomicrograph of the liver of Cow 4 showing severe periportal to bridging fibrosis and bile ductule hyperplasia typical of that seen in residual chronic sporidesmin toxicity (facial eczema). Note the apparently functional bile duct near top center that has peribiliary edema. Many of the remaining islands of hepatic parenchyma show areas of necrosis. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 636 U/L, 720 U/L, 45 µM, and 1.61 µM, respectively. H&E 10X.

more subtle, despite high GGT and GDH activities in serum collected shortly before euthanasia. Mild bile ductule proliferation and mild periductular edema and fibrosis, with the latter having a loose, concentric arrangement resembling the rings of an onion, as seen in the liver biopsies described above, were consistent features. Occasional epithelial cells within bile ducts had pycnotic nuclei, and a few mononuclear cells were sometimes visible within the periductular connective tissue. Overall, hepatocytes appeared diffusely swollen, with occasional binucleate cells and a variation in nuclear size. There was frequently a marked variation in staining intensity between groups of hepatocytes and neighboring cells. Sheets of hepatocytes showed hydropic to multilocular fatty change that progressed to foci of lytic necrosis in some instances (figure 13). Adjacent cells often had smaller, more darkly stained nuclei and increased cytoplasmic eosinophilia. In the latter parts, scattered mitotic figures were sometimes seen. The grossly visible discrete foci on the diaphragmatic surface of the liver of Cow 2 comprised coagulative necrosis accompanied by hemorrhage that appeared to have a centrilobular distribution (possibly hypoxic necrosis). These foci of coagulative necrosis were more severe in the dorsal lobe, while some of those in the caudate lobe were obscured by inflammatory cells. In the liver of Cow 5, which was euthanized in extremis 9 days after the start of clinical photosensitivity, hepatocytes varied in staining intensity (described above) and appeared dissociated (figure 14). Conspicuous bile duct lesions were present in this animal. They were characterized by dilation of some that were lined by attenuated epithelium and surrounded by concentric rings of fibrosis (figure 15), lysis of small ducts (figure 16), evidence of asymmetrical epithelial regeneration in other small ducts (figures 17 and 18), suppurative inflammation (figure 19), and obliteration of other ducts by fibrotic scars (figure 20).

Rare inflammatory foci that appeared to contain bile and that were adjacent to portal triads (most likely biliary infarcts) were present. Occasional bile plugs were evident in canaliculi. Beneath the mucosa of the cystic duct, larger veins were sometimes thrombosed, and adjacent arterioles showed fibrinoid necrosis of their walls. The gallbladder wall often had submucosal edema and occasional thrombi within capillaries.

In the ulcerated abomasums (Cows 2 and 5) and in the infarcted omasal laminae (Cow 5), the lesions were accompanied by proprial and submucosal thrombosis and vasculitis, occasional fungal hyphae in affected blood vessels, as well as coagulative necrosis and associated inflammation. The rumens of all 5 cows were normal.

Macrophages containing hemosiderin were prominent in the red pulp of the spleen and in the lamina propria of the small intestine. In the kidneys of Cow 2, some tubules in the medulla contained hemoglobin casts, while Cows 2 and 3 had hemosiderin granules within tubular epithelium. The urinary bladder, pancreas, and brain were normal. The zona fascicularis of the adrenal glands of Cow 5 were hyperplastic.

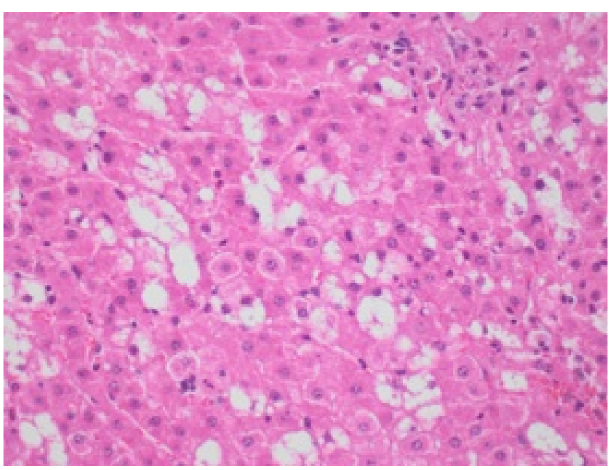

Figure 13. Photomicrograph of a liver biopsy from an acutely photosensitized cow showing swollen hepatocytes with individual cells and groups containing fatty vacuoles. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentration at the time were 537 U/L, 381 U/L, 18 µM, and 0.61 µM, respectively. H&E 40X.

Treatment and prevention

Provision of adequate shade, injection of analgesic and anti-inflammatory drugs, and application of zinc-containing ointments and balms to severely affected skin and teats were the treatments most commonly insituted.

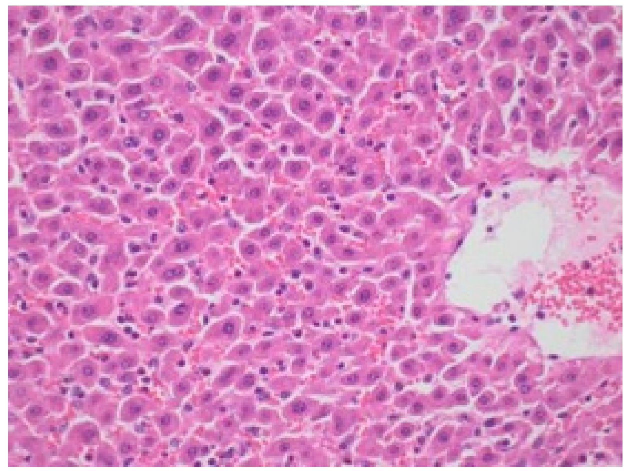

Figure 14. Photomicrograph of the liver from Cow 5 showing dissociation of hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

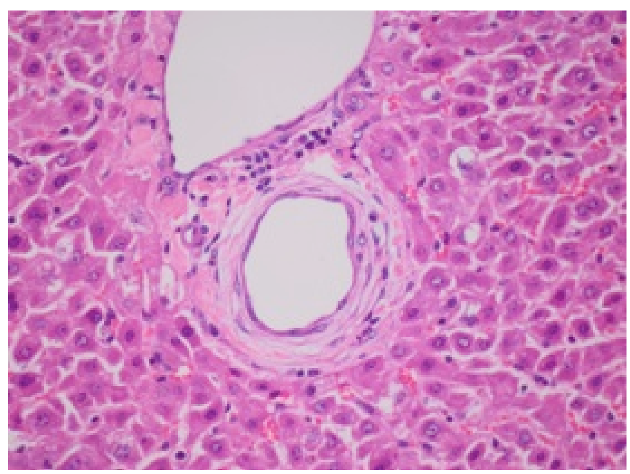

Figure 15. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. Note the bile duct in the center that is lined by attenuated squamous epithelium and surrounded by concentric rings of fibrosis. Note also the randomly scattered darker hepatocytes with smaller dark nuclei, similar to those in the liver biopsy in figure 6. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

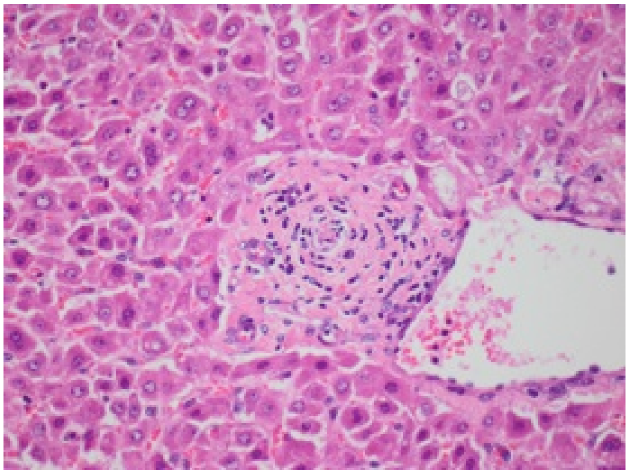

Figure 16. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a small bile duct is necrotic and surrounded by mild concentric fibrosis and some inflammatory cells. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

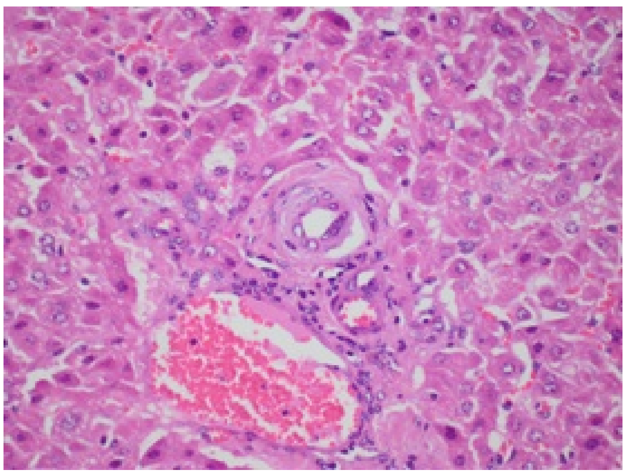

Figure 17. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, the epithelium of a small bile duct shows asymmetrical epithelial regeneration. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

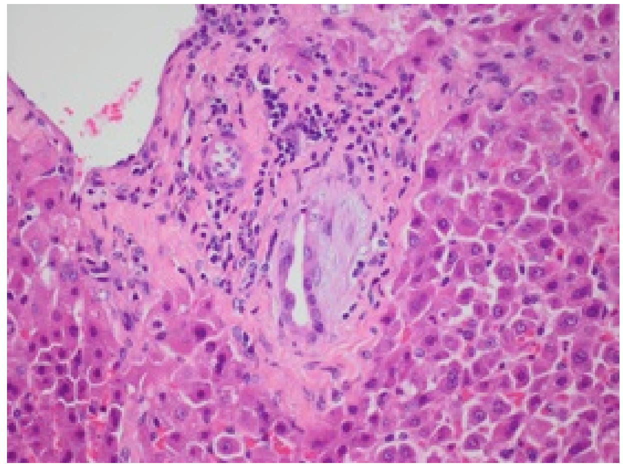

Figure 18. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a small bile duct is regenerating adjacent to a scar (on the right) that appears to be an obliterated bile duct. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

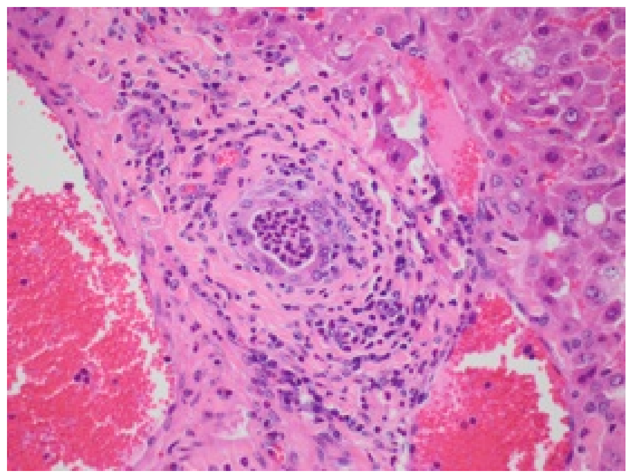

Figure 19. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a bile duct lined by regenerating epithelium contains a plug of neutrophils. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

Discussion

The microscopic appearance of the skin lesions in *Brassica* photosensitivity (as depicted in figure 4), as in many other forms of photosensitivity, resemble partial-thickness (second-degree) and/or full-thickness (third-degree) thermal burns (Rubin and Farber 1994). Sebaceous gland and other adnexal epithelial cells presumably contribute to the reepithelialization and healing of such lesions

Contrary to expectations, the dermal vasculature in pigmented skin can also manifest fibrinoid necrosis, thrombosis, and/or hemorrhage. The unusual intraepidermal vesicles (figure 10) seen in Cow 4 resemble those seen in pemphigus vulgaris, a rare autoimmune disease of the mucosae, mucocutaneous junction, and skin, reported in dogs, cats, and some other species, but not cattle (Ginn et al. 2007). In this disease, autoantibodies react with cell-adhesion desmosomal proteins in the basal layer of squamous epithelium in mucosae and skin (Ginn et al. 2007).

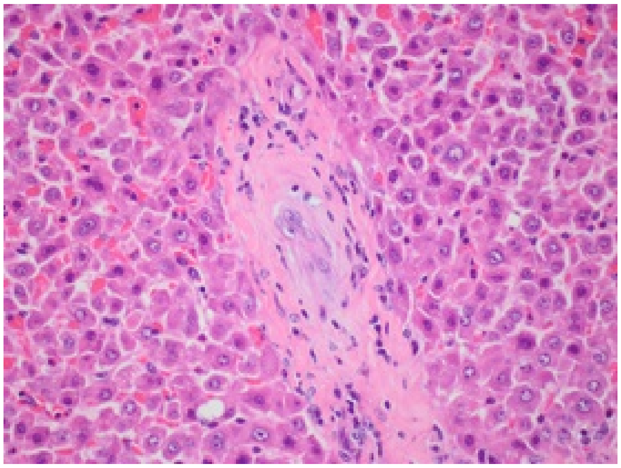

Figure 20. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, the bile duct has been obliterated by scar tissue in which residual, possibly regenerating, epithelial cell nuclei appear embedded. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

Although not examined histologically, the mucous membranes and mucocutaneous junctions of this animal appeared grossly normal. To our knowledge, pemphigus vulgaris has not been reported in cattle.

The clinical biochemistry findings in *Brassica* photosensitivity in cattle closely resemble those seen in facial eczema. The two diseases often occur on the North Island at about the same time, adding to the conundrum that farmers and their veterinarians face when it comes to prophylaxis and treatment. The absence of spores in *Brassica* leaf litter associated with 8 outbreaks of photosensitivity concurs with previous observations (Thornton and Sinclair 1960).

In acute cases of facial eczema, lesions of the medium-size and larger bile ducts are often conspicuous (Cunningham et al. 1942). The biliary epithelium in recovered animals seems to have a strong hyperplastic tendency. Bile ductule proliferation and associated peribiliary fibrosis are prominent lesions in animals previously exposed to sporidesmin a year or more prior. In some animals, the bile ductule hyperplasia and fibrosis seem to be relentlessly progressive, leading to eventual biliary obstruction and failure of excretion of phytoporphyrin, such that cattle (or sheep) can become photosensitive at any time of the year.

When examining liver biopsies from clinical cases of *Brassica* photosensitivity in cattle, the bile duct and parenchymal lesions are frequently mild, and their subtlety makes it difficult to explain the marked elevations of GGT and GDH activities and occasional jaundice. GGT is an inducible enzyme in biliary epithelium, so there is generally a lag period of 10 to 14 days before the enzyme activity rises (Smith and Gravett 1986). Since it is not possible to reproduce clinical cases of *Brassica* photosensitivity at the present time, it is not known how long it takes for GGT activities to rise. However, on the basis that clinical photosensitivity often manifests itself just a few days after animals are introduced to *Brassica,* it is speculated that GGT is more rapidly induced than it is in the case of facial eczema. On the other hand, the rise in GDH activity would reflect mitochondrial damage and hepatocellular leakage (necrosis).

A possible explanation for the failure to find significant lesions that could satisfactorily explain the clinical chemistry in liver biopsies could be the fact that percutaneous biopsies are only accessible in the dorsolateral liver. It is well known that liver lesions in facial eczema are often more prominent in the ventral (left) lobe (Cunningham et al. 1942) and that liver biopsies from the dorsal lobe can be misrepresentative. Perhaps the same is true for *Brassica* hepatotoxicity. The finding of widespread but subtle bile duct lesions in Cow 5, which was euthanized and necropsied 9 days after the start of clinical photosensitivity, could provide evidence of *Brassica*-induced biliary epithelial damage. Such conspicuous bile duct lesions were not seen to the same degree in any of the other cases, possibly because the latter were examined in the more acute phase. Another feature is that bile duct lesions in *Brassica* hepatotoxicity conceivably heal and regress and do not lead to a progressive obliterative cholangitis as seen in chronic cases of facial eczema. This is borne out by the findings in the liver biopsies of the same cow that developed *Brassica* photosensitivity in 2 successive seasons.

So, in light of the above, what toxin(s) in *Brassica* could possibly cause both the biliary and hepatocellular damage? Because of the generally beneficial role that consumption of *Brassica* spp., such as broccoli *(B. oleracea* ssp. *italica),* has in cancer prevention in humans, considerable literature exists on the chemistry of their principal secondary compounds, the sulfur-containing glucosinolates. Glucosinolates, and their breakdown products, contribute to the characteristic odors and flavors of the respective *Brassica*. To date, more than 100 glucosinolate compounds have been identified, and one or more of these compounds characteristically occur in profiles that are distinct for the various species, interspecies hybrids, and cultivars (Fahey et al. 2001). Glucosinolates are found throughout the plant (seeds, roots, stems, leaves, and flowers). The highest concentration, 30 to 110 times more than in the vegetative portions, is in the seeds (Tookey et al. 1980, Carlson et al. 1987, Zukalová et al. 2002, Cartea and Velasco 2008). The same glucosinolates are found in the phylloplane, or leaf surface (Griffiths et al. 2001). Genetic factors, developmental stage of the plant, part of the plant, environmental temperature, season, plant health (including plant-fungal and plant-insect interactions), and sulfur and nitrogen fertilization all affect the concentrations of both individual and total glucosinolates in *Brassica* (Carlson et al. 1981, Forss and Barry 1983, Zukalová et al. 2002, Cartea and Velasco 2008, Westwood and Nichol 2009). Dry weather conditions lead to increased concentrations of glucosinolates (Barry 2013).

Turnips, rape, and swedes contain aliphatic (glucoiberin, progoitrin/*epi*-progoitrin, glucoraphanin, sinigrin, gluconapin, glucobrassicanapin, gluconapoleiferin), indole (4-hydroxyglucobrassicin, glucobrassicin, neoglucobrassicin), and aromatic (gluconasturtiin) glucosinolates (Carlson et al. 1981, 1987, McDanell et al. 1988, Matthäus and Luftmann 2000, Griffiths et al. 2001, Kim et al. 2001, Zukalová et al. 2002, Padilla et al. 2007, Cartea and Velasco 2008). Progoitrin/*epi*-progoitrin, gluconapin, glucobrassicanapin, glucobrassicin, and gluconasturtiin are the glucosinolates that generally occur in the highest concentrations, although ratios and concentrations can vary considerably, and a number of other glucosinolates are usually also present (Carlson et al. 1981, 1987, Matthäus and Luftmann 2000, Kim et al. 2001, Zukalová et al. 2002, Padilla et al. 2007, Barry 2013). In New Zealand, progoitrin is the dominant glucosinolate in turnips, forage rape, and swedes, while the highest total values for glucosinolates occur in turnips and forage rape, followed by swedes, then kale (Barry 2013). Glucosinolates are inactive anionic compounds that occur as potassium salts in intact plant cells and that are accompanied by the endogenous enzyme myrosinase (= thioglucoside glucohydrolase) that enables hydrolysis when the raw, wet, unheated plant cells are ruptured during mastication (Tookey et al. 1980). Hydrolysis releases glucose and the acid sulfate ion from the unstable aglycone thiohydroxamate-*O*-sulfonate, which contains a variable amino acid-derived side chain. Intramolecular spontaneous and nonenzymatic “Lossen”-type rearrangement of the aglycone may form isothiocyanates, thiocyanates, oxazolidinethiones, nitriles, elemental sulfur, epithionitriles, alkanes, or indolyl compounds, depending on pH, availability of ferrous ions, and the activities of myrosinase, epithiospecifier protein, epithiospecifier modifier protein, and nitrile-specifier protein when plant cells are injured (Daxenbichler et al. 1964, Paik et al. 1980, Tookey et al. 1980, Cartea and Velasco 2008, Hayes et al. 2008, Kissen and Bones 2009). Isothiocyanates are regarded as the most active compounds but are often volatile, highly reactive, and unstable; nitriles, on the other hand, are less reactive but more stable (Bellostas et al. 2008). All of the derivatives are potentially toxic (Tookey et al. 1980).

At pH 6 to 7, the predominant isothiocyanate metabolites of glucosinolates in turnips and rape are 2-hydroxy-3-butenyl (from progoitrin), 3-butenyl (from gluconapin), 4-pentenyl (from glucobrassicanapin), and 2-phenylethyl (from gluconasturtiin) (Cole 1976, Carlson et al. 1987, Kim et al. 2004, Cartea and Velasco 2008). The concentration of the acclaimed human-health-beneficial sulforaphane (4-methylsulfinylbutyl isothiocyanate), derived from the glucosinolate glucoraphanin in broccoli, is low to absent in turnips and rape (Carlson et al. 1987, Song et al. 2006, Cartea and Velasco 2008). Isothiocyanates are readily absorbed from the intestine and are conjugated to glutathione within hepatocytes (Zhang 2000); corresponding mercapturic acids are secreted in urine, and these can be used as biomarkers of *Brassica* consumption (Vermeulen et al. 2003).

Isothiocyanates, both naturally occurring and synthetic, have received a lot of research attention in laboratory animals. One of the most intensively studied, 2-phenylethyl isothiocyanate from gluconasturtiin, shows no measurable hepatotoxicity in rats (Gray et al. 1995). A synthetic isothiocyanate that has been extensively studied in rats, mice, and guinea pigs, and which causes massive hyperplasia of small bile ducts that is reversible on cessation of dosing, is α-naphthyl isothiocyanate (ANIT) (Lopez and Mazzanti 1955, Steiner and Carruthers 1963). An interesting feature of ANIT toxicity in rats is that serum GGT activities have been shown to increase dramatically 1 to 2 days after a single oral dose of 20 mg/100 g body weight (Bulle et al. 1990). Other laboratory animal species such as hamsters, rabbits, and dogs are less sensitive to the effects of ANIT (Amin et al. 2006). Administration of ANIT to sheep and calves as single or multiple daily doses, at a much greater magnitude than those given to rodents, caused a marked hepatocellular response – swelling, vacuolation and single cell necrosis was seen in liver biopsies – with a corresponding increase of serum GDH activity and bilirubin concentration. In contrast to the progressive bile duct hyperplasia seen in rodents, there was only “slight evidence” of biliary hyperplasia and periportal fibrosis (Gopinath and Ford 1970). In this study, photosensitivity was not reported, and the serum activity of GGT was not measured.

Heat-treatment of rapeseed meals causes the glucosinolate progoitrin to be predominantly metabolized to 5-vinyl-2-oxazolidinethione (goitrin) (Paik et al. 1980). Goitrin inhibits iodine incorporation into thyroxine and interferes with thyroxine secretion. These effects are not negated by iodine supplementation (Cheeke 1998). In rats, goitrin, administered at 40 to 100 mg/kg subcutaneously, has been shown to increase thyroid and liver weights (Nishie and Daxenbichler 1982). Thiocyanates also inhibit iodine uptake by the thyroid, but iodine supplementation can overcome this (Cheeke 1998).

On the other hand, conditions conducive to the formation of organic cyanides (nitriles) from glucosinolates, at the expense of the corresponding isothiocyanates, include autolysis (endogenous enzyme hydrolysis without heat), heating, and acidic pH (pH 5 to 6), as well as nonenzymatic catalysis by Fe^{2+} in ferrous sulphate (VanEtten et al. 1969a, Cole 1976, Daxenbichler et al. 1977, Paik et al. 1980, Forss and Barry 1983, Bellostas et al. 2008). The majority (up to 90%) of the degradation products that result from the presence of intact glucosinolates at body temperature in the acid pH of the stomach or abomasum and in the presence of as little as 0.25 M excess Fe^{2+} are nitriles (Forss and Barry 1983, Bellostas et al. 2008). Under such conditions, the following nitriles are potentially derived: 1-cyano-2-hydroxy-3,4-epithiobutane and 1-cyano-2-hydroxy-3-butene (crambene) from progoitrin; 1-cyano-3,4-epithiobutane and 1-cyano-3-butene from gluconapin; and 1-cyano-4,5-epithiopentane and 1-cyano-4-pentene from glucobrassicanapin (VanEtten and Daxenbichler 1971, Kirk and Macdonald 1974, Paik et al. 1980). Additional nitrile metabolites potentially derived from turnips and rape include 2-phenylproprionitrile from gluconasturtiin and indole-3-acetonitrile from glucobrassicin (Cole 1976, Daxenbichler et al. 1977, McDanell et al. 1988). Of these nitriles, the most stable is 1-cyano-2-hydroxy-3-butene from progoitrin (Paik et al. 1980).

Rats fed diets containing mixed nitriles developed liver lesions (bile duct hyperplasia, fibrosis, megalocytosis, and zonal necrosis) and megalocytosis of renal tubular epithelial cells (VanEtten et al. 1969b). Similar dose-dependent lesions, associated with serum biochemical alterations indicative of hepatocellular damage and cholestasis, were induced in rats that were fed diets containing 10 to 22 mg/kg 1-cyano-2-hydroxy-3,4-epithiobutane for 90 days (Gould et al. 1980). The nitriles responsible for the nephrotoxicity in rats include 1-cyano-2-hydroxy-3,4-epithiobutane and 1-cyano-3,4-epithiobutane; doses of 50 to 125 mg/kg given by gavage once daily for 3 days are toxic (Nishie and Daxenbichler 1980, Gould et al. 1985, Wallig et al. 1988b). Another less potent nitrile derived from progoitrin, 1-cyano-2-hydroxy-3-butene, is a selective pancreatotoxin (causing apoptosis and necrosis in individual exocrine acinar cells) in rats at daily gavage doses of 200 mg/kg for up to 4 days (Wallig et al. 1988a). Subcutaneous injections of this nitrile into pregnant rats induced liver necrosis and bile duct hyperplasia after 12 days (Nishie and Daxenbichler 1980). In mice, the nitrile metabolites of the glucosinolate progoitrin are about 8 times as toxic as the oxazolidinethione metabolite, goitrin (VanEtten et al. 1969a). The oral administration to sheep of allyl cyanide, the nitrile metabolite of the glucosinolate sinigrin, found in *B. oleracea* (cabbage, cauliflower, broccoli, Brussels sprouts, and kale) and *B. nigra* (black mustard), and in small amounts in turnips and rape (Kim et al. 2001), caused minor liver damage as indicated by slightly raised GGT activities, but the lesions were not characterized histologically (Duncan and Milne 1992, 1993).

In ruminants grazing *Brassica* crops, the effects of derived nitriles will depend on the amount produced following enzymatic autolysis during chewing; the amount produced nonenzymatically by low pH and the presence of ferrous ions; the degree of their microbial degradation in the rumen; and the nature, concentration, reactivity, and host tolerance of absorbed nitriles (Forss and Barry 1983, Bellostas 2008). An aspect that will need investigation in future cases is that of rumen pH (Barry 2013). Rumen pH was not measured in the cows with gross omasal and abomasal lesions described above.

Apart from glucosinolates and their metabolites, there is an amino acid, S-methyl cysteine sulfoxide, that is converted during rumen fermentation into dimethyl disulphide, the compound responsible for the hemolytic anemia in some *Brassica* – notably kale – poisonings. Erucic acid (found mainly in rapeseed oils, flavonoid polyphenolic compounds (such as quercetin, kaempferol, isorhamnetin, and cyanidin), nonflavonoid phenolic compounds (hydroxycinnamic acids such as *p*-coumaric, sinapic, and ferulic acids), tannins, sinapine and related phenolic choline esters, phytic acid, ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids, and terpenes are also found in *Brassica* (Bouchereau et al. 1991, Lajolo et al. 1991, Cheeke 1998, Abdel-Farid et al. 2006, 2007, Cartea et al. 2011). On hot, cloudy days and following rainfall at the end of a drought, nitrate levels in *Brassica* can reach toxic levels (Barry 2013).

Many of the glucosinolates, terpenes, and phenylpropanoids found in *Brassica* function as phytoanticipins (antimicrobial and pesticide compounds present in plants *before* challenge by phytopathogenic microorganisms). In addition, phytoalexins (antimicrobial and pesticide compounds synthesized by and accumulated in plants *after* exposure to phytopathogens), which comprise sulfur-containing indoles and indole-3-acetonitrile, are produced by *Brassica* (Lichtenstein et al. 1962, Ames et al. 1990, VanEtten et al., 1994, Pedras et al. 2002, Abdel-Farid et al. 2006).

The fact that certain cultivars of turnip (Barkant) and rape x kale (Titan) seem overrepresented in *Brassica* photosensitivity outbreaks in New Zealand probably reflects farmer preference for the respective cultivar characteristics (i.e., market share) rather than innate toxic potential. At this stage, the only things that can be suggested in terms of prevention are limiting time on, and/or limiting intake of, the available crop. For downer animals, the possibility that hypocalcemia plays a complicating role needs further investigation.

Apart from the as-yet-unexplained photosensitizations seen in cattle and sheep grazing *Brassica* forage crops, a few weeds belonging to the Brassicaceae family have also been associated with photosensitivity in cattle in the United States. *Descurainia pinnata* (tansymustard, which closely resembles *D. sophia*, flixweed) and *Thlaspi arvense* (field pennycress, fanweed, or stinkweed) have been implicated in Montana and Colorado (Pfister et al. 1989) and in Oklahoma (Martin and Morgan 1987), respectively. Tansymustard grown under certain conditions and fed for 3 weeks was hepatotoxic to hamsters (Pfister et al. 1990). These weeds contain gluconapin and sinigrin, from which 3-butenyl and 2-propenyl (allyl) isothiocyanates, 1-cyano-3,4-epithiobutane, and 3-phenylproprionitrile are derived following hydrolysis (Daxenbichler et al. 1964, Afsharypuor and Lockwood 1985, Smith and Crowe 1987, Fahey et al. 2001, Knight and Stegelmeier 2007).

In conclusion, none of the secondary compounds found in turnips, rape, swedes, kale, or their various hybrids have so far been shown to be either hepatotoxic (in cattle) or photodynamic (in lambs with rape scald). It is possible, however, that special circumstances and unique combinations of plant (with or without phytopathogenic fungi, bacteria, or viruses), rumen, and/or liver metabolites could produce derivatives that have severe hepatotoxic and/or cholangiotoxic effects, or that could enter the bloodstream and react with light. As noted above, a number of nitrile derivatives are hepatotoxic, nephrotoxic, or pancreatotoxic in rats. At this stage, therefore, nitriles derived from glucosinolates would seem to be the most likely candidates for culpability in the hepatotoxicity that sometimes occurs in cattle and that manifests as photosensitivity. Research on nitrile concentrations in rumen fluid, serum, and liver tissue of affected cattle is warranted. Further work to characterize the effects of oral doses of purified isothiocyanates and nitriles in laboratory animals is required. Conclusive evidence will hopefully be obtained when research using purified derivatives is extended to susceptible ruminants.

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