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Preface

We are pleased to publish this 3rd issue of the International Journal of Poisonous Plant Research (IJPPR). The objectives of the IJPPR include providing a forum for publishing original research, case reports, and scientific reviews of poisonous-plant-related investigations or research. Additionally, we want this journal to provide a central outlet to share new information and technology from around the world on analytical techniques, tools to enhance diagnosis, and methods to reduce or prevent animal poisonings from plants. There is currently no journal, electronic or printed, that specifically focuses on poisonous plant research, and IJPPR aims to fill this critical role for scientists, veterinarians, educators, and the general public with an interest in toxic plants and their associated impact on animals and people throughout the world. We have employed an electronic publication system to make the papers as widely accessible as possible on the USDA-ARS Web site at no charge. It is our intent for *IJPPR* to be an interdisciplinary source bringing together all disciplines with a common interest in poisonous plants.

In this issue the manuscripts provide diverse research information and photographs of plant poisonings from the United States and other parts of the world. The cover and first article represent an interesting case of poisoning in camels by the plant *Zygophyllum coccineum* (Tartir) growing in the Al-Najaf desert of Iraq in the Middle East. Also in this issue are reports of the photosensitizing effects of *Brassica* forages in cattle in New Zealand, a case report of halogeton poisoning in cattle, and the impact of protein supplementation on cattle grazing lupine pastures.

The Editors-in-Chief thank those who have assisted in the production of the third issue, particularly Terrie Wierenga, Editorial Assistant, USDA-ARS Poisonous Plant Research Laboratory, Logan, UT, Mark Boggess, former National Program Leader, Office of National Programs, Sue Kendall, Editor, Information Staff, Beltsville, MD, and others responsible for editing this online journal. We also thank the *IJPPR* Editorial and Advisory boards for peer review and recommendations for publication.

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Diarrhea Associated with Ingestion of *Zygophyllum coccineum* (Tartir) in Camels in the Al-Najaf Desert in Iraq

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Abstract

Zygophyllum coccineum is a common plant found in areas of the Eastern (Arabian) Desert. It is rich in saponins, and its extracts have a variety of pharmacological actions, including acting as a diuretic and antipyretic. After several years of drought, during which many forbs and shrubs disappeared, heavy rains in 2012 and 2013 resulted in profuse growth of *Z. coccineum* (called "tartir" in Arabic). Field outbreaks of diarrhea in camels due to ingestion of *Z. coccineum* were reported in the Al-Najaf desert in Iraq. The clinical signs observed in the field cases resembled those reproduced in experimental rats and were consistent with acute diarrhea due to poisoning. Relocation of the camels to a different grazing area led to remission of the clinical signs.

Keywords: Al-Najaf desert, camel, Iraq, poisoning, *Zygophyllum coccineum*

Introduction

Camels *(Camelus dromedarius)* are important and versatile animals found in arid and semi-arid areas of the world. According to statistics from the Food and Agriculture Organization of the United Nations (FAO), there are approximately 25 million camels in the world. There were a total of 58,000 camels in Iraq, according to the FAO statistics of 2011 (Omer 2011). This estimation agrees with the statistical information of the Iraqi Veterinary General Company, which in 2008 had recorded a total of 58,893 camels in Iraq. This is an increase of 21.28% when compared to the Comprehensive Agrarian Census Data from 2001, which had recorded 23,413 camels.

The split upper lip of camels means that they can graze selectively up to a recorded height of 3.5 meters and utilize thorny plants. Camels have a reported preference for plants that have high water and salt content. It has been recorded that camels eat toxic plants avoided by other mammals (Dörges and Heucke 1997, Lapidge et al. 2008).

The Al-Najaf desert suffered from an absence of rainfall for several years, which led to the absence of pastures and disappearance of many grasses and other desert plants. But at the end of 2012 and in early 2013, all of Iraq's provinces experienced heavy rainfall, which led to growth of shrubs and forb plants in most desert areas. Consequently, many of the Bedouins moved to the areas flourishing with grass and green plants to graze their camels.

Z. coccineum has rarely grown in the past few years in the Iraqi desert due to the scarcity of rain. However, there was profuse growth of *Z. coccineum* after the heavy rain in 2012 and 2013 in the Al-Najaf desert. The plant is very common in the limestone "wadis" and plains of the Eastern (Arabian) Desert and is tolerant of saline soils. In the Egyptian deserts, *Z. coccineum,* from the family Zygophyllaceae, grows wild (Batanouny and Ezzat 1971, El-Hadidi 1972) and has been traditionally used in medicines as an antihelminthic, diuretic, antidiabetic, antiasthma, antigout, antirheumatic, and antihypertensive agent (Saber and El-Moghazy 1960, Gibbons and Oriowo 2001).

Morphologically, *Z. coccineum* appears as a low shrub, perennial herb, or desert succulent undershrub, is up to 75 cm high, and is characterized by numerous stems and erect, young, green branches (Soliman 1939, Reed 1997). The leaves are 2-foliolate, over 10 mm long, cylindrical, bright green, glabrous, fleshy, and carried on a fleshy, long petiole. The plant is common, but it is unpalatable, and animals generally do not graze on it. It does not make good fuel. The plant is, therefore, neither used for grazing nor is it cut for fuel.

Many studies have shown the pharmacological action and toxicity of *Z. coccineum*. The aqueous extract of the plant is documented to cause lowering of blood pressure, and it acts as a diuretic, antipyretic, and local anesthetic. It has antihistamine activity and helps in the stimulation of an isolated amphibian heart, stimulation of a guinea pig's intestine, relaxation of an isolated intestine, contraction of the uterus, and vasodilation. The extract antagonized acetylcholine's action on skeletal muscle and acted additively with the muscle-relaxant effect of d-tubocurarine (Saad et al. 1967a,b, Aclinou et al. 1988, Gibbons and Oriowo 2001, Doligalska et al. 2011, Bhattacharya and Haldar 2012, Das et al. 2012).

Z. coccineum is a saponin-rich plant. Phytochemical investigations have revealed that the major secondary metabolites in *Z. coccineum* are a class of quinovic acid compounds belonging to the ursane-type triterpene saponins, including zygophylloside S, together with a known flavonoid glycoside and a sterol glycoside (Ghafoor 1974, Ahmad et al. 1990, 1992, 1993, Ghazala 1992, Elgamal et al. 1995, Amin et al. 2011).

The authors report here severe watery diarrhea, polyuria, and conjunctivitis in a herd of camels that grazed accidentally on *Z. coccineum*.

History of Outbreak

In May 2013, a herd of 40 adult camels and 10 young camel calves was moved to a new, flourishing grazing area in the Al-Hidaya/Al-Najaf/Iraqi desert. Green plants and low shrubs (figure 1) were growing in this area, and the camels readily grazed them. After 24 hours, all the adult camels started showing signs of severe diarrhea (figure 2A), excessive voiding of urine, restlessness, severe lacrimation, and photosensitization. One pregnant female died after severe bloating, respiratory distress, and polyuria with dark red urine. The diarrhea became worse as the camels continued to graze, and most animals became dehydrated. A few camels were in sternal recumbency and expressed high-pitched bleats, loud bellows, and roars in addition to straining and suffering during the passing of soft-formed green feces (figure 2B). Fecal samples and swabs were collected and sent for bacterial and parasitic investigations. Relocation of the camels to a different grazing area led to remission of these clinical signs.

Figure 1. *Zygophyllum coccineum.*

Figure 2. A: Tail and legs contaminated by feces in a camel with diarrhea. B: Camel passing soft green feces, which contaminate the surrounding thighs and tail.

Materials and Methods

In order to do the preliminary evaluation of the plant, a pilot study was designed. The plant is well known in pastoralism and is locally called "tartir". The plant was sent to the Herbal Center for scientific identification. Scientifically, the plant is called *Z. coccineum*. Soon after collection, 100 g of the fresh plant were washed thoroughly with water, separated into pieces, and immediately crushed thoroughly in tepid water (~ 40 °C) using a mechanical grinder.

After cooling to room temperature (24 ± 2 °C), the extract was separated from the remaining vegetable debris by pressing the material through a muslin cloth. The resulting liquid was filtered and kept as aliquots and considered as water suspension of the fresh *Z. coccineum* (WSFZC).

Four adult Sprague-Dawley male rats weighing 180 to 250 g were used in this study. The rats were obtained from the Laboratory Animal Unit, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine (FVM), Kufa University (KU), Iraq. The rats were grouped randomly into 2 groups, housed in polyacrylic cages, and maintained under standard laboratory conditions (temperature 25 ± 2 °C with a dark/light cycle of 12/12 hours). They were allowed free access to the standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory conditions for 3 days before starting the experiment. All the procedures described were reviewed and approved by the Kufa University Research and Animal Ethical Committee (KU.FVM.AEC number 0706-2013). All rats were fasted for 12 hours with water ad libitum and placed in individual observation cages. The first group (treatment) was orally gavaged 5 mL WSFZC/rat for 2 days; the second group (control) was orally gavaged distilled water. The rats were observed to see whether they produced formed or unformed stool. Formed stool referred to stool that was in a pellet form or a rugby-ball shape. Unformed stool referred to feces that were muddy or watery, and this was judged as diarrhea. After 3 days, the rats were euthanized via an intraperitoneal injection of ketamine:xylazine. Subsequently, the rats were necropsied, and the liver, gastrointestinal tract, spleen, kidney, pancreas, and lymph nodes were collected. All the tissues were fixed in neutral buffer formalin (10%) and submitted to the Anatomy and Histology Department, Faculty of Veterinary Medicine, Kufa University, for histopathology processing.

Results

Testing of fecal samples from the camels revealed neither pathogenic bacteria nor parasitic infestation.

Two hours after oral gavage with WSFZC, the treatment group of rats showed diarrhea and passing of unformed stool, which was soft and green. Severe diarrhea continued the 2nd day, and the rats became dehydrated. They developed rough coats, aggressive behavior, and polyuria. They had an abnormally high consumption of water in comparison to the control group, which continued to pass formed stools.

Histopathological analysis did not reveal any specific pathological changes in the intestine or other tissues from the rats.

Discussion

Before the occurrence of diarrhea, the herd of camels was allowed to graze on dry grass shrubs. Different green desert plants grew in the Al-Najaf desert after a heavy rain, which encouraged the Bedouins to move their herds of camels to these areas. The camels grazed readily and were observed eating toxic plants normally avoided by other mammals (Dörges and Heucke 1997). The abundant presence of *Z. coccineum* in the region encouraged the camels to consume it quickly, which led to the occurrence of diarrhea, polyuria, and lacrimation in all adult animals. The young animals did not show any clinical signs, because they were suckling milk and did not eat the plant.

The clinical signs that appeared in these animals were compatible with the pharmacological and toxic effects of the *Z. coccineum* saponin compound that was identified in previous studies (Saad et al. 1967a,b, Johnson, et al. 1986, Aclinou et al. 1988, Gibbons and Oriowo 2001, Doligalska et al. 2011, Bhattacharya and Haldar 2012, Das et al. 2012). Ghazala (1992) isolated 5 triterpenoidal saponins from *Z. coccineum*. Several studies agree that saponin compounds affect animals and humans in both a positive and negative manner. Saponins play a biological role as they have membrane-permeabilizing, immunostimulant, and hypocholesterolemic properties, and they have been found to significantly affect growth and feed intake in animals. These compounds have been observed to kill protozoa, impair protein digestion and the uptake of vitamins and minerals in the gut, and act as hypoglycemic agents (Das et al. 2012).

A triterpenoid was found to be the most active compound and caused diarrhea in mice due to an increase in the motor activities of the normal bowel (Bhattacharya and Haldar 2012). In the present study, diarrhea was found to be one of the very prominent clinical signs in the camels as well as in the experimental rats. The symptom of diarrhea was compatible with the previous studies that demonstrated the gastrointestinal effects of the triterpenoidal saponin extract; it was a remarkable stimulant laxative that increased the motor activities of a normal bowel along with having prokinetic effects in normal as well as constipated Swiss albino mice, while inducing diarrhea with higher doses (Bhattacharya and Haldar 2012). It was due to this fact that the severity of diarrhea was dose-dependent and some camels in the present outbreak showed severe diarrhea, as they had consumed large quantities of *Z. coccineum,* which contained triterpenoidal saponin. The diarrheal action due to the triterpenoidal compound could cause toxic effects when consumed in appreciable amounts and could be life-threatening in contraindicated subjects (Pasricha 2006).

Bhattacharya and Haldar (2012) investigated the gastrointestinal effect of triterpenoids on normal nonconstipated (naive) mice, to determine the increase in motor activities of the normal bowel, and in drug-induced constipated mice, to determine the excretory bowel activities to counteract the constipation. They found that oral administration of a triterpenoid produced a laxative effect in both naive and constipated mice in a dose-dependent manner. Triterpenoids modified the excretory bowel activities, as evidenced by an increase in fecal output, number of wet feces excreted, frequency and weight of stools, fecal water content, and diarrhea episodes in both naive and constipated mice. They agreed that triterpenoids accelerated the gastrointestinal propulsion rate and acted as stimulants for gastrointestinal motility, as it occurred in the case of putative stimulant laxatives, thereby confirming its action as a stimulant laxative. They also found that triterpenoids elicited gastrointestinal activity similar to that affected by the reference drug castor oil. An accelerated transit of the liquid digestive contents through the small intestine and colon did not permit adequate time for reabsorption of water and electrolytes and resulted in watery stool and diarrhea.

In the present outbreak, 1 pregnant female camel died after severe bloating, respiratory distress, polyuria, and dark red urine. These physiological disorders might be due to a *Z. coccineum* saponin compound. This was compatible with a previous observation, which found that saponin could play a role in the pathophysiology of bloating and was associated with bloat in ruminants (Wilkes and Godwin 1995). Saponin had the ability to form stable foams in low concentrations that led to reduced microbial protein production and protozoal numbers (Wilkes and Godwin 1995). In addition, saponins caused severe physiological disorders, such as hemolysis, gastroenteritis, paralysis, and death (Bondi et al. 1973). Other researchers found that saponins reduced the digestibility of forage in ruminants (Oleszek 1996, Small 1996, Oleszek et al. 1999).

Rats were used in the pilot investigation to determine the effects of *Z. coccineum*. Rats are more sensitive to plant compounds and toxins and have been used in screening techniques for detection of the toxicity of plant compounds. Several workers have used a rat bioassay to test for the presence of toxins in tropical pasture legumes (Strickland et al. 1987, Bindon and Lamond 1966). The treated rats showed diarrhea; however, neither a histopathological change in the intestine nor in any other tissue of the experimental rats was seen. These results supported the stimulant laxative mechanism of diarrhea that led to an increase in the motor activities of the digestive tract. The polyuria that appeared in the experimental rats as well as in the camels confirmed that *Z. coccineum* had a diuretic effect, as had been shown previously (Amin et al. 2011).

The severe lacrimation and photosensitization that has appeared in all camels may be due to the hypersensitivity reaction and the effect of the saponin compound on the immune system. Oda et al. (2000) demonstrated that saponin has the unique ability to stimulate the cell-mediated immune system as well as to enhance antibody production. The other advantage is that only a low dose of saponin is required for adjuvant activity (Oda et al. 2000). Saponins reportedly induce the production of cytokines, such as interleukins and interferons, that might mediate immunostimulant effects (Kensil 1996). The camels in this case were exposed to a high dose of saponins, which might have increased the uptake of other antigens from the gut and other membranes, leading to stimulation of the cell-mediated immune system, which further led to the development of lacrimation and photosensitization.

This is the first reported case of acute poisoning diarrhea in a herd of camels due to *Z. coccineum* with a high concentration of the saponin compound. The prominent clinical signs were severe diarrhea, polyuria, and photosensitization, which occurred due to the pharmacological action and toxicity effects of *Z. coccineum* saponins. A similar clinical presentation was reproduced in rats by giving them an oral gavage of the plant extract in water. This field outbreak showed the toxicity of *Z. coccineum*, although this plant has been traditionally used as a hypertensive, anthelminthic, diuretic, and for reducing blood sugar.

Precautions must be taken to prevent misuse by humans when making traditional medicines. More studies are highly recommended for further investigation.

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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

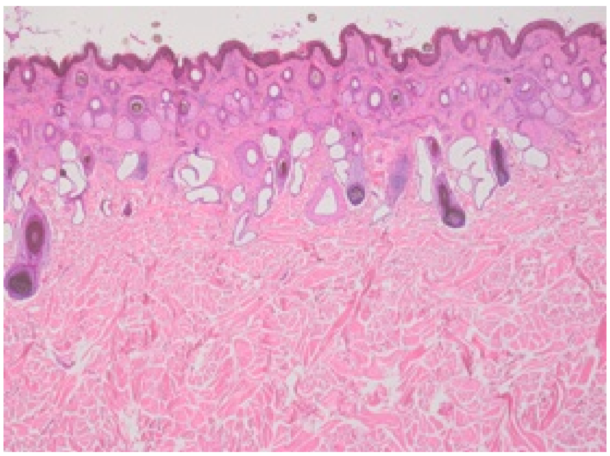

Figure 3. Photomicrograph of a biopsy of normal pigmented (black) skin from the cow in figure 1. H&E 4X.

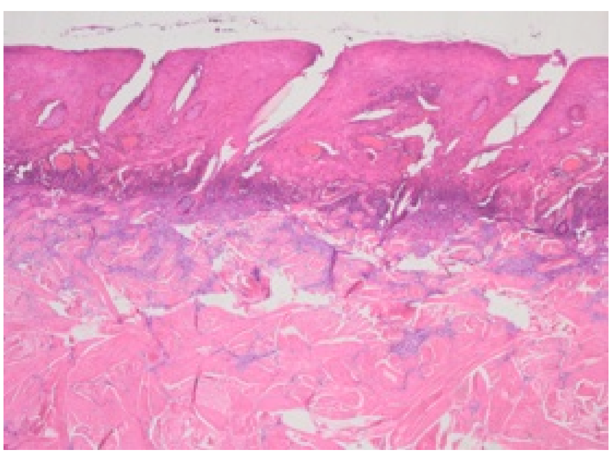

Figure 4. Photomicrograph of a biopsy of affected nonpigmented (white) skin from the cow in figure 1, showing diffuse necrosis of the epidermis and superficial dermis to the depth of the apocrine sweat glands and hair follicle bulbs, while sebaceous glands are still recognizable. Involved blood vessels 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.

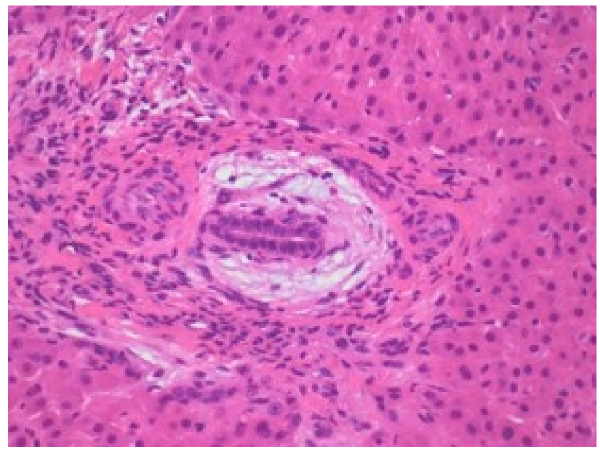

Figure 5. Photomicrograph of a liver biopsy from an acutely photosensitized cow, showing characteristic peribiliary oedema and mild loose concentric fibrosis. This cow had recovered from turnip photosensitivity a year prior. The serum GGT and GDH activities at the time were 867 U/L and 515 U/L, respectively.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

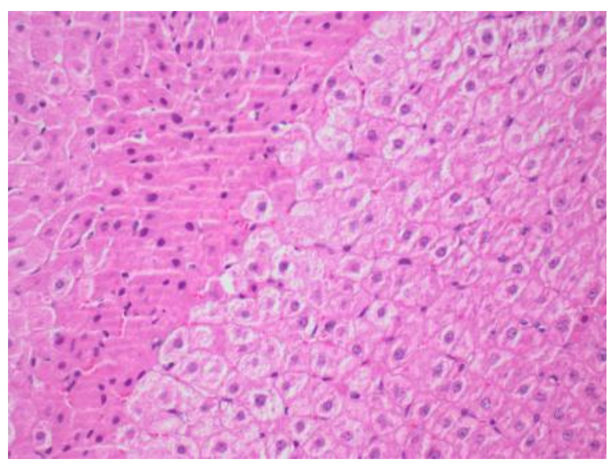

Figure 6. Photomicrograph of a liver biopsy from an acutely photosensitized cow, showing marked delineation in staining intensity between more eosinophilic hepatocytes with small, dark nuclei (top left) and cells with more vesicular cytoplasm and normal nuclei (right and bottom). Serum GGT and GDH activities 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

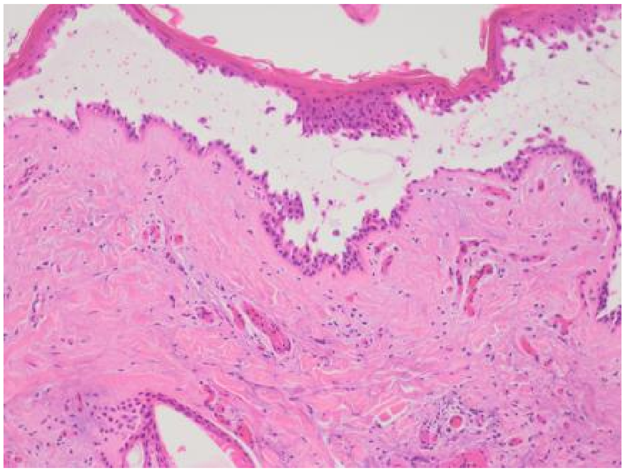

Figure 10. Photomicrograph of a section of nonpigmented skin from Cow 4 showing a suprabasilar intraepidermal cleft containing free-floating acanthocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 636 U/L, 720 U/L, 45 µM, and 1.61 µM, respectively.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

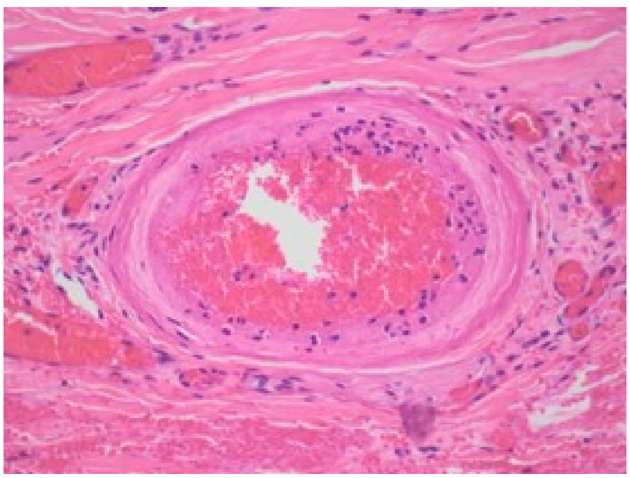

Figure 11. Photomicrograph of the dorsal dermis of a pigmented (black) ear of Cow 4 showing fibrinoid necrosis of the wall of a medium-sized blood vessel. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations at the time were 636 U/L, 720 U/L, 45 µM, and 1.61 µM, respectively.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

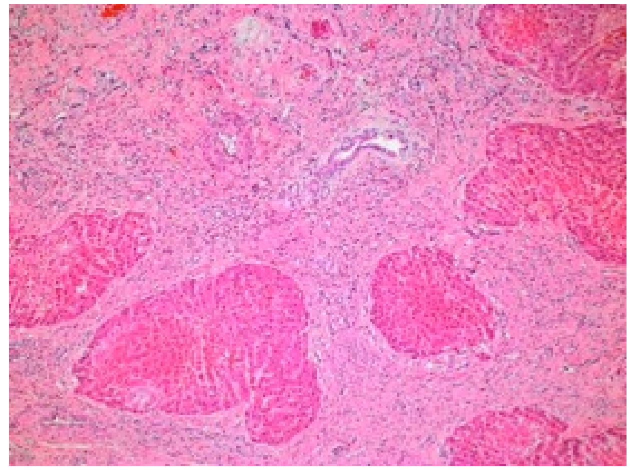

Figure 12. Photomicrograph of the liver of Cow 4 showing severe periportal to bridging fibrosis and bile ductule hyperplasia typical of that seen in residual chronic sporidesmin toxicity (facial eczema). Note the apparently functional bile duct near top center that has peribiliary edema. Many of the 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.

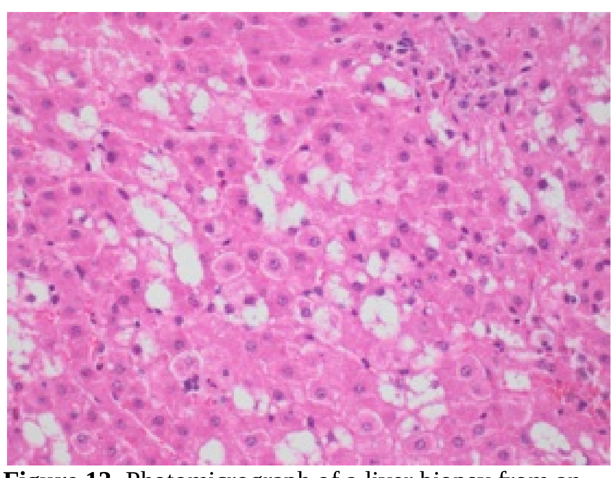

Figure 13. Photomicrograph of a liver biopsy from an acutely photosensitized cow showing swollen hepatocytes with individual cells and groups containing fatty vacuoles. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentration at the time were 537 U/L, 381 U/L, 18 µM, and 0.61 µM, respectively.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

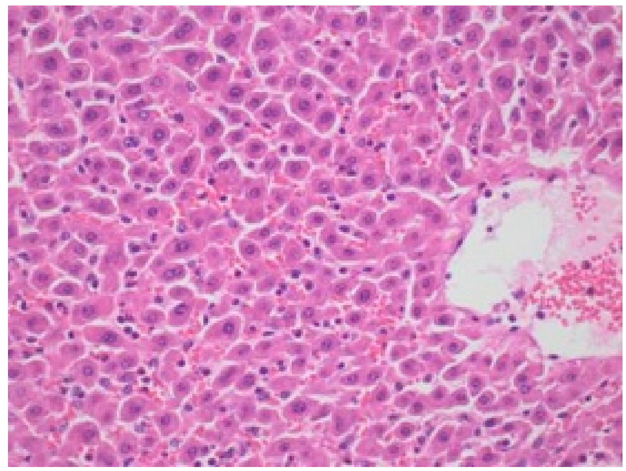

Figure 14. Photomicrograph of the liver from Cow 5 showing dissociation of hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 1,045 U/L, 159 U/L, 99 µM, and 1.65 µM, respectively. H&E 40X.

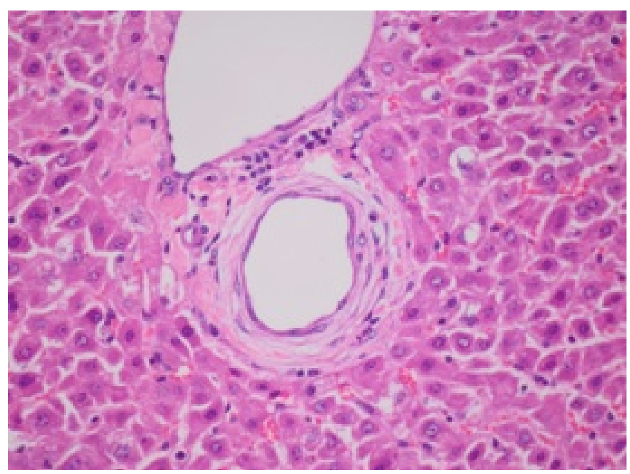

Figure 15. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. Note the bile duct in the center that is lined by attenuated squamous epithelium and surrounded by concentric rings of fibrosis. Note also the randomly scattered darker hepatocytes with smaller dark nuclei, similar 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.

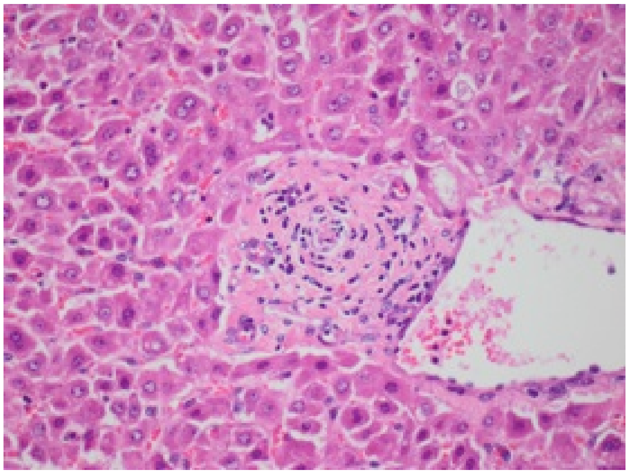

Figure 16. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a small bile duct is necrotic and surrounded by mild concentric fibrosis and some inflammatory cells. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin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

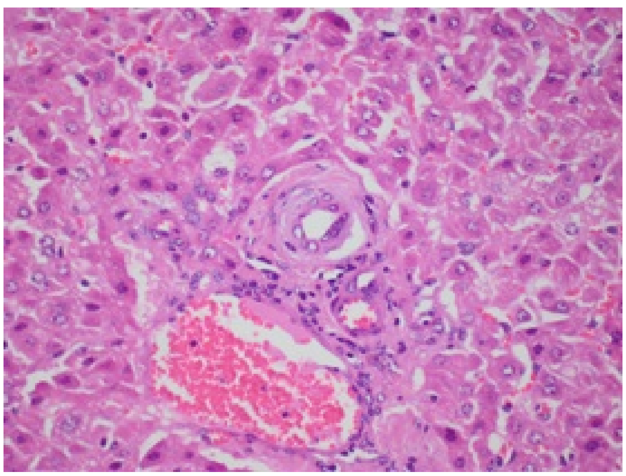

Figure 17. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, the epithelium of a small bile duct shows asymmetrical epithelial regeneration. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations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

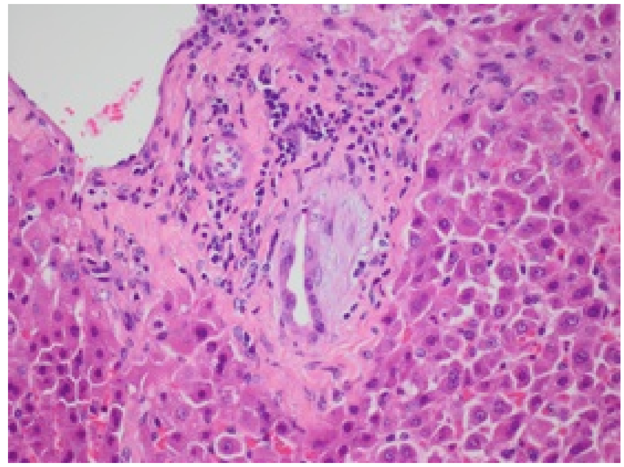

Figure 18. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a small bile duct is regenerating adjacent to a scar (on the right) that appears to be an obliterated bile duct. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and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

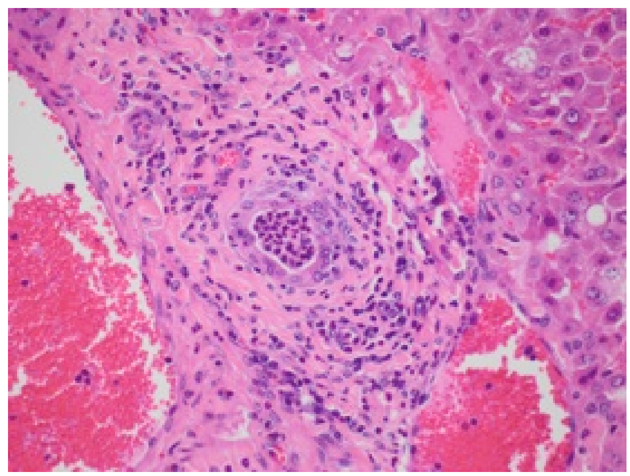

Figure 19. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, a bile duct lined by regenerating epithelium contains a plug of neutrophils. Note the scattered dark hepatocytes. Serum GGT and GDH activities and bilirubin and phytoporphyrin concentrations were 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).

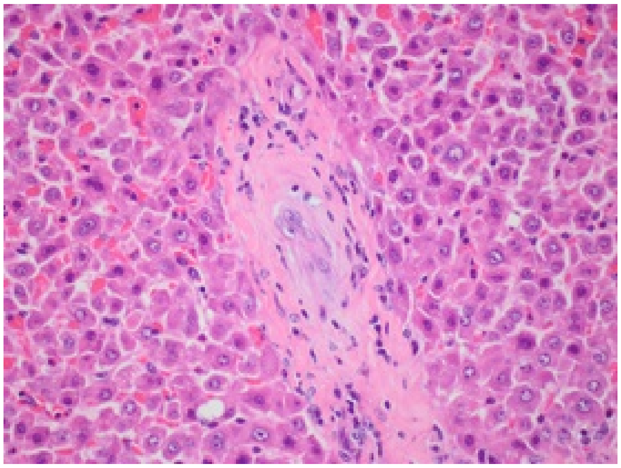

Figure 20. Photomicrograph of the liver from Cow 5 that had been photosensitive for 9 days. In the center, the bile duct has been obliterated by scar tissue in which residual, possibly regenerating, epithelial cell nuclei appear embedded. Note the scattered dark hepatocytes. Serum GGT and GDH activities 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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Halogeton (*H. glomeratus*) Poisoning in Cattle: Case Report

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Abstract

Historically, the most significant losses from halogeton poisoning have been reported in sheep, with multiple catastrophic deaths documented. While recorded death losses in cattle from halogeton poisoning are less common than in sheep, recent cases, including 2 reported here, and anecdotal reports from other ranchers suggest that the impact of halogeton losses in cattle herds in the western United States is much more widespread than originally thought. Halogeton may accumulate up to 30% oxalates; a small amount of the plant (300 g) is enough to cause death in sheep. Oxalates precipitate calcium from the blood, resulting in hypocalcemia, formation of calcium oxalate crystals, and uremia. In this report, 2 cases of halogeton poisoning in cattle are documented, including a history of halogeton grazing, supportive oxalate analysis of plants collected from the poisoning locations, and histological evidence of classic oxalate nephropathy and calcium oxalate crystal nephrosis.

Keywords: cattle, halogeton, oxalates

Introduction

Originally from Eurasia, halogeton (*Halogeton glomeratus*; figure 1) is an invasive, noxious weed in many parts of the world. In North America, it was first collected and identified in the United States in Wells, Nevada, in 1934 (Cook and Stoddart 1953). It is unclear how this weed was introduced, but it displaced native plants due to overgrazed and depleted desert range conditions, and within 40 years it infested 11.2 million acres throughout the intermountain west and Colorado Plateau (figure 2; James et al. 2005). Numerous cases of acute sheep death attributed to halogeton grazing were reported during the ensuing years, culminating in a very public Idaho case involving the death of nearly 1,300 head of sheep. In 1952, federal funds were allocated for eradication and control with the passage of a halogeton control bill (Young 1988).

The mechanism of halogeton toxicity was found to be soluble oxalates, sodium oxalate and potassium oxalate, which bind calcium, forming calcium oxalate monohydrate and insoluble calcium oxalate. Calcium oxalate monohydrate damages mitochondria, increases reactive oxygen species, and decreases tricarboxylic acid enzymes (succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, and other respiratory enzymes), resulting in mitochondrial dysplasia and reduced oxidative phosphorylation (Chungang and McMartin 2005). Calcium oxalate may precipitate, forming crystals that mechanically injure renal tubules, interfere with rumen function, and affect specific metabolic pathways, including calcium homeostasis. Clinically, this is seen as renal failure with severe calcium oxalate crystalline nephropathy. Oxalate form, dose, and duration are important in disease progression, as certain acute poisonings can produce sudden death prior to the development of crystalline nephropathy.

Halogeton and greasewood *(Sarcobatus vermiculatus)* are the principal toxic oxalate-producing range plants found in North America. Halogeton oxalate content generally ranges between 15% and 18%, with reports as high as 36% dry weight. Acute toxicity has historically been

Figure 1. Halogeton with close-up of leaves (inset).

Figure 2. Halogeton in high desert pasture.

associated with sheep, but occasional cases have been reported in cattle (McMartin and Wallace 2005).

Case 1

Twenty-six mature Black Angus cows, in a herd of 350, were found dead in 2012 by a northwestern Utah producer. Cattle were in poor body condition (BCS 3.5/9) coming off of summer grazing and had been trucked 50 km to winter pastures. Death occurred within 1 week of moving. A necropsy was performed on 1 of the deceased animals, and the referring veterinarian submitted tissues. Histologic evaluation identified severe oxalate nephrosis, with intratubular material consistent with calcium oxalate crystals.

The producer submitted plant samples, and subsequent plant samples were obtained and submitted during a farm visit by the author. Submitted plants were identified as *H. glomeratus*.

A major significant growth area of halogeton with evidence of halogeton grazing was found in one of the pastures where a gate was left open. Halogeton plant growth seemed to be associated with disturbance along an old irrigation canal. Oxalate content of the plants collected at this site was 29%. Halogeton plants were identified and collected from a neighboring ranch 24 km away and found to contain 17% oxalates.

Case 2

In 2006, a rancher in southeastern Idaho had 6 cows die over a 2-day period, followed by 3 more deaths in the subsequent 2 days. Two of the cows were submitted for necropsy evaluation at the Utah Veterinary Diagnostic Laboratory. Cows were in average body condition (BCS 5/9). Upon opening the rumen, it was noted that the rumen was full of plant material unfamiliar to the pathologist. Inspection by the toxicologist identified the plant material as *H. glomeratus*. Histologic evaluation found oxalate nephrosis with intratubular crystalline material consistent with calcium oxalate crystals in both cows.

Subsequent investigation with the rancher revealed that the cattle had been moved from a high mountain range 24 hours prior to the first death. The cattle had been unloaded and were being trailed to a new pasture. Inspection of the site where the cows were bedded the first night revealed a large stand of halogeton that had been extensively grazed. The rancher described the cows as actively going to those plants. Further discussions with the rancher found that the cows had not been receiving any salt or mineral supplements for an extended period prior to the halogeton exposure.

In total, 9 cows out of 85 that were bedded next to the halogeton stand died over a period of 4 days. Oxalate nephrosis with intratubular oxalate crystals supported the diagnosis of *H. glomeratus* poisoning.

Discussion

Deaths were attributed to either acute nephrosis and renal crisis or severe hypocalcemia secondary to grazing halogeton. Because the cattle in these 2 cases were in poor to average body condition and perhaps dehydrated from being trucked some distance, the toxic effects of oxalic acid ingestion (sodium and potassium) from the halogeton were exacerbated. Food and water deprivation was previously reported as a contributor to the severity of oxalate toxicosis (Lincoln and Black 1980). The binding of calcium in the formation of calcium oxalate and crystallizing in the kidney during excretion caused renal tubular epithelial degeneration and necrosis. While hypocalcemia was not determined in these cases, it has been reported as a common consequence of oxalate formation, as the binding of calcium depletes serum calcium levels, and it likely played a role in these cases (James

1968, Sebastian et al. 2007). In ruminants, hypocalcemia is a major contributor to rumen stasis, paresis, and death (Ruckebusch 1983, Cheng et al. 1998, Van Winden and Kuiper 2003, Russell and Roussel 2007).

Halogeton growth in Case 1 seemed to be associated with previously disturbed land, with the majority of growth near an old ditch bank. On the neighboring ranch, halogeton was growing in the corner of a pasture used for cultivation. In Case 2, the halogeton growth was also associated with disturbed areas, a livestock trail in this case. This is consistent with previously reported investigations of halogeton toxicities (Stoddart et al. 1951). The variation in oxalate levels seen here between neighboring ranches validates previous reports of large variations in plant oxalate content (Stoddart et al. 1951, James 1972, Lincoln 1980, James et al. 2005).

The following are recommendations for avoiding oxalate toxicity:

1. Avoid introducing hungry, thirsty, or salt-deprived animals into halogeton-infested areas.
2. Ensure that plenty of fresh water, adequate forage, and calcium-enriched trace mineral salt are available.
3. Provide supplements with calcium carbonate, calcium chloride, or dicalcium phosphate, e.g., 83% alfalfa pellet with 15% calcium carbonate and 2% molasses (Cook and Stoddart 1953).
4. Treat affected animals with parenteral calcium (intravenous calcium borogluconate); this treatment is reported to be more effective in cattle than sheep.
5. Halogeton may be controlled with 2,4-D herbicide. Seeding with perennial grasses, such as crested wheatgrass, will compete with the halogeton and help prevent reinvasion.
6. Avoid overgrazing, and practice good range-management techniques to slow halogeton invasion. Soil disturbance may result in a rapid spread of halogeton.

The cases reported here document that halogeton continues to be a problem for livestock ranchers in the intermountain United States region. Furthermore, while cases have historically been associated with grazing sheep, halogeton toxicoses in cattle are possible and should be considered when cattle are grazing in areas where halogeton is endemic. Precautions should be taken accordingly.

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Crude Protein Supplementation To Reduce Lupine Consumption by Pregnant Cattle in the Scablands of Eastern Washington

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Abstract

Lupinus leucophyllus (velvet lupine) is prevalent in eastern Washington State, and when consumed by pregnant cows, it can cause "crooked calf syndrome." Rangelands in this region are dominated by poor-quality annual grasses. The objective of this study was to determine whether feeding supplemental crude protein (CP) would reduce cattle consumption of velvet lupine during midsummer. Twelve pregnant cows were divided into 2 treatments: 6 controls received no supplement, and 6 cows received a supplement of 44% soybean meal at 4 g/kg body weight per day for 19 days in July 2010. Lupine density was >10 plants/m^2. There was no supplement effect on lupine consumption (P = 0.68), nor was there a day x supplement interaction (P = 0.88). Supplemented cattle took 6.9 ± 0.7% of daily bites as lupine compared to 5.0 ± 0.6% of bites for controls. Dry grass and dry forbs were the major diet components selected by cattle. Cattle ate mostly dry grass during the first 8 days of the trial, but then their consumption of dry forbs increased substantially. Cattle switched from eating green forbs to lupine after about 1 week; this change coincided with a decrease in green forb biomass. Five calves from both treatments were born with mild to moderate birth defects. We conclude that CP supplementation had no effect on cattle consumption of lupine under these supplement and abundant-forage conditions. The combined effect of supplementation and drought (i.e., forage scarcity) on lupine consumption by cattle has not been determined.

Keywords: cattle, lupine, protein supplementation

Introduction

Lupines (*Lupinus* spp.) are prevalent in the rugged Scabland region of eastern Washington State. Rangelands in this region are dominated by annual grasses, including *Bromus tectorum* (cheatgrass) and *Taeniatherum caput-medusae* (medusahead), and a number of weedy forbs, such as *Chondrilla juncea* (skeletonweed) and *Amsinckia lycopsoides* (fiddleneck). Lupines are deep-rooted legumes that are grazed in mid- to late summer, when annual grasses are dormant and other forbs have been depleted by grazing. One dominant lupine species is *Lupinus leucophyllus* (velvet lupine), which contains the teratogenic alkaloid anagyrine. When pregnant cows graze velvet lupine between days 40 and 100 of gestation, they are at risk of having deformed offspring, so-called "crooked calves." The incidence of crooked calf syndrome on ranches where velvet lupine is prevalent is estimated to be about 5% per year. Some years the incidence is much higher, typically when lupine populations are very dense from increased winter and spring precipitation (Panter et al. 2009).

Our studies on this type of rangeland indicate that grazing cattle prefer graminoids, particularly green grass when available (Lopez-Ortiz et al. 2007, Pfister et al. 2008). However, at times cattle will consume relatively large amounts of forbs (>60% of the diet) depending on the forage milieu (Lopez-Ortiz et al. 2007, Pfister et al. 2008). Some studies have suggested that cattle may include large amounts of forbs in their diets because of forbs' high protein content (Pieper and Beck 1980, Holechek 1984).

Altering the nutritional status of cattle may influence their forage preference (Heady 1964). Protein supplementation can influence an animal's nutritional status (Kartchner 1980). However, protein supplementation has had limited success in influencing botanical diet selection by grazing cattle on grass-forb rangelands (Judkins et al. 1985). In contrast, Odadi et al. (2013) reported that protein supplementation reduced forb grazing by cattle in African savanna during the dry season. For ruminants grazing on shrub-dominated rangelands (e.g., sagebrush), providing supplemental protein and/or energy to detoxify terpenes may greatly enhance shrub use by ruminants (Villalba et al. 2006, Dziba et al. 2007).

If protein supplementation could reduce selection of plants such as toxic lupine by grazing livestock, then it could be a useful tool to reduce the risk of crooked calf syndrome on Scabland rangelands. During a typical summer in the Scablands, all grasses and many forbs become dry and senescent, with resultant decreases in concentrations of crude protein, compared to forages that remain green later into the summer. Grazing cattle typically increase their consumption of lupine in midsummer (Ralphs et al. 2006). Pregnant heifers require approximately 9% to 10% crude protein (CP) in their diet to gain 0.75 kg/day (NRC 2000). Our hypothesis was that cattle would reduce consumption of still-green lupine in midsummer after the grasses had senesced if the cattle were supplemented with high-quality protein. Thus, the purpose of this trial was to determine whether providing a CP supplement to pregnant cattle would alter their selection of velvet lupine during midsummer grazing.

Materials and Methods

The grazing study was conducted from July 8 to July 26, 2010, near Washtucna, WA (46° 48' 12.8" N 118° 17' 08.5" W; 529 m elevation). Vegetation on the study site was dominated by cheatgrass and medusahead; other annual species included fiddleneck, tumblemustard *(Sisymbrium altissimum)*, tansy mustard *(Descurainia pinnata)*, prickly lettuce *(Lactuca serriola)*, and black mustard *(Brassica nigra)*. Velvet lupine and vetch *(Vicia cracca)* were abundant in all pastures.

All procedures were approved by the Utah State University Institutional Animal Care and Use Committee and were conducted under veterinary supervision. Twelve 2-year-old Hereford x Angus (479 kg ± 13 kg body weight) cows were time bred so that they were between 40 and 60 days pregnant at the start of this study. They were verified pregnant by rectal palpation before the study began and palpated upon return to Logan, UT, shortly after the end of the study. The 12 cows were divided into 2 treatments: 6 controls receiving no supplemental feed and 6 cows receiving a supplement of 44% soybean meal at 4 g/kg body weight per day. Supplemental feeding began 10 days before the grazing trial was started.

A lupine-infested pasture was subdivided into 6 smaller pastures such that lupine was abundant in all pastures, and 2 cows from the same treatment were placed into each of the 3 replicate pastures. Cattle were placed into a corral at night at 1900 h each day, with water and salt provided ad libitum. Supplement was fed to each animal individually in the corral during the evening. Lupine consumption was determined using bite counts. Each morning at 0530 h, cattle were released in pairs into their respective pastures to graze, and diets were determined using bite counts. Bite counts were taken on individual animals during active grazing periods; categories for bite counts were lupine (subcategories: whole plant, leaves, flowers, and pods), dry grass, green grass, dry forbs (other than lupine), and green forbs. Each animal was observed for a 5-min period, then the observer moved to the next animal. In this manner, the observer rotated through the entire group of animals several times each day. Animals were accustomed to the procedure, and the observer could remain within 1 to 2 m of the animals without disturbing their grazing. Typically, about 30 min of daily observation time was recorded for each animal.

Forage availability and quality were evaluated by weekly clipping of plants to ground level in 10 plots (0.25 m^2) within each of the 6 pastures. Clipped material was sorted into dry grass, green grass, lupine, other green forbs, and other dry forbs. The clipped material was dried at 40 °C for 48 hours and weighed to determine available forage (kilograms per hectare). The same plant material was also retained for nutritional analysis. Lupine density was determined at the beginning of the study by placing 150 quadrats, 0.5 m^2, along 3 pace transects that trisected all of the pastures, and counting all lupine plants within each plot.

Five individual clipped samples of each forage category for each time point were ground to pass a 1-mm screen in a Wiley mill and analyzed for dry matter, CP (N x 6.25; LECO FP-528 Nitrogen

Analyzer, LECO Corp., St. Joseph, MI), neutral detergent fiber (NDF) (ANKOM Fiber Analyzer system), and in vitro true digestibility (ANKOM Daisy II system). The NDF procedure was modified by addition of heat-stable amylase (Sigma Chemical, St. Louis, MO).

The alkaloid composition of lupine (Lee et al. 2007) was determined on 10 randomly selected whole plants collected weekly. Extraction of *L. leucophyllus* was done by weighing 100 mg of ground plant material into a 10-mL screw-top test tube equipped with Teflon-lined caps. Five milliliters of 1*N* hydrochloric acid (HCl) and 4 mL chloroform ($CHCl_3$) were added to the test tubes, which were placed in a mechanical shaker for 15 min, then centrifuged to separate the aqueous and organic phases. The aqueous fraction was transferred to a clean test tube and basified to a pH of between 9 and 9.5 by drop-wise addition of concentrated ammonium hydroxide (NH_4OH). The basified solution was extracted twice with $CHCl_3$ (4 mL, then 2 mL). The combined $CHCl_3$ fractions were filtered through anhydrous sodium sulfate (Na_2SO_4) and then dried under a stream of nitrogen (N_2) at 50 °C to yield the crude alkaloid fraction.

The alkaloid fraction extracted from *L. leucophyllus* was reconstituted in 4 mL of methanol containing 1.3 µg/mL caffeine (internal standard). A portion (~1 mL) was transferred to 1.5-mL gas chromatograph (GC) autosample vials, and 2 µL were injected into an HP 5890 GC (HP 5890, Agilent, Palo Alto, CA) equipped with a split/splitless injector, FID detector, and a J&W DB-5 (30 m x 0.33 mm i.d.) capillary column. Injector temperature was 250 °C and operated in the splitless mode. Split vent flow rate was 60 mL/min and purged after 1 min. Oven temperature was programmed: 100°C for 1 min; 100 to 200°C at 50°C/min; and 200 to 320°C at 5°C/min. Caffeine was used as an internal standard, and plant alkaloid peaks were quantified against a 6-point anagyrine standard curve over the range of 25 to 800 µg/mL anagyrine in methanol prepared by serial dilution.

Climatic data before and during the study were taken from a National Climate Data Center weather station in Endicott, WA, and a local meteorological station in Washtucna, WA. Analysis of all data was performed using SAS (SAS Institute Inc., Cary, NC). The bite-count data were checked for normality and transformed using an arcsine square root transformation typically used for proportions ranging from 0 to 100%. All means shown here are nontransformed means. The bite-count data were analyzed using a mixed linear model (Proc Mixed in SAS), with pasture as the experimental unit; animals as a random factor nested within pasture and treatment; treatment; days; and interactions. The probability-of-difference option was used for preplanned comparisons.

Results

The mean maximum, minimum, and average temperatures during the study period were 33, 18, and 25 °C, respectively. There was no precipitation during the study. Normal January-to-June precipitation in Washtucna is 14.5 cm (NOAA 2002); during 2010, precipitation during this period was 14% above normal.

Lupine was abundant and uniformly distributed across the pastures. Mean (± SE) lupine density for plants of all phenological growth stages and across all 6 small pastures was 10.8 ± 1.0 plants/m^2. There was ample dry grass during the study, but virtually no green grass in the pastures (table 1). Conversely, there was an abundance of green forbs. Much of the green forb component was *V. cracca*, which eventually changed to the dry-forb category, with a few other weedy plants such as rush skeletonweed also present.

The anagyrine concentration in the lupine population was near or above 0.2% during the study

Table 1. Forage availability (kg/ha ± SEM) of green and dry grasses and forbs, and lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010.

Date	Dry Grass	Green Grass	Dry Forbs	Green Forbs	Lupine
July 8	701 ± 102	18 ± 9	29 ± 15	524 ± 63	766 ± 126
July 15	497 ± 39	0	85 ± 14	206 ± 37	372 ± 68
July 22	210 ± 33	0	156 ± 27	185 ± 22	281 ± 53

Table 2. Alkaloid concentration (%) of lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010.

Date	Anagyrine	Lupanine	Total alkaloids
July 8	0.21	0.09	1.06
July 15	0.24	0.03	0.85
July 22	0.19	0.03	0.81

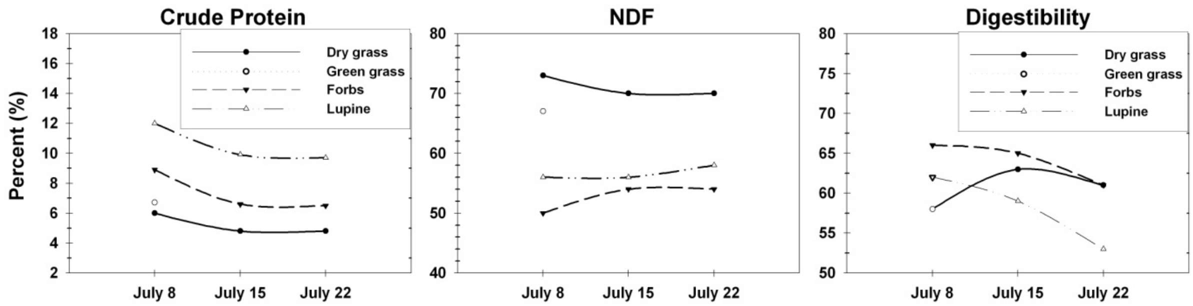

Figure 1. Nutrient content (% of dry matter) in dry grass, green grass, other forbs, and lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010. Crude protein is N x 6.25; NDF = neutral detergent fiber; digestibility = in vitro true digestibility.

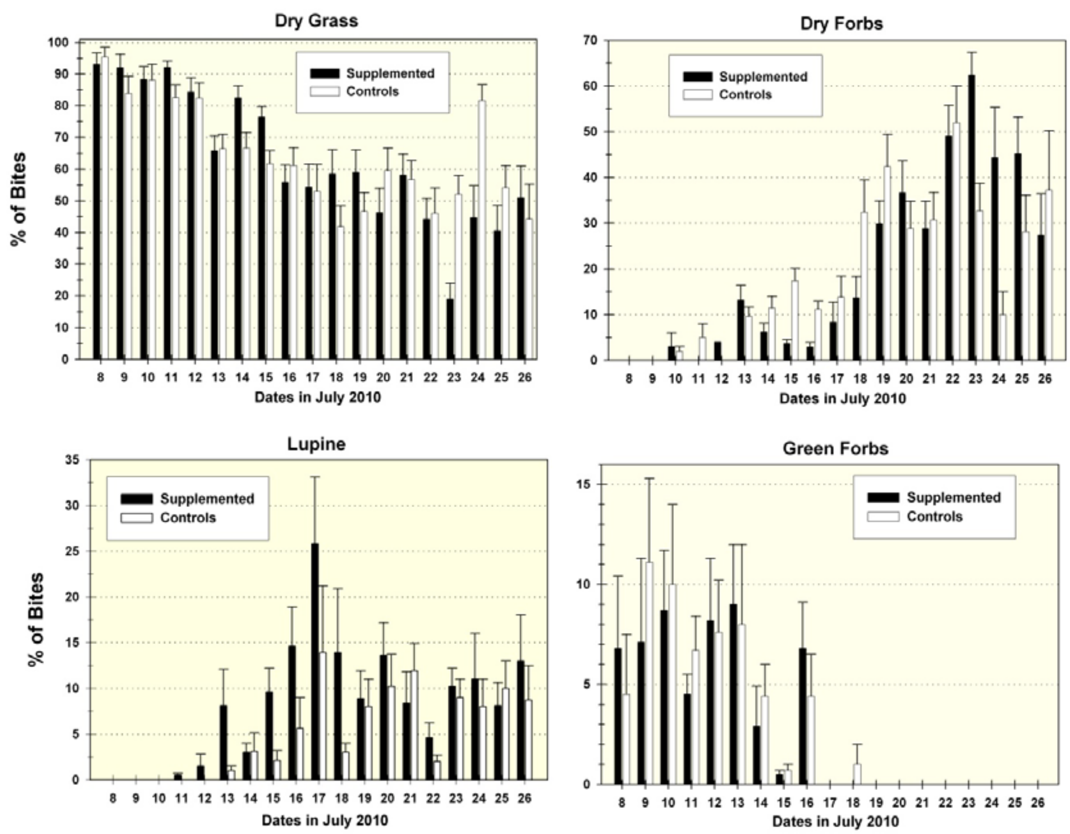

Figure 2. Diet selection (% of bites ± SE) by cattle during July 2010 when receiving either a protein supplement (soybean meal) or no supplement (controls) while grazing a lupine-infested rangeland in eastern Washington.

period (table 2). Total alkaloid concentrations exceeded 0.85% on the three sampling dates.

Over the study period, the average CP content was 5.3% for dry grass, 6.7% for dry forbs, and 7.7% for green forbs (including vetch). These values are in contrast to the average CP content of vetch alone (8.4%) and lupine (10.5%). Dry grass contained the highest concentrations of NDF (>70%), whereas lupine and green forbs contained less NDF (50–60%). As the lupine matured during the study, digestibility decreased from >60% to slightly above 50%; Digestibility of green forbs and dry grass was >60% at the start of the study and decreased only slightly during the study period (figure 1).

Dry grass and dry forbs were the major diet components during much of the study (figure 2). Cattle ate mostly dry grass during the first 8 days of the trial, then dry grass consumption became more variable (figure 2). There was a day x supplement interaction (*P* = 0.04), with control animals eating more dry grass on days 23 and 24. The consumption of forbs such as *V. cracca* increased substantially in the latter portion of the trial, but there was no treatment effect or treatment x day interaction (P = 0.08). There was no treatment (i.e., supplement) effect on lupine consumption (P = 0.68), nor was there a day x supplement interaction (P = 0.88). Numerically, supplemented animals ate substantially more lupine on several days during the trial (figure 2), but animal-to-animal variability was high, and no statistical differences were found in lupine consumption between supplemented and control animals. Overall, supplemented cattle took 6.9 ± 0.7% of daily bites as lupine compared to 5.0 ± 0.6% of bites for control cattle. Interestingly, cattle began to switch from green forbs to lupine in their diets after about 1 week on the study pastures, and this switch coincided with a decrease in available green forb biomass about midway through the trial.

One pregnant cow resorbed her fetus during the grazing study. Five other calves were born with mild to moderate arthrogryposis, scoliosis, and/or rotational defects (i.e., impaired leg movement) in the front legs (table 3). No cleft palates were noted. There was no apparent relationship between supplementation and the occurrence of birth defects.

Table 3. Deformities in calves born to pregnant cows which grazed on lupine-infested pasture from July 8 to 26, 2010, within the gestational day 40 to 70 window for birth defects. + signifies slight defects, and ++ indicates moderate defects. S= Supplement; C=Controls. No cleft palates were noted.

		Deformity		
Cow #	Treat-ment	Arthro-gryposis	Scoliosis	Leg movement
62	S	+		+
59	S			+
61	S			+
36	C		++	
57	C	++		+

Discussion

There was ample plant biomass for cattle diet selection throughout the entire study period; near the end of the study, there were declining but still-sufficient amounts of green and dry forbs, dry grass, and lupine across the pastures. It was apparent that the vegetation, including lupine and other forbs, responded favorably in terms of biomass to the wetter-than-normal winter and spring conditions. This site was selected specifically because of the abundant lupine population during some years. Because of the intentional timing of the study, the grasses were nearly all senesced when the study began. By the last week of the study, virtually all lupine plants on the study site were at least partially dry from the seasonally hot temperatures (>38 °C) and desiccating wind. Under these forage and climatic conditions, there was no effect of CP supplementation on lupine consumption by pregnant cattle.

As noted in other studies (Ralphs et al. 2006, Pfister et al. 2008), lupine is not a preferred forage in these annual rangelands, but cattle eat lupine after the grasses are senesced. Further, in this study, cattle increased lupine consumption and greatly diminished consumption of green forbs about midway through the study period. This dietary switch coincided with increased consumption of dry forbs, particularly vetch, the majority of which senesced during this period on the study site. Lopez-Ortiz et al. (2007) and Ralphs et al. (2011) also found that cattle selected lupine only after availability of annual grasses and other forbs declined. Our observations indicate that cattle typically began to graze lupine plants near the top, eating floral parts, pods, leaves, and stem, then often regrazed the same plants several times, eventually eating the plants down to short, bare stalks.

One can speculate that the cattle may have responded differently to the supplement if lower precipitation had resulted in reduced biomass for other forbs, particularly the relatively palatable vetch. The abundant vetch was consumed when green, and cattle continued to consume the vetch after it senesced. However, our previous experience in this region suggests that lupine populations are also depressed during drought (Pfister et al. 2008). Lupine density at this site was >10 plants/m^2, which contrasts greatly with lupine density measured at another nearby site during a moderate drought (1-2 plants/m^2; Pfister et al. 2008). The effectiveness of any supplementation regime to reduce lupine consumption by cattle during drought in the Scablands region is not known. However, recurring drought conditions in the Scablands region since 1997 have reduced lupine populations and diminished the incidence of crooked calf syndrome (Panter et al. 2009). Gay et al. (2007) also noted an association between lupine density in pastures and the reported incidence of “crooked calves.”

It is interesting to note that some “weedy” forbs such as rush skeletonweed are relatively palatable for cattle on these degraded annual rangelands; the stemmy skeletonweed, for example, is listed as a noxious weed in eastern Washington and elsewhere. However, at times cattle consumed relatively large quantities of this weed. On these annual rangelands, cattle also consumed large, but declining, quantities of cheatgrass and also some of the very coarse medusahead. We did not distinguish between consumption of the various annual grasses in our bite counts, but in the absence of other, more palatable green grasses, cattle readily accepted the dry annual grasses, primarily cheatgrass, as forage.

The CP content of lupine and vetch was numerically higher than other available forages such as dry grass or dry forbs. However, it is important to note that these CP concentrations are taken from clipped plant material, and numerous studies have shown that cattle can select a diet substantially higher in nutritional composition than is shown in available plant material by clipping (Weir and Torell 1959, Galt et al. 1969, Coleman and Barth 1973). That was undoubtedly true for cattle in this study as well. Thus, we speculate that even unsupplemented cattle in this study were able to meet their minimal nutritional needs for CP (about 9% CP in diet) without grazing toxic lupine on this annual grassland site during midsummer.Soybean meal is a rich source of protein, with both rumen degradable protein (RDP, 65% of CP) and rumen undegradable protein (RUP, 35% of CP). Many forage grasses contain mostly RDP (Buckner et al. 2013), at levels similar to those in soybean meal. There is no information available on the relative RDP and RUP concentrations of Scabland forbs or lupine. It is unclear whether using a protein supplement that would provide more RUP (e.g., blood meal and feather meal) would have altered the results of this study.

The anagyrine concentrations in lupine of about 0.20% found in this study are either similar or slightly higher than those noted in other studies in this area for whole plant material (Ralphs et al. 2006, Pfister et al. 2008). Floral parts and pods are typically highest in anagyrine (Ralphs et al. 2006), but these were not analyzed separately in this study. At the start of this study, pods were present but were rapidly drying and shattering. Even though cattle ate considerable lupine over a 14-day period, the birth defects we noted in calves the following spring were mild to moderate, suggesting that the lupine exposure was perhaps too brief in this study to cause severe birth defects. Another consideration is that the cattle were penned overnight during the study in order for us to use the bite-count method, and it may be that such interruptions in lupine grazing allowed circulating anagyrine concentrations to decline sufficiently to reduce the severity of defects. There is currently little information available about the anagyrine dose and duration necessary to cause defects.

We conclude that supplemental protein had no effect on lupine consumption by pregnant cattle. This study was conducted under conditions of abundant forage, including lupine and other forbs, and these conditions undoubtedly had a major impact on the findings. With normal precipitation patterns in conjunction with overgrazing, it is possible that the depletion of forbs by grazing may not go hand in hand with lupine depletion, and under this scenario the likelihood of a positive effect from protein supplementation may increase. It is unknown whether cattle grazing on lupine-infested rangelands during drought would alter their lupine consumption if given a CP supplement, because lupine populations also decline during drought along with other forage classes.

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