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Preface

We are very pleased to publish this inaugural issue of a new all-electronic journal, *International Journal of Poisonous Plant Research* (*IJPPR*), through the assistance of the USDA-ARS Information Staff, Beltsville, MD. *IJPPR* is an online-only, peer-reviewed journal published semi-annually (two issues per calendar year). The primary objective of *IJPPR* is to provide timely research results and new technology to all those working in various disciplines involving poisonous plants. The *Journal* will encompass all aspects of poisonous plant research including original research, case reports, field observations in domestic and wild animals, and scientific reviews. There currently is not any journal, electronic or printed, that specifically targets toxic plant research, and *IJPPR* aims to fill this critical gap. We've employed an electronic publication system to make the papers as widely accessible as possible on the USDA-ARS website. It is our sincere hope that *IJPPR* will contribute in a meaningful manner toward facilitating and enhancing poisonous plant research and communication around the globe.

This inaugural issue of *IJPPR* consists of papers submitted by various research scientists around the world, reflecting our objective of making *IJPPR* a truly international journal. We thank those who have assisted in the production of this inaugural issue, particularly Terrie Wierenga, Editorial Assistant, USDA-ARS Poisonous Plant Research Laboratory, Logan, UT; Sandy Miller Hays, Director, USDA-ARS Information Staff, Beltsville, MD; and Mina Chung, Supervisory Editor, USDA-ARS Information Staff, Beltsville, MD. We also thank all the individuals who have agreed to serve on the *IJPPR* Editorial Board and Editorial Advisory Board.

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Contents

Toxic Plants of Veterinary and Agricultural Interest in Colombia

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Abstract

Colombia has the second largest plant biodiversity of any country in the world, with about 25,000 species of vascular plants. This is due in part to its equatorial location, and large variation in elevation and associated gradients in temperatures and rainfall. Livestock in Colombia graze vast tracts of land with a wide variety of herbaceous and woody plants. Although the annual cattle mortality from plant poisoning in Colombia is estimated at 130,000, the economic impact on the entire livestock industry has not been fully evaluated. Information on toxic plants is scarce in Colombia, and livestock poisoning by plants is seldom documented. This review presents the current knowledge on the identity of plants known to have poisoned livestock in Colombia and on research conducted into these toxic plants. To the extent known, the toxic component(s), major clinical signs and circumstances of poisoning, location, and environmental factors are discussed. Many of the plants identified in Colombia are considered toxic on the basis of world literature, but toxicosis in Colombia has not always been documented. The information on toxic plant chemistry in Colombia is mostly limited to the plant's nitrate or cyanide content. Research is needed to determine not only which plants represent a potential risk for animal health and production but also their phytochemistry and toxicology. It is strongly recommended that veterinarians document plant poisoning cases through government reporting services and that university and government veterinarians, scientists, and extension agents investigate episodes of plant toxicosis and publish their findings. This would help identify toxic species for further phytochemical and toxicological studies and possibly pharmacological activity.

Keywords: toxic plants, Colombia, plant poisoning, livestock, pets

Introduction

Toxic plants affecting both large and small animals are a major concern for the practicing veterinarian and livestock producer in every country. In countries with higher plant biodiversity, the number of problematic toxic plants may be greater. Plant biodiversity in Colombia is very high, as there are about 25,000 species of vascular plants in Colombia, both native and naturalized (Bernal et al. 2006). This biodiversity corresponds to about 8 percent of the total vascular plants on earth, which makes the country the second largest in plant biodiversity in the world, the largest being Brazil. However, information on toxic plants in Colombia is scarce and is usually published only in local, Spanish-

language journals. Further, it is not customary among local veterinarians to write case reports, thus most of the plant poisonings that occur in Colombia are not documented in the literature.

The impact of toxic plants on Colombian livestock production has not been fully evaluated. It is estimated that more than 40 million hectares of the country are used for livestock production, with a bovine population of about 26 million animals (Ministerio de Agricultura y Desarrollo Rural, 2005). Colombia ranked ninth in the world for cattle population. However, livestock production is mostly extensive with a very low population of animals kept under intensive production systems. Land used for cattle grazing contains complex mixes of native and invasive plants (Peña et al. 1980), which may increase the risk of exposure to toxic plants, many of which have not been characterized or have only been partially characterized. Conservative estimates indicate that in Colombia, toxic plants cause an annual mortality rate of about 0.5 percent (Peña et al. 1980), which is currently equivalent to about 130,000 cattle. This percentage is in agreement to that reported by Tokarnia et al. (2002) in Brazil, who estimated that between 800,000 to 1,120,000 cattle of the 160-million population die annually from plant poisoning, a mortality rate corresponding to 0.5 to 0.7 percent.

The aim of the present review is to briefly describe the most important native and introduced toxic plants present in Colombia that affect animals and to summarize the published research on these toxic plants, highlighting research conducted in Colombia.

Major Toxic Plants Affecting Animal Health and Production in Colombia

Plants were grouped based on the major organ system affected by consumption of the plant. Common names given to these plants in Colombia are provided in parentheses after the Latin botanical name or indicated in one of the tables. It is important to note that Colombia is a tropical country located on the Equator, which means that there are no seasons such as winter, spring, summer, or fall, and that annual plants behave as perennials under these conditions. The average environmental temperature is mostly determined by elevation with distinct temperature gradients from low- to high-elevation rangelands. There are, however, "dry" and "rainy" seasons when low or high precipitation is expected every year. The times of the year with higher precipitation rates are April through May and October through November. The start of the rainy period is accompanied by intense growth of some plant species used for animal feed (especially grasses), a situation that is usually associated with increased accumulation of potentially toxic compounds such as nitrates. However, some plants accumulate more toxins during the dry periods, for example, the native plant *Mascagnia concinna*, which accumulates more cyanogenic glycosides during these periods. Colombia is politically divided into "departments" (states), and sometimes this word is used to indicate a specific geographical region.

Plants That Affect the Digestive System

Plants That Cause Irritation of the Oral Cavity

Many plants belonging to the Araceae family contain needle-shaped calcium oxalate crystals in their leaves. These crystals are known as raphides and are housed inside specialized cells known as idioblasts (Genua and Hillson 1985). When the plant leaves are chewed, the idioblasts are broken and the oxalate crystals are expelled, causing an immediate burning sensation in the oral cavity tissues. Plants that accumulate calcium oxalates are fairly common in Colombia and some of them are even native to the country, such as *Dieffenbachia picta* (cucaracho), recognized as the most toxic of all Araceae plants (Cao 2003). The genus *Dieffenbachia* comprises about 135 species, most of them present in South America. Colombia has the highest biodiversity with 37 species, followed by Ecuador with 34, Peru with 30, Brazil with 27, Panama with 20, and Costa Rica with 13 (Croat 2004). Although toxicosis by *D. picta* in livestock is rare, the ingestion of its leaves has caused intoxication in humans and pets. In dogs, the oxalates of *D. picta* can cause severe inflammation and necrosis of the epithelium of the tongue and oral cavity and may even cause death (Loretti et al. 2003). Besides calcium oxalates, *D. picta* contains proteolytic enzymes that induce histamine release causing a severe inflammatory response that may lead to asphyxia and death (Loretti et al. 2003).

Other plants of the Araceae family common in Colombia are *Alocasia macrorrhiza* (rascadera, bore, taro gigante), *Caladium* spp. (caladio, rascadera), *Monstera deliciosa* (abalazo, balazo), and *Philodendron* spp. (balazo). With the exception of *Alocasia macrorrhiza*, these plants are all native

to tropical America. These plants contain reduced concentrations of calcium oxalate compared with *D. picta*, and they are rarely associated with toxicological problems.

Plants That Affect the Gastrointestinal Tract

Ricinus communis (castor, higuerilla, palmacristi, ricino) is a naturalized plant common in Colombia from sea level to 2600 m elevation. *R. communis* seeds contain ricin, one of the most potent lectins known. In general, lectins cause necrosis of the cells lining the gastrointestinal tract. Ricin is comprised of two subunits: Unit B (for binding) is the actual lectin that binds to galactosyl residues in cellular membranes, whereas unit A (for activity) is an enzyme capable of inactivating ribosomes in eukaryotic cells (Barbieri et al. 1993). All animal species are sensitive to the effects of ricin. The toxicosis, however, is uncommon and it is usually associated with feeding garden clippings or with contamination of forage grasses with *R. communis* trimmings (Aslani et al. 2007). Clinical signs include weakness, salivation, profuse aqueous diarrhea, dehydration, mydriasis, teeth grinding, hypothermia, and recumbence; the major postmortem finding is severe gastroenteritis (Aslani et al. 2007). Other plants present in Colombia that contain potentially toxic lectins in their seeds are *Jatropha curcas* (piñón de fraile, purga de fraile), *Abrus precatorius* (chochos de pinta negra, jetiriquí) and *Canavalia ensiformis* (canavalia, fríjol blanco). The lectins present in these plants correspond to curcin, abrin, and concanavalin A, respectively. However, toxicosis with these plants has not been documented in Colombia.

Another plant compound highly irritating to the gastrointestinal mucosa is ricinoleic acid, a fatty acid present in *Ricinus communis* seeds, considered to be responsible for the cathartic properties of ricin oil. Ricinoleic acid is an irritant that alters the intestinal epithelium causing loss of water and electrolytes, increased loss of luminal DNA, and decreased enzymatic activity of enterocytes (Bretagne et al. 1981).

Plants That Affect the Blood

Plants Causing Hemolytic Anemia

Feeding culled onions has been associated with hemolytic anemia in cattle and other animal species. *Allium cepa*, which includes all types of onions, is capable of causing toxicosis in both large and small animals due to its content of organic sulfoxides, especially alkyl or alkenyl cysteinyl sulfoxides (Rae 1999, Parton 2000). After ingestion, the organosulfoxides are transformed into a complex mixture of organic sulfur compounds, some of which are capable of causing intravascular hemolysis in cattle, sheep, and horses. Onion toxicosis, which occurs sporadically in cattle in Colombia, has been extensively documented in the literature with the first case reported in 1909 (Goldsmith 1909). The toxicosis occurs because cattle readily eat onions and usually prefer them to high-quality forages or grains (Rae 1999). The excessive intake of onions leads to hemolytic anemia and methemoglobinemia, which develops within a week of onion ingestion. Clinical signs in cattle include diarrhea, hemoglobinuria, ataxia, and coma. Cattle are more sensitive than horses, and goats are the most resistant. The hemolytic anemia caused by onion ingestion can also occur in dogs and cats (Parton 2000).

Another plant that causes intravascular hemolysis is *Brassica oleracea* (col silvestre), several varieties of which are used as forage for ruminants. *B. oleracea* contains the non-protein amino acid *S*-methyl cysteinyl sulfoxide (SMCO), which is reduced in the rumen to dimethyl disulfide, a hemolysin (Duncan and Milne 1993). The anemia induced by the intravascular hemolysis may be lethal in cattle, which are very sensitive to the hemolytic effects of SMCO (Prache 1994).

Plants Causing Methemoglobinemia

The nitrite ion, which is formed by bacteria in the rumen from plant nitrate, is the major cause of methemoglobinemia in ruminants. Methemoglobin is an abnormal form of hemoglobin in which its normal ferrous moiety (Fe^{2+}) oxidized to the abnormal ferric form (Fe^{3+}). The oxidized form is not capable of transporting oxygen and there is a decrease in the oxygenation capacity of the blood. The severity of the clinical signs and effects depends on the amount of methemoglobin formed. Signs of hypoxia develop when 20 to 30 percent of the hemoglobin is converted to methemogloblin, and death can occur at 70 to 80 percent methemoglobin levels (Vermunt and Visser 1987). Many plants have been identified as accumulators of toxic nitrate levels in Colombia (table 1), this being one of the most common plant toxicosis recognized in cattle.

As shown in table 1, the most important group of plants responsible for nitrate poisoning in cattle are forage grasses with at least nine species known to be associated with nitrate poisoning. An example of the high nitrate levels present in Colombian grasses is the study of Trheebilcock et al. (1978), who analyzed samples of *Panicum maximum* from the northern part of the country (Departments of Córdoba and Sucre). The average nitrate levels found were 1209 and 5260 ppm for fresh plants collected during the dry season and after the onset of the rainy season, respectively. Also in Colombia, Guzmán et al. (1978) reported a case in the Valle del Cauca Department that caused acute mortality in 19 of 64 steers that were fed cut *Pennisetum purpureum*. The grass was found to contain 445 ppm nitrate and 971 ppm nitrite; the high nitrite content was attributed to microbial processes.

The Amaranthacea family also contains plants associated with nitrate poisoning in cattle. *Amaranthus dubius* and *A. spinosus* are two species of *Amaranthus* common in Colombia that have been associated with nitrate intoxication, especially during the transition between the dry and the wet seasons (Torres 1984a). Another Amaranthacea is *Chenopodium album*, a plant recently reported in Colombia (Fernández-Alonso and Hernández-Schmidt 2007), which can cause lethal intoxication in ruminants because of its high nitrate levels (although it can also accumulate soluble oxalates). Levels of 2,500 ppm nitrate-nitrogen were reported in *Chenopodium album* hay associated with mortality in cattle (Ozmen et al. 2003). Other *Chenopodium* spp. such as *C. ambrosoides* and *C. quinoa* are considered native to Colombia but have not been reported as toxic.

Another plant associated with high nitrate content is *Mascagnia concinna*, a vine of the Malpighiaceae family native to the Magdalena Valley of Colombia. Nitrate concentrations ranging from 5,300 to 29,200 ppm dry matter were reported by Torres (1984a) and from 1,555 to 10,763 in fresh material by Trheebilcok et al. (1978).

The Phytolaccaceae *Petiveria alliacea* can also contain toxic levels of nitrate. Studies conducted in Colombia with fresh plants showed that during the dry season, the plant accumulates an average of 1,155 ppm nitrate but during the rainy season, the average levels are 7,867 ppm (Trheebilcock et al. 1978). *Heliotropium indicum* is a Boraginaceae also known to accumulate toxic concentrations of nitrates. In samples collected in the northern part of the country, Trheebilcock et al. (1978) found average nitrate levels of 178 and 7,195 ppm in fresh material collected before the rainy season and immediately after the start of the rainy season, respectively.

Plants That Affect the Coagulation of Blood

Cumarinic glycosides can be found in *Anthoxanthum odoratum* (Poaceae) and in *Melilotus* spp. (Fabaceae). *Anthoxanthum odoratum* (oloroso) was introduced in Colombia during colonial times,

Table 1. Major nitrate-accumulating plants affecting livestock in Colombia

Family	Latin name	Common name
Amaranthaceae	*Amaranthus dubius*	Adormidera, bledo liso
	Amaranthus hybridus	Amaranto, bledo chico
	Chenopodium album	Quenopodio
Boraginaceae	*Heliotropium indicum*	Verbena, rabo de alacrán
Malpighiaceae	*Mascagnia concinna*	Mindaca, mataganado
Poaceae	*Andropogon bicornis*	Barba de indio, cola de zorro
	Brachiaria mutica	Pasto pará
	Lolium perenne	Balico, raigrás inglés, raigrás perenne
	Panicum maximum	Pasto guinea, siempreverde
	Paspalum paniculatum	Paja brava, paja del camino
	Paspalum virgatum	Gramalote, yerba peluda
	Penisetum purpureum	Pasto elefante
	Sorghum bicolor	Sorgo, sorgo forrajero
	Sorghum halepense	Pasto Johnson, capim argentino
Phytolaccaceae	*Petiveria alliacea*	Anamú
Solanaceae	*Solanum nigrum*	Campano, yerbamora

and it is common in cold regions of the country at altitudes from 2,600 to 3,500 m above sea level (Fernández-Alonso and Hernández-Schmidt 2007). *Melilotus albus* and *M. officinalis* (trébol dulce) were also introduced, and they are currently considered part of the naturalized flora of Colombia (Bernal et al. 2006, Fernández-Alonso and Hernández-Schmidt 2007). When hay from these plants becomes moldy, the cumarinic glycosides can produce dicumarol, an anticoagulant that causes depletion of active vitamin K in the liver resulting in reduced clotting factors being released into the blood (Hallak and Wedlund 1991). The toxicosis by *A. odoratum* and *Melilotus* spp. in cattle has been well documented in the world literature (Pritchard et al. 1983, Puschner et al. 1998, Runciman et al. 2002). Affected animals are weak and reluctant to move, show petechial hemorrhages in mucosal surfaces, may bleed from natural orifices, and show increased prothrombin and partial thromboplastin time. At postmortem examination, multiple petechial and ecchymotic hemorrhages are seen. Uncoagulated blood may also be seen in any of the body tissues and cavities including the chest and abdomen, depending on the activity of the animal.

Cardiotoxic Plants

Cardiac glycosides are a specific type of toxic glycosides that affect the cardiac muscle, sometimes causing fatal toxicosis. Cardiac glycosides increase the contraction force of the heart by inhibiting the myocardial Na-K ATP-ase, which can lead to cardiac arrest (Poindexter et al. 2007). Two types of cardiac glycosides are recognized depending on their chemical characteristics, namely, cardenolide and bufadienolide glycosides. At least four plants containing cardenolide cardiac glycosides are present in Colombia: *Digitalis purpurea* (dedalera, digital, guargüeron), *Nerium oleander* (oleander, delfa, adelfa, azuceno de La Habana), *Thevetia peruviana* (catapis, oleander amarillo), and *Asclepias curassavica* (bencenuco, mataganado). All have sporadically caused toxicosis in herbivores.

Digitalis purpurea was introduced into Colombia as an ornamental by British engineers around 1856; and it currently grows wild in highlands, including the high plateau where Bogotá is located, at 2,640 m above sea level. *D. purpurea* contains cardiac glycosides in all parts of the plant but the concentration is higher in the leaves.

Nerium oleander is a perennial bush native to the Mediterranean region and Asia but now is common in all tropical and subtropical regions of the world. In Colombia it is cultivated as an ornamental for its colourful flowers, which can be white, pink, yellow, or red. All parts of the plant contain cardiac glycosides with oleandrin being the most abundant. Toxicosis has occurred in horses and cattle usually due to contamination of pastures with plant clippings from *N. oleander* bushes. *Thevetia peruviana* (= *T. nereifolia*, *Cascabela peruviana*, and *C. thevetia*) is a bush native to South America, similar to *Nerium oleander* but smaller, with narrower leaves and only yellow flowers. This plant contains cardenolide cardiac glycosides, primarily thevetin A and thevetin B, especially in the seeds (Roberts et al. 2006). There are reports of human toxicosis caused by this plant, generally associated with the intake of the seeds. Intake of one or two seeds causes gastrointestinal symptoms, and intake of three or four seeds affects the heart and may cause death (Roberts et al. 2006). In the city of Medellín (Department of Antioquia), seeds of *T. peruviana* were sold as a weight loss aid and several women died after eating the seeds (López 2002).

Asclepias curassavica is a plant native to the Caribbean but now is commonly found in Colombia at elevations up to 1,600 m. This plant contains asclepine, a cardiac glycoside with higher potency than strophantin, digoxine, digitoxine, and digitoxigenine (Patnaik and Köhler 1978), which are some of the most potent cardiac glycosides known. Even though the plant is not palatable for herbivores, it has been associated with sporadic cases of toxicosis in cattle.

Hepatotoxic Plants

The main hepatotoxic plants present in Colombia affect the liver by causing either hepatocellular necrosis or intrahepatic cholestasis. Pyrrolizidine alkaloids (PAs) are a large group of hepatotoxins characterized by the presence of a pyrrolizidine nucleus in their structure and are capable of causing hepatocellular necrosis. Compounds in plants known to cause intrahepatic cholestasis are the lantadenes from *Lantana* spp.; sporidesmin, a mycotoxin formed by a fungus on grasses; and the steroidal saponins present in several grasses. All hepatotoxins may cause secondary photosensitization in ruminants due to an alteration in the metabolism of

chlorophyll leading to skin damage when ruminants are exposed to the sun.

Plants Containing Substances That Cause Hepatocellular Necrosis

The PAs are a large group of hepatotoxins present in plants found in Colombia, and PA toxicosis has been reported in livestock, poultry, pigs, and humans in Colombia. Extensive literature reviews on the chemistry, mechanism of action, and effects of PAs in animals and humans have been published (Mattocks 1986, Diaz 2001, Fu et al. 2004, Rietjens et al. 2005). In general, PAs induce hepatocyte necrosis that progresses to the destruction of the parenchymal cells of the organ and eventually to liver failure. PAs are also potent carcinogens at levels below those causing hepatic necrosis. Even though PAs are mainly hepatotoxic, some of them can also affect the lungs, especially monocrotaline.

More than 6,000 plants are believed to contain PAs, many of which are present in Colombia in all kinds of ecosystems. The most important PA-producing plants from the toxicological standpoint belong to one of the families Asteracea, Fabaceae, or Boraginaceae. Table 2 summarizes the major PA-containing plants present in Colombia.

Among the Asteraceae family (formerly known as Compositae) the most important hepatotoxic genera are *Senecio* and *Eupatorium*. Two toxic species of *Senecio* common in Colombia are *Senecio formosus* and *S. madagascariensis*. The former is a plant native to the highlands between 3,000 and 4,000 m above sea level and commonly found in the Colombian Andean regions of Cundinamarca, Cauca, and Nariño. There are no reports of toxicosis in animals caused by this plant; however, *Senecio formosus* has caused irreversible hepatic damage in human patients who ingested infusions made with its dry leaves. The clinical history, symptoms, signs, lesions, and postmortem findings of almost 20 fatal cases reported in Bogotá were documented by Toro et al. (1997).

Senecio madagascariensis is an annual or perennial herb native to South Africa reported for the first time in Colombia in the 1980s. It is an aggressive weed that propagates rapidly, and it has already colonized all the high plateau of the departments of Cundinamarca and Boyacá (Fernández-Alonso and Hernández-Schmidt 2007).

Horses are highly sensitive to the PA of *S. madagascariensis* and can even be intoxicated in utero. In Australia, Small et al. (1993) reported a case where a foal exhibited growth retardation and jaundice at birth and died at 2 months of age with liver damage. During gestation the mare was kept in a field heavily infested with *S. madagascariensis*, which resulted in fetal exposure in utero. In Colombia, *S. madagascariensis* has been associated with sudden death in cows immediately after parturition. The cause of this sudden death syndrome is unknown, but it is possible that the metabolic changes associated with parturition and the onset of lactation pose an extra load to a liver that has been severely affected by the chronic ingestion of the plant. Burgueño-Tapia et al. (2001) analyzed *S. madagascariensis* plants collected in Colombia and found that the plants contain chemical substances known as calolides. However, the toxicological with plants from Australia or Hawaii although the concentration was lower. The total concentration of PAs in samples from Australia, Hawaii, and Colombia was 3,089, 2,133, and 805 µg/g, respectively. The major PAs found in the Colombian samples were senecivernine, senecionine, integerreimine, mucronatinine, and usaramine (D.R. Gardner and G.J. Diaz, 2009, unpublished data). The other genus of the Asteracea family reported to accumulate PA is *Eupatorium*. Several species of this genus have been reported in Colombia (Powell

Table 2. Major pyrrolizidine alkaloid-producing plants reported in Colombia

Family	Latin name	Common name
Asteraceae	*Eupatorium spp.*	Amarguero, chilico, hierba de chivo
	Senecio formosus	Arnica, árnica de páramo, árnica de Bogotá
	Senecio madagascariensis	Manzanilla del llano
Boraginaceae	*Borago officinalis*	Borraja
	Cynoglossum spp.	Cinoglosa, lengua de perro
	Heliotropium europeum, H. indicum	Verbena, rabo de alacrán
	Symphytum officinale	Consuelda, consuelda mayor
Fabaceae	*Crotalaria spp.*	Crotalaria, cascabel, cascabelito

and King 1969), including *E. inulaefolium*, which has been reported as hepatotoxic for cattle in other countries (Sharma et al. 1998). Another toxic *Eupatorium* species in Colombia is *E. stochaedifolium*, whose leaves and flowers were reported by Pérez-Arbeláez (1931) as toxic. However, no information on the toxic components of the plant or its effects in animals or humans was provided.

Within the Fabaceae family, the genus *Crotalaria* is notorious for the high PA content of some of its plants. In Colombia, *Crotalaria* spp. grow from sea level to about 3,000 m above sea level, especially in areas with clearly defined dry periods such as the inter-Andean valleys, the northern part of the country, and the eastern savannas known as the llanos. These plants grow as weeds in well-fertilized soils used to grow corn, sorghum, or soybeans, and their seeds may contaminate these agricultural crops. At least 19 species of *Crotalaria* are present in Colombia (Bernal 1986) and some are recognized as toxic, including *C. spectabilis*, *C. retusa*, *C. sagittalis*, and *C. pallida*. *Crotalaria* poisoning in Colombia has been reported in pigs, goats, laying hens, and broiler chickens. In 2001 large losses were caused to the poultry and pig industry when sorghum grain contaminated with *C. retusa* seeds was used to prepare mixed rations for monogastric animals. The level of contamination in sorghum lots with *C. retusa* seeds ranged from 2 to 5 percent (G.J. Diaz, 2009, unpublished data). These levels are extraordinarily high since a level of just 0.05 percent (equivalent to one *C. retusa* seed per 65,000 sorghum seeds) was associated with lethality in pigs (Hooper 1978). *Crotalaria pallida* is another toxic *Crotalaria* sp. present in Colombia. A natural outbreak of *C. pallida* poisoning was reported in goats in the Department of Santander (Canchila 2001), and experimentally, *C. pallida* seeds were found to be highly toxic to broiler chickens (Diaz et al. 2003).

The third family of plants known to accumulate high levels of PAs is the Boraginaceae . A total of 13 genera of this family have been reported in Colombia, including the toxic genera *Heliotropium*, *Symphytum*, and *Cynoglossum* (Barajas-Meneses et al. 2005). The *Heliotropium* genus is represented by at least 9 species, which are widely distributed from 0 to 3,200 m above sea level (Barajas-Meneses et al. 2005). The main toxic *Heliotropium* species reported in Colombia are the introduced species *H. europaeum* and *H. indicum*, the latter containing not only PAs but also toxic concentrations of nitrates. The other *Heliotropium* species present in Colombia (e.g. *H. angiospermum*, *H. peruvianum*, *H. procumbens*, *H. salicioides*, and *H. ternatum*.) have not been studied to determine their potential adverse effects in animals or humans. Another toxic Boraginaceae present in Colombia is *Symphytum officinale*, a perennial herb native to Europe and recently reported in Colombia (Fernández-Alonso et al. 2007). Similar to *Senecio formosus*, *Symphyum officinale* represents mainly a risk for humans and no cases of toxicosis in animals have been reported. The ingestion of tea made with *S. officinale* leaves has caused liver damage in human patients, and the sale of the dry leaves of the plant has been banned in countries such as Germany and Canada (Stickel and Seitz 2005). *Cynoglossum officinale* is another Boraginaceae known to contain hepatotoxic PA. Calves dosed with 60 mg/kg of PA from *C. officinale* died within 24 h with massive hepatocellular necrosis and liver hemorrhages (Baker et al. 1991). *C. officinale* has not been reported in Colombia but two other species of *Cynoglossum* (*C. amabile* and *C. trianaeum*) were reported by Barajas-Meneses et al. (2005). The presence of *C. officinale* in Colombia cannot be ruled out because it is considered a cosmopolitan plant. The toxicology of *C. amabile* and *C. trianaeum* has not been investigated.

Plants That Cause Intrahepatic Cholestasis

Lantana camara (venturosa, sanguinaria, lantana) is a tree or bush of the Verbenaceae family native to tropical America. In Colombia, it is a common plant in all ecosystems from sea level to 2,500 m elevation. The phytochemistry of *L. camara* is complex as it contains a wide variety of chemical substances, including triterpenes, mono and sesquiterpenes, iridoid and phenyl ethanoid glycosides, nafthoquinones, and flavonoids, among other compounds (Ghisalberti 2000, Sharma and Sharma 2007). The hepatotoxic action of *L. camara* has been attributed to two pentacyclic triterpenes known as lantadene A and B. The lantadene content in *L. camara* plants is variable, and potentially toxic plants contain at least 80 and 200 mg/kg of lantadenes A and B, respectively (Ghisalberti 2000). In practice, this is equivalent to a dosage of 40 g of

fresh material per kilogram of weight. Lantadenes are biotransformed by hepatic cytochrome P-450 enzymes into toxic compounds that damage the bile canaliculi, producing intrahepatic cholestasis and impairment of the normal flow of bile (Sharma and Sharma 2007). The primary toxic action of the lantadenes may result in secondary photosensitization due to the reduced excretion of phylloerythrin, a natural metabolite product of the anaerobic fermentation of chlorophyll and normally excreted in bile (Johnson 1982). Disruption in the biliary elimination of phylloerythrin increases its blood level and deposition in subcutaneous tissues. In non-pigmented areas of the skin or in areas without dark hair, phylloerythrin reacts with solar light, forming reactive molecules that damage the local tissue causing erythema, edema, inflammation, and necrosis of the epidermis. *Lantana camara* toxicosis can affect cattle, sheep, goats, horses, and buffaloes. Apart from *L. camara,* there are at least 14 species of *Lantana* present in Colombia (Bernal et al. 2006), whose toxicology and potential adverse effects in animals have not been investigated.

Plants that contain steroidal saponins may also cause intrahepatic cholestasis in cattle but through a different mechanism of action than lantadenes. The toxic effect of the steroidal saponins is related to their normal metabolism in the rumen (Graydon et al. 1991). The first step in the metabolism of steroidal saponins is a rapid hydrolysis in the rumen that releases the corresponding sugars and aglycones (sapogenins). The sapogenins are then absorbed and transported to the liver where they are conjugated with glucuronic acid and excreted in the bile. Once in the bile, they form insoluble calcium salts of sapogenin glucuronate that precipitate inside and around the biliary ducts (Graydon et al. 1991). These glucuronate crystals block the normal secretion of bile, which in turn disrupts the normal secretion of phylloerythrin, the compound responsible for the secondary photosensitization. The major sapogenin responsible for hepatogenous photosensitization is epismilagenine (Miles et al. 1992). Most of the plants that contain toxic levels of steroidal saponins in Colombia belong to the Poaceae family (grasses) and include *Brachiaria brizantha* (pasto alambre), *Brachiaria decumbens* (braquiaria), *Panicum coloratum* (pasto Klein), *Panicum maximum* (pasto guinea), and *Pennisetum clandestinum* (kikuyo). Toxic effects have been reported but not confirmed. Alternatively, *B. brizantha* and *B. decumbens* can also induce secondary photosensitization in cattle, sheep, and goats due to hepatic damage from the hepatotoxic compound sporidesmin, a mycotoxin produced by the fungus *Pithomyces chartarum*. This toxicosis has been observed sporadically in Colombia. The mechanism of action of sporidesmin involves the formation of reactive oxygen species that damage the biliary canaliculi (Morris et al. 2004). *Sapindus saponaria* (chambimbe, jaboncillo, pepo) is a tree native to the tropical humid forests of Colombia (600-2,000 m above sea level) that grows up to 12 m in height. In Colombia, ingestion of *S. saponaria* by cattle has been associated with hepatotoxicity and photosensitization (Torres 1984b), which could be explained by its content of saponins (Tsuzuki et al. 2007). However, the toxic component of *S. saponaria* to cattle is still not confirmed. Phytochemical studies conducted by Wahab and Selim (1985) showed that this plant contains flavonoids (in leaves and twigs), tannins, essential oils, anthraquinones (in twigs), β-sitosterol, α and β-amirin (in seeds), rutin, luteolin, and 4'-methoxyflavon (in seeds and leaves). The saponins of *S. saponaria* are toxic to fish and have traditionally been used by indigenous people for fishing (Quigley 1956).

Trema micrantha (Ulmaceae), a plant reported as hepatotoxic for horses and ruminants in Brazil (Gava et al. 2010), occurs in Colombia where it is known as zurrumbo, majagua, verraquillo, and other names depending on the geographical region (Bernal et al. 2006). *Xanthium* spp. (Asteraceae) containing the hepatotoxic compound carboxyatractyloside (Witte et al. 1990) are also found in Colombia: *X. cavanillesii* (cadillo), *X. spinosum* (casamarucha), and *X. strumarium* (cadillo, cardo) (Bernal et al. 2006). However, no cases of toxicosis associated with these plants have been documented.

Plants That Affect the Urinary System

Urinary bladder tumors in cattle have been associated with the intake of *Pteridium aquilinum* (helecho macho, helecho liso). This weedy plant found worldwide grows in well-drained, acid soils and open lands and is common in the eastern part of Colombia. Cattle readily eat the plant when it is still young; old plants are normally not eaten unless there are no other plants in the pasture. This plant contains at least two important toxic components: a thiaminase capable of destroying vitamin B1 and a mutagenic carcinogenic glycoside known as ptaquiloside (Smith 1997). In Colombia the toxicosis

by *P. aquilinum* has been mainly associated with a disease in cattle known as bovine enzootic hematuria, which causes economic losses in some Departments where dairy cattle are raised (Pedraza et al. 1983). The toxicosis results from the chronic intake of ptaquiloside and its major sign is hematuria caused by the development of multiple bleeding tumors in the bladder mucosa (Pedraza et al. 1983, Smith 1997). The glycoside can be excreted in the milk (Alonso-Amelot et al. 1997) and in Costa Rica and Venezuela, the intake of milk from cows feeding on *P. aquilinum* has been associated with an increased incidence of gastric cancer (Alonso-Amelot 1997). The incidence of gastric cancer in humans who consume milk from cows exposed to *P. aquilinum* has not been investigated in Colombia.

High levels of soluble oxalates that chemically correspond to sodium or potassium salts of oxalic acid (Diaz 2001) are a common cause of plant-induced nephrotoxicity. Soluble oxalates are readily absorbed in the systemic circulation where they can react with blood calcium, causing hypocalcemia and tetania. Oxalates eventually form insoluble calcium oxalate crystals that block the renal tubules (James and Butcher 1972). Precipitation of calcium oxalate crystals in the kidney leads to anuria, uremia, and acute renal failure. Soluble oxalate toxicosis is more common in ruminants because the plants that contain them are usually more palatable and readily eaten compared with plants containing insoluble oxalates. At postmortem examination there are edema and hemorrhages of the ruminal mucosa and kidney inflammation (James and Butcher 1972). Most of the soluble oxalate-accumulating plants of toxicological interest in Colombia belong to the Poaceae (grasses), Amaranthaceae, and Polygonaceae families.

Native or naturalized grasses known to accumulate potentially toxic levels of soluble oxalates include *Brachiaria humidicola* (braquiaria alambre), *Cenchrus ciliaris* (pasto buffel), *Digitaria decumbens* (pasto pangola), *Panicum maximum* (pasto guinea, india, siempreverde), *Pennisetum clandestinum* (kikuyo), *Pennisetum purpureum* (pasto elefante), and *Setaria sphacelata* (setaria, pasto miel). In horses, prolonged intake of tropical grasses containing soluble oxalates can lead to secondary hyperparathyroidism or osteodystrophia fibrosa (Cheeke 1995). This problem is caused by reduced calcium absorption from the gut due to the reaction of the soluble oxalate with the dietary calcium, forming calcium oxalate. Levels of 0.5 percent or more soluble oxalate in forage grasses may cause nutritional hyperparathyroidism in horses, while levels of 2 percent or more may cause acute toxicosis in ruminants (Cheeke 1995). The oxalate content in grasses is highest during rapid growth, such as after the onset of the rainy season, and may reach levels of 6 percent or more dry weight. However, soluble oxalate toxicosis has not been documented in Colombia.

From the Amaranthaceae family, the highly toxic plant *Halogeton glomeratus* (James and Butcher 1972) has not been reported in Colombia, but there are about 20 *Amaranthus* species including *A. retroflexus* and *A. hybridus*, two introduced invasive and toxic weeds. These two weeds contain both soluble oxalates and nitrates although the toxicosis is generally associated with their oxalate content. Acute renal failure and perirenal edema have been reported worldwide in cattle, sheep, pigs, and horses that ate these plants (Last et al. 2007). Signs and lesions in cattle include weakness, ataxia, high blood urea levels, proteinuria, perirenal edema, and nephrosis.

Another common plant in Colombia that accumulates potentially toxic levels of soluble oxalates is the Polygonaceae *Rumex crispus* (lengua de vaca, romaza). The toxicosis by *R. crispus* affects mainly sheep although it can also affect cattle, which can die acutely after eating high amounts of the plant. The soluble oxalate content in *R. crispus* can be as high as 6.6 to 11.1 percent dry matter (Panciera et al. 1990); however, levels of soluble oxalate in *R. crispus* in Colombia have not been investigated.

Plants That Affect the Nervous System

Plants That Block the Neuromuscular Junction

Conium maculatum is native to Europe and naturalized in Colombia and is commonly found along roadsides and close to irrigation waters, usually between 1,200 and 2,800 m above sea level. *Conium maculatum* contains at least five main piperidine alkaloids, of which the most important are coniine (mainly in the seeds) and γ-coniceine (in vegetative tissue). The other three alkaloids are *N*-methylconiine, conhydrine, and pseudoconhydrine. In world literature, the toxicosis has been reported in horses, pigs, sheep, and cattle, with cattle the most sensitive species. The clinical signs of *Conium maculatum* poisoning in domestic animals and humans were reviewed by Panter et al. (1988) and more recently by Vetter (2004). Coniine, γ-

coniceine, and *N*-methylconiine cause paralysis of the musculature due to the blockade of the neuromuscular junctions. The initial signs of the acute toxicosis include muscle weakness, tremors, incoordination, and mydriasis, followed by bradycardia, depression, coma, and death from respiratory failure. Poultry species (turkeys, geese, and quail) show ataxia and inability to fly (Frank and Reed 1987). The closely related toxic plant of the same family (Apiaceae), known as waterhemlock (*Cicuta* spp.), has not been reported in Colombia.

Plants That Affect the Central Nervous System (CNS)

Ipomoea carnea (batatilla, tapabotija, bejuco pupú, campanuela) is native to tropical and subtropical America and grows spontaneously in the eastern part of Colombia and other warm parts of the country. It is used as an ornamental and can become a weed in pastures, especially in the eastern region of the country. Antoniassi et al. (2007) and Armién et al. (2007) showed that *Ipomoea carnea* subsp. *fistulosa*, a subspecies present in Colombia, affects the central nervous system of cattle, sheep, and goats in Brazil. The toxic compound of this plant was found to be the indolizidine alkaloid swainsonine that inhibits lysosomal hydroxylases, particularly the enzyme α-mannosidase. Swainsonine causes a cellular alteration known as lysosomal storage disease, characterized by excessive carbohydrate accumulation within the lysosomes (Jolly and Walkley 1997). Livestock exposed to the toxin fail to gain weight and exhibit neurological alterations including failure to apprehend and swallow feed, hypermetria, and ataxia (Antoniassi et al. 2007). Postmortem examination reveals no macroscopic changes but histological lesions in neurons can be seen. In Colombia, *Ipomoea carnea* is considered to be one of the most important toxic plants for cattle in the Arauca river valley (Vargas et al. 1998). *Ipomoea* spp. can also accumulate ergot alkaloids and calystegines. Other plants known to accumulate swainsonine include species of the genera *Astragalus*, *Oxytropis*, *Swainsona*, and *Sida*. Of these genera, only *Sida* has been reported in Colombia (Bernal et al. 2006) but not the toxic species *Sida carpinifolia* (Seitz et al. 2005).

Grasses That Cause Neurological Signs

Phalaris aquatica (formerly known as *P. tuberosa*) and *P. arundinacea* are two species of *Phalaris* naturalized in Colombia. These grasses are of low palatability, and consumption causes diarrhea. In cattle and sheep these grasses may cause acute toxicosis with sudden death, subchronic toxicosis with transient neurological signs, or chronic toxicosis with permanent neurological damage (Bourke et al. 2003). Clinical signs are mostly neurological and include ataxia, aimless walking, muscular fasciculation, tremors, opisthotonus, excessive salivation, tetanic spasms, and limb paddling (Bourke et al. 2005). The toxic effects of *P. aquatica* and *P. arundinacea* are attributed to their alkaloid content. These plants contain at least five indole alkaloids, three β-carboline alkaloids, and several phenolic amines including hordenine, tyramine, and *N*-methyl-tyramine (Bourke et al. 2006).

Another grass sporadically associated with nervous signs in Colombian cattle is the introduced species *Pennisetum clandestidum* (kikuyu). Mejía (1985) reported high mortality in dairy cattle foraging on *P. clandestinum* in the high plateau of Bogotá and the valleys of Ubaté and Chiquinquirá. The main signs included tremors, ataxia, ruminal stasis, recumbence, decreased milk production, and piloerection. Sporadic episodes of kikuyu poisoning similar to the one reported in Colombia have been reported in the literature but the cause of the problem is still unknown (Cheeke 1998, Bourke 2007).

Cynodon dactylon is an introduced grass recently reported in Colombia by García-Ulloa et al. (2005) that can be found from sea level to 2,000 m elevation. This grass causes a disease in cattle known as bermuda grass tremor, a form of convulsive ergotism (Porter 1997). The main clinical signs include fasciculations of the neck and chest muscles and inability to move or stand due to paralysis of the rear limbs. The disease is caused by ergot alkaloids, mainly ergonovine and its epimer ergonovinine, which are produced by toxic strains of *Claviceps* sp. growing in the grass (Porter et al. 1974). Another grass reported to cause ergot-induced toxicosis, mainly due to the alkaloid ergovaline, is *Festuca arundinaceae* (Tor-Agbidye et al. 2001). Although this grass is common in Colombia, the toxicosis has not been reported, which means that either the disease has been overlooked or not documented. Alternatively, since the grasses that do not contain the endophyte fungus *Neotyphodium coenophialum* are not toxic, it is possible that the

varieties introduced in Colombia are not toxigenic. The same is true for *Lolium perenne*, a naturalized forage grass native to Europe, Asia, and North Africa. This grass may contain tremorgenic mycotoxins called lolitrems when infected with the endophyte *Neotyphodyum lolii* (Hovermale and Craig 2001), but this toxicosis has not been documented in Colombia. *Lolium perenne*, however, has been associated with high levels of nitrate in Colombia (table 1).

Plants That Induce Thiamine Deficiency

Plants that accumulate thiaminases may induce thiamin deficiency with the subsequent development of neurological signs, especially in horses. Ruminants are highly resistant to thiaminases because they synthesize the vitamin in the rumen. At least two plants present in Colombia have been reported to contain thiaminases: the previously mentioned *Pteridium aquilinum* and *Equisetum* spp. (cola de caballo). Several species of *Equisetum* are found in Colombia including *E. bogotense*, *E. myriochaetum*, and *E. giganteum* (Hauke 1969). *Equisetum* spp. are not palatable and therefore the toxicosis is infrequently reported worldwide. The toxicosis is subchronic and has been associated with the intake of hay contaminated with 20-percent *Equisetum* or more during 2 to 5 weeks (Cheeke 1998). Initial signs in horses include anorexia, weight loss, and emaciation that lead to progressive motor incoordination and posterior paralysis or prostration. In terminal cases there are opisthotonus, convulsions, and death.

Other Neurotoxic Plants

Bambusa vulgaris (bamboo, guauda amarilla) is a Poaceae reported to have caused neurologic disorders in horses in Brazil (Barbosa et al. 2006); major signs include motor incoordination, paresis of the tongue, depression, ataxia, and incoordination. Although this plant is common in Colombia, no reports of toxicosis have been documented.

Hypochaeris radicata (Asteracea) is present in Colombia under the common names of centella, chicoria, chicria, and diente de león (Bernal et al. 2006). This plant should not be confused with the "true" diente de león, *Taraxacum officinale* (Asteraceae). *H. radicata* produces a toxicosis in horses known as stringhalt, whose major clinical signs are high stepping with hyperflexion of the hind limbs (Araújo et al. 2008). The disease was recently diagnosed in horses imported from Argentina at the College of Veterinary Medicine of the National University of Colombia in Bogotá but has not been documented in native horses. Two plants of the Fabaceae family known to produce neurological signs in horses and other animals are present in Colombia: *Indigofera spicata* (añil, añalito, azul) and *Lathyrus sativus* (arveja de monte) (Diaz 2010). However, no cases of toxicosis caused by these plants have been reported yet.

Plants That Affect the Musculoskeletal System and Connective Tissue

The genus *Senna* (formerly known as *Cassia*) includes several species of plants known to induce myopathy in cattle, horses, and pigs that graze on them or that eat feed contaminated with their seeds. *Senna* toxicosis causes myocardial degeneration, congestive heart failure, and generalized degeneration of skeletal muscles. The muscle damage is accompanied by high serum activity of the enzymes aspartate amino transferase (AST) and creatine kinase (CK) and myoglobinuria. At postmortem examination, the affected muscles look pale and show whitish striations (Barth et al. 1994). The *Senna* spp. recognized as toxic that have been reported in Colombia include *S. occidentalis* (bicho de café, café de brusca, cafelillo), *S. obtusifolia* (bicho, chilinchil), *S. reticulata* (bajagua, dorancé), *S. tora*, and *S. roemeriana*. The weed *S. obtusifolia* is commonly seen in corn, sorghum, and soybean fields in Colombia, and the seeds have been found contaminating harvested crops. Studies conducted in Colombia with broiler chickens and laying hens have demonstrated the adverse effects of *Senna* seeds on poultry production (Torres et al. 2003).

Petiveria alliacea (anamú) is an herb native to tropical America known in some places as "garlic weed" because of its strong garlic odor. Reported only in Colombia, *P. alliacea* produces a unique subchronic toxicosis in young cattle known as dystrophic muscular emaciation. The disease is observed mainly in calves 2 to 12 months old and sometimes even in calves suckling lactating cows that eat the toxic plant, which suggests that the toxin (or toxins) is excreted in the milk. The toxicosis has been reproduced by feeding 3 g of the plant daily for 30 days (Torres 1984a). Experimental intoxication of cattle and sheep shows decreased activity of serum cholinesterases, incoordination, severe flexion

of the fetlock, and severe muscle atrophy. Also, the meat from these animals develops a strong garlic odor and is usually rejected by the consumer. The compound responsible for the toxic effects of *P. alliacea* has not been identified but it could potentially be dibenzyltrisulfide (DBTS), a bioactive compound with insecticidal activity isolated from the plant by Johnson et al. (1997). Two *Phytolacca* spp. have been reported as toxic in Colombia: *P. icosandra* (altasara, cargamanta, yerba de culebra) and *P. bogotensis* (altasara, cargamanta, guaba, yerba de culebra). Pérez-Arbeláez (1931) indicated that the roots, leaves, and fruits of these two species are toxic but did not detail either the signs of the toxicosis or the animal species affected.

Two plants of the Malpighiaceae family native to Colombia have been associated with a disease of cattle and sheep characterized by the deposition of an abnormal pink or violet pigment in connective tissues (including bones and teeth): *Bunchosia pseudonitida* (mamey, tomatillo, pateperro, cuatrecasas) and *Bunchosia armeniaca* (mamey de tierra fría, manzano de monte). Mortality is usually low (less than 5 percent) but morbidity, represented by animals abnormally pigmented, can be higher than 90 percent (Peña 1982). This disease occurs in the departments of Tolima and Huila, particularly during periods of drought, and is known as bovine chromatosis. Besides producing a discoloration of the connective tissues, *B. pseudonitida* has been associated with ataxia and incoordination, excretion of discoloured urine, and hepatic toxicity characterized by increased activity of serum gamma glutamyl transpeptidase (GGT) and mild degenerative histologic lesions (Mejía 1984). The disease causes losses to the cattle producer not only because of the mortality but also because pigmented carcasses are confiscated by the authorities. The chemical composition of the pigment is still unknown but it is suspected that the alkaloid 2,3-dehydro-4β-piperidone might play a role. This alkaloid was isolated from *B. pseudonitida* leaves and takes on a violet color on contact with air. The pigment is eliminated very slowly, and pigmented animals require at least 9 months of uncontaminated food to completely eliminate the pigment from their tissues (Torres 1984b).

Conium maculatum may affect the musculo-skeletal system. In addition to being neurotoxic, the alkaloids from *Conium maculatum* can also cause congenital malformations in calves, particularly contractures of the musculoskeletal system and cleft palate. The sensitive periods for congenital malformations during gestation are days 40 to 100 for contractures and cleft palate and days 40 to 50 for cleft palate only (Panter et al. 1988). However, these malformations have not yet been documented in Colombian livestock.

Plants That Affect the Skin

Toxic plant-induced primary and secondary photosensitization is a common cause of skin lesions in livestock throughout the world. Secondary photosensitization results from impaired excretion of phylloerythrin, as previously discussed under hepatotoxic plants. *Fagopyrum esculentum* (alforjón, trigo sarraceno, trigo negro), formerly known as *Polygonum fagopyrum*, is a Polygonaceae native to central Asia and naturalized in Colombia. The flowers and seeds of *F. esculentum* contain a conjugated photo-reactive quinone known as fagopyrin (Hagels et al. 1995), which has been known to induce primary photosensitization in cattle, sheep, goats, and other animal species for many years (Sheard et al. 1928). Primary photosensitization is caused by the reaction of the photoreactive compound in non-pigmented skin when it is exposed to solar radiation in the ultraviolet range. The photoactive compounds absorb solar energy, forming reactive molecules (free radicals) that react with nearby macromole-cules, causing inflammation, erythema, edema, serous exudation, scar formation, and skin necrosis. *Hypericum perforatum* (St. Johnswort) contains a pigment similar to fagopyrin known as hypericin, also capable of inducing primary photosensitization. Although *H. perforatum* has not been reported in Colombia, there are about 20 species of *Hypericum* present in the country (Bernal et al. 2006) whose phytochemistry has not been investigated.

Several plants of the Euphorbiaceae family are potentially toxic due to their content of phorbol type diterpene esters, which are highly irritating to the skin and mucosa, and some are tumor promoters (Goel et al. 2007). *Croton* spp. are among the Euphorbiaceae known to contain skin-irritating substances. Some of the *Croton* spp. reported in Colombia include *C. argenteus* (hierba de cotorra), *C. bogotanus* (croto, tapamucro), *C. eluteria* (cascarilla, quina aromática), *C. funckianus* (croto, drago), *C. hirtus* (= *C. glandulosus*, come-mano, drago, guacamayo), *C. lechleri* (drago, drago sangregado), *C. leptostachyus* (drago, mosquerillo),

C. magdalenensis (drago, guacamayo), and *C. schiedeanus* (almizclillo, sabaleto).

Another genus of Euphorbiaceae family is *Euphorbia* from which at least two species have been reported as toxic in Colombia: *E. dichotoma* (teología, tafura) and *E. prostrata* (golondrina, leche de sapo). *Euphorbia dichotoma* was reported as toxic by Pérez-Arbeláez (1931), but there are no toxicological studies for this species. *Euphorbia prostrata* has been reported to produce a latex that irritates the skin of the animals and produces diarrhea and colic when ingested. The latex of *Hura crepitans* (ceiba blanca, ceiba lechosa, tronador), a huge tree of the Euphorbiaceae family, is also considered toxic and highly irritating to the skin and mucosa. In humans, the intake of *H. crepitans* seeds produces a burning sensation in the mouth, vomition, diarrhea, dyspnea, and headache (Fowomola and Akindahunsi 2007). The phorbol esters from another Euphorbiaceae tree known as *Jatropha curcas* (piñón de fraile, purga de fraile, tártaro emético) are also highly irritating to the skin, and its seeds contain a caustic oil (Pérez-Arbeláez 1931). Other *Jatropha* spp. reported as toxic in Colombia are *J. multifida* (avellano purgante) and *J. urens* (Pérez-Arbeláez 1931), but their phytochemistry and toxicology are unknown.

Solanum verbascifolium (miao de perro) is a bush used sporadically in Colombia as a forage plant during the dry season. The leaves of this plant are rough and cause skin irritation. Steroidal and triterpenoid saponins have been isolated from *S. verbascifolium* (Shultes and Raffauf 1990), as well as the glycoalkaloid solasonine, a cinnamic acid derivative, p-coumaramide, and vanillic acid (Zhou and Ding 2002). Although empirically this plant is considered toxic for cattle in Colombia, the toxicosis by *S. verbascifolium* has not yet been documented. In a review on calcinogenic plants, Mello (2003) indicates that *S. verbascifolium* is a calcinogenic plant and cites a study by Tustin et al. (1973); however, Tustin et al. (1973) do not mention *S. verbascifolium* anywhere in their publication and there are no reports in the literature indicating that this plant is, in fact, calcinogenic.

Plants That Affect Reproduction

Phytoestrogens are compounds present in some plants that mimic the action of estradiol and may cause adverse reproductive effects. *Medicago sativa* (alfalfa) and *Trifolium pratense* (trébol rojo) are two of the phytoestrogen-containing plants that have been associated with reproductive problems in cattle in Colombia. Alfalfa contains at least four phytoestrogens: one derived from coumestan (coumestrol) and three from isoflavone (apigenin, luteolin, and quercetin). The concentration of each phytoestrogen varies from 15 to 225 µg/g dry matter with reduced concentrations at the beginning of the flowering period (Seguin et al. 2004). *Trifolium pratense* contains isoflavone-type phytoestrogens including genistein, daidzein, biochanin A, and formononetin. Genistein and daidzein are also present in soybeans. Even though phytoestrogens in general are considered to be low to moderately estrogenic, they can affect reproduction in cattle and have been associated with impairment of ovarian function, cystic ovaries, irregular estrous cycles, decreased conception rates, embryonic mortality, and mammary gland development in heifers (Adams 1995).

Solanum aphyodendron (mata de tinto, tinto, frutillo) is a common species found on roadsides in Colombia, which often forms large monospecific stands in open areas (Knapp 1985). In Colombia, this plant has been reported to cause abortion in cows (Torres 1984b), and in the Boyacá Department aqueous extracts of the plant are used to treat retained placentas in cattle, which usually results in the expulsion of the retained placenta within hours. Sarmiento et al. (1982) investigated the effect of aqueous extracts of *S. aphyodendron* on female dogs and found that it produces uterine contractions and other effects such as mydriasis, hypotension, tachycardia, increased respiratory rate, and depression. The compound responsible for the effect on the uterine muscle is still unknown.

Plants That Contain Systemic Poisons

Systemic poisons interfere with biochemical processes common to all cells and usually do not have a particular target organ. Examples of these poisons are monofluoroacetic acid, a compound capable of blocking the Krebs cycle, and cyanide, an inhibitor of the respiratory chain in the mitochondria. The Rubiaceae *Palicourea margravii*, also known as *P. crocea* (café de monte, cafecillo, café bravo, flor de muerto), contains monofluoroacetic acid and is considered one of the main toxic plants for cattle in the Arauca River valley (Vargas et al. 1998). This plant typically

Table 3. Major cyanogenic glycoside-accumulating plants in Colombia

Family	Latin name	Common name
Adoxaceae	*Sambucus canadensis, S. nigra*	Sauco, saúco
Bignoniaceae	*Tanaecium exitiosum, T. jaroba**	Mataganado, bejuco blanco
	*Tanaecium nocturnum**	Unknown
Euphorbiaceae	*Manihot esculenta*	Yuca agria, yuca blanca
Fabaceae	*Lotus spp.*	Trébol pata de pájaro, trébol de cuernos
	Phaseolus lunatus	Fríjol lima
	Trifolium repens	Trébol blanco
Linaceae	*Linum usitatissimum*	Lino, linaza
Malpighiaceae	*Mascagnia concinna*	Mindaca, mataganado
Poaceae	*Cynodon dactylon*	Pasto argentina, pasto bermuda
	Digitaria sanguinalis	Guardarocío, pata de gallina
	Sorghum bicolor	Sorgo, sorgo forrajero
	Sorghum halepense	Pasto Johnson, capim argentino
	Zea mays	Maíz
Rosaceae	*Prunus spp.*	Cerezo, duraznillo, manzano criollo

*It is thought that these plants contain cyanogenic glycosides but this has not been demonstrated.

causes sudden death, especially if the poisoned animals are forced to walk or run.

Several plant species containing cyanogenic glycosides are present in Colombia, some of which are known to cause livestock losses (table 3). Upon hydrolysis in the rumen, cyanogenic glycosides release cyanide (HCN), which is readily absorbed into the systemic circulation. In the plasma, HCN dissociates into hydrogen and nitrile ion (CN^-), the latter of which binds strongly to the terminal cytochromes of the mitochondrial respiratory chain, thereby inhibiting tissue cellular respiration and causing anoxia. Levels of 20 mg HCN/100 g of fresh plant or higher are considered potentially toxic.

Sambucus canadensis and *S. nigra* are two trees of the Adoxacea family naturalized in Colombia. They can accumulate toxic amounts of cyanogenic glycosides, especially in their leaves, roots, and green fruits. The major cyanogenic glycosides present in *Sambucus* spp. are sambunigrine, holocaline, and prunasine (Dellagreca et al. 2000). Though the toxicosis by *Sambucus* spp. has not been documented in Colombia, it has been reported in both animals and humans in other countries (Atkinson and Atkinson 2002). *Tanaecium exitiosum* (bejuco blanco, mataganado) and *T. jaroba* (calabacillo, bejuco blanco) are two plants of the Bignonaceae family native to Colombia that are toxic to livestock. *Tanaecium exitiosum* is a vine native to the Magdalena River valley that was reported for the first time by the Colombian botanist Armando Dugand in 1942 (Dugand 1942). Even though the plant is known to be toxic and highly palatable to cattle (hence the common name "mataganado," which means "cattle killer"), no studies have been conducted to determine its phytochemistry. In the only study so far conducted with this plant, Mora (1943) found that 70 g of the plant is lethal for cows and goats when given in two separate dosages within 24 h. Mora (1943) postulated that the plant accumulates cyanogenic glycosides, but no attempt was made to isolate these compounds.

Alwyn Gentry, a U.S. botanist who studied extensively the Bignonacea from Colombia and other Andean countries, reported that *T. exitiosum* is currently virtually extinct in the Magdalena River valley due to the systematic destruction to prevent cattle mortality (Gentry 1992). There are no references to *T. exitiosum* in the literature after the one published by Gentry in 1992. *Tanaecium jaroba* is a plant native to the northern region of the country also known to be toxic by cattle growers; however, no studies have been conducted to determine its chemical composition.

Another *Tanaecium* spp. reported to be toxic is *T. nocturnum*. It is native to the Amazon Basin, where the Tikuna Indians report it as toxic, as it apparently contains cyanogenic glycosides (Schultes and Raffauf 1990). Another native Colombian plant known to contain cyanogenic glycosides is *Mascagnia concinna*, a plant also known to accumulate toxic concentrations of nitrate. Torres (1984a) reported that this plant may accumulate levels of HCN greater than 40 mg of HCN/100 g. In a study conducted by Gómez (1975), it was found that less than 2 g of fresh leaves per kg body weight is lethal for cattle during the dry season. He also found that the plants accumulate more cyanogenic glycosides during the dry season as compared with the rainy season.

Conclusions

The present review describes some of the potentially toxic plants present in Colombia, a country that possesses the second-largest botanical biodiversity in the world. Toxic plants can adversely affect every organ system and pose a risk to animal health and production. Even though many of the toxic plants present in Colombia have been described and studied in other countries, it is important to investigate if the same toxic compounds reported elsewhere are present in the plants that actually grow in Colombia. Some of the plants, however, are only known in Colombia and their toxicology needs to be further investigated. These include, for example, three *Tanaecium* spp. reported as toxic (*T. exitiosum*, *T. jaroba*, and *T. nocturnum*), two *Bunchosia* spp. (*B. pseudonitida* and *B. armeniaca*), two *Phytolacca* spp. (*P. bogotensis* and *P. icosandra*), and *Mascagnia concinna*.

The information on toxic plant chemistry in Colombia is mostly limited to their nitrate or cyanide content. Research is needed not only to determine which plants represent a potential risk for animal health and production but also to investigate their phytochemistry and toxicology. It would be very useful if veterinarians were able to document plant poisoning cases through government reporting services. Furthermore, university and government scientists, veterinarians, and extension personnel could fully investigate the various toxicoses and publish their findings in specialized journals. This would help to identify toxic species for further phytochemical and toxicological studies and possibly pharmacological activity.

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References

Adams, N.R. 1995. Detection of the effects of phytoestrogens on sheep and cattle. *Journal of Animal Science* 73:1509-1515.

Alonso-Amelot, M.E. 1997. The link between bracken fern and stomach cancer: milk. *Nutrition* 13:694-696.

Antoniassi N.A.B., E.V. Ferreira, C.E.P. dos Santos, et al. 2007. Intoxicação espontânea por *Ipomoea carnea* subsp. *fistulosa* (Convolvulaceae) em bovinos no Pantanal Matogrossense. *Pesquisa Veterinaria Brasileira* 27:415-418.

Araújo, J.A.S., B. Curcio, J. Alda, et al. 2008. Stringhalt in Brazilian horses caused by *Hypochaeris radicata*. *Toxicon* 52:190-193.

Armién, A.G., C.H. Tokarnia, P. Vargas Peixoto, and K. Frese K. 2007. Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoeae carnea* subsp *fistulosa* (Convolvulaceae). *Veterinary Pathology* 44:170-184.

Aslani, M.R., M. Maleki, M. Mohri, et al. 2007. Castor bean (*Ricinus communis*) toxicosis in a sheep flock. *Toxicon* 49:400-406.

Atkinson, M.D., and E. Atkinson. 2002. Biological flora of the British Isles. *Journal of Ecology* 90:895-923.

Baker, D.C., J.A. Pfister, R.J. Molyneux, and P. Kechele. 1991. *Cynoglossum officinale* toxicity in calves. *Journal of Comparative Pathology* 104:403-410.

Barajas-Meneses, F., J.L. Fernández-Alonso, and R. Galindo-Tarazona. 2005. Diversidad y composición de la familia Boraginaceae en el Departamento de Santander (Colombia). *Caldasia* 27:151-172.

Barbieri, L, M.G. Battelli, and F. Stirpe. 1993. Ribosome-inactivating proteins from plants. *Biochimica et Biophysica Acta* 1154:237-282.

Barbosa J.D., C.M.C. de Oliveira, M.D. Duarte, et al. 2006. Poisoning of horses by bamboo, *Bambusa vulgaris*. *Journal of Equine Veterinary Science* 26:393-398.

Barth, A.T., G.D. Kommers, M.S. Salles, et al. 1994. Coffee senna (*Senna occidentalis*) poisoning in cattle in Brazil. *Veterinary and Human Toxicology* 36:541-545.

Bernal, H.Y. 1986. Flora de Colombia. 4. *Crotalaria*. Instituto de Ciencias Naturales, pp. 18-19, Universidad Nacional de Colombia, Bogotá, Colombia.

Bernal, R., G. Galeano, Z. Cordero, et al. 2006. Diccionario de nombres comunes de las plantas de Colombia. Versión en línea. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia. http://biovirtual.unal.edu.co/diccionario/

Bourke, C.A. 2007. A review of kikuyu grass (*Pennisetum clandestinum*) poisoning in cattle. *Australian Veterinary Journal* 85:261-267.

Bourke, C,A, S.M. Colegate, and R.A. Culvenor. 2006. Evidence that N-methyltyramine does not cause *Phalaris aquatica*-related sudden death in ruminants. *Australian Veterinary Journal* 84:426-427.

Bourke, C.A., S.M. Colegate, D. Rendell, et al. 2005. Peracute ammonia toxicity: a consideration in the pathogenesis of *Phalaris aquatica*. Australian Veterinary *Journal* 83:168-171.

Bourke, C.A., D. Rendell, and S.M. Colegate. 2003. Clinical observations and differentiation of the peracute *Phalaris aquatica* poisoning syndrome in sheep known as "polioencephalomalacia-like sudden death." *Australian Veterinary Journal* 81:698-700.

Bretagne, J.F., N. Vidon, C. L'Hirondel, and J.J. Bernier. 1981. Increased cell loss in the human jejunum induced by laxatives (ricinoleic acid, dioctyl sodium sulphosuccinate, magnesium sulphate, bile salts). *Gut* 22:264-269.

Canchila, A. 2001. Intoxicación en cabras por consumo de *Crotalaria pallida* en Santander. *Revista Colombiana de Ciencias Pecuarias* 14(Supl. 2001):65.

Cao, H. 2003. The distribution of calcium oxalate crystals in genus *Dieffenbachia* Schott and the relationship between environmental factors and crystal quantity and quality. University of Florida. Master's thesis.

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *Journal of Animal Science* 73:909-918.

Cheeke, P.R. 1998. Natural Toxicants in Feeds, Forages, and Poisonous Plants, 2nd ed., Interstate Publishers, Inc., Danville, IL.

Croat, T.B. 2004. Revision of *Dieffenbachia* (Araceae) of Mexico, Central America, and the West Indies. *Annals of the Missouri Botanical Garden* 91:668-772.

Dellagreca, M., A. Fiorentino, P. Monaco, et al. 2000. Cyanogenic glycosides from *Sambucus nigra*. *Natural Product Letters* 14:175-182.

Diaz, G.J. 2001. Naturally occurring toxins relevant to poultry nutrition. *In* S. Leeson and J.D. Summers, eds., Scott´s Nutrition of the Chicken, 4th ed., pp. 544-591, University Books, Guelph, Ontario, Canada.

Diaz, G.J. 2010. Plantas tóxicas de importancia en salud y producción animal en Colombia. Editorial, pp. 113-117, Universidad Nacional de Colombia, Bogotá, Colombia.

Diaz, G.J., L.P. Roldán, and A. Cortés. 2003. Intoxication of *Crotalaria pallida* seeds to growing broiler chickens. *Veterinary and Human Toxicology* 45:187-189.

Dugand, A. 1942. Dos nuevas Bignonaceas del Valle del Magdalena. *Caldasia* 1:29-35.

Duncan, A.J., and J.A. Milne. 1993. Effects of oral administration of brassica secondary metabolites allyl cyanide, allyl isothiocyanate and dimethyl disulphide, on the voluntary food intake metabolism of sheep. *British Journal of Nutrition* 70:631-645.

Fernández-Alonso, J.L., and M. Hernández-Schmidt. 2007. Catálogo de la flora vascular de la cuenca alta del río Subachoque (Cundinamarca, Colombia). *Caldasia* 29:73-104.

Fernández-Alonso, J.L., A. Galindo, and J.M. Idrobo. 2007. Las plantas como evidencia legal. Desarrollo de la botánica forense en Colombia. *Revista de la Academia Colombiana de Ciencias* 31:181-198.

Fowomola, M.A., and A.A. Akindahunsi. 2007. Nutritional quality of sandbox tree (*Hura crepitans* Linn). *Journal of Medicinal Food* 10:159-164.

Frank, A.A., and W.M. Reed. 1987. *Conium maculatum* (poison hemlock) toxicosis in a flock of range turkeys. *Avian Diseases* 2:386-388.

Fu, P.P., Q. Xia, G. Lin, and M.W. Chou. 2004. Pyrrolizidine alkaloids–Genotoxicity, metabolism enzymes, metabolic activation and mechanisms. *Drug Metabolism Reviews* 36:1-55.

García-Ulloa, J.A., C. Lastra, C. Salas, and M. Medina. 2005. Estudios de gramíneas (Poaceae) de Colombia: veinte novedades corológicas. *Caldasia* 27:131-145.

Gava, A., J. Lucioli, F.H. Furlan, et al. 2010. Intoxicação por *Trema micrantha* (Ulmaceae) em caprinos no Estado de Santa Catarina. *Pesquisa Veterinaria Brasilera* 30:191-194.

Gentry, A.H. 1992. A synopsis of Bignoniaceae ethnobotany and economic botany. *Annals of the Missouri Botanical Garden* 79:53-64.

Genua, J.M., and C.J. Hillson. 1985. The occurrence, type and location of calcium oxalate crystals in the leaves of fourteen species of Araceae. *Annals of Botany* 56:351-361.

Ghisalberti, E.L. 2000. *Lantara camara* L. (Verbenaceae). *Fitoterapia* 71:467-486.

Goel, G., H.P.S. Makkar, G. Francis, and K. Becker. 2007. Phorbol esters: structure, biological activity, and toxicity in animals. *International Journal of Toxicology* 26:279-288.

Goldsmith, W.W. 1909. Onion poisoning in cattle. *Journal of Comparative Pathology and Therapeutics* 22:151.

Gómez, B. 1975. *Mascagnia concinna*, (Morton), planta tóxica al ganado vacuno. *Revista ICA* 10:513-514.

Graydon, R.J., H. Hamid, P. Zahari, and C. Gardiner C. 1991. Photosensitization and crystal-associated cholangiohepatopathy in sheep grazing *Brachiaria decumbens*. *Australian Veterinary Journal* 68:234-236.

Guzmán V.H., G.A. Morales, and R. Ochoa. 1978. Intoxicación en bovinos por nitratos acumulados en pasto elefante (*Pennisetum purpureum*, Shum). *Revista ICA* 13:113-118.

Hagels, H., D. Wagenbreth, and H. Schilcher. 1995. Phenolic compounds of buckwheat herb and influence of plant and agricultural factors (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gärtner). *In* T. Matano and A. Ujihara, eds., Current Advances in Buckwheat Research: Proceedings of the 6th International Symposium on Buckwheat , pp. 801-809, Shinshu University Press, Shinshu, China.

Hallak, H.O., and P.J. Wedlund. 1991. Vitamin K epoxide reductase activity and its inhibition by warfarin in young and old rats. *Drug Metabolism and Disposition* 19:278-279.

Hauke, R.L. 1969. Gametophyte development in Latin American horsetails. *Bulletin of the Torrey Botanical Club* 96:568-577.

Hooper, P.T. 1978. Pyrrolizidine alkaloid poisoning–pathology with particular reference to differences in animal and plant species. *In* R.F. Keeler, K.R. van Kampen, and L.F. James, eds., Effects of Poisonous Plants on Livestock, pp. 161-176, Academic Press, New York, NY.

Hovermale, J.T., and A.M. Craig. 2001. Correlation of ergovaline and lolitrem B levels in endophyte-infected perennial ryegrass (*Lolium perenne*). *Journal of Veterinary and Diagnostic Investigation* 13:323-327.

James, L.F., and J.E. Butcher. 1972. Halogeton poisoning of sheep, effect of high level oxalate intake. *Journal of Animal Science* 35:1233-1238.

Johnson, A.E. 1982. Toxicological aspects of photosensitization in livestock. *Journal of the National Cancer Institute* 69:253-258.

Johnson, L., L.A.D. Williams, and E.V. Roberts. 1997. An insecticidal and acaricidal polysulfide metabolite from the roots of *Petiveria alliaceae*. *Pesticide Science* 50:228-232.

Jolly, R.D., and S.U. Walkley. 1997. Lysosomal storage diseases of animals, an essay in comparative pathology. *Veterinary Pathology* 34:527-548.

Knapp, S. 1985. New species of *Solanum* Section Germinata (G. Don) Walp. (Solanaceae) from South and Central America. *Annals of the Missouri Botanical Garden* 72:558-569.

Last, R.D., J.H. Hill, and G. Theron. 2007. An outbreak of perirenal oedema syndrome in cattle associated with ingestion of pigweed (*Amaranthus hybridus* L.). *Journal of the South African Veterinary Association* 78:171-174.

López, N.A. 2002. "Dieta / Intoxicación con catapis." News article published in the newspaper "El Tiempo" on September 19, 2002.

Loretti, A.P., I.M.R. da Silva, and R.E. Ribeiro. 2003. Accidental fatal poisoning of a dog by *Dieffenbachia picta* (dumb cane). *Veterinary and Human Toxicology* 45:233-239.

Mattocks, A.R. 1986. Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, San Diego, CA.

Mejía, A.B. 1985. Intoxicación por nitratos en los bovinos. *Ganados y Praderas* 4:43-45.

Mejía, B. 1984. Toxicología de la *Bunchosia pseudonitida*, estudio clinico-patológico de la cromatosis bovina. Programa Universidad Nacional de Colombia / Instituto Colombiano Agropecuario, Bogotá, Colombia. Master's thesis.

Mello, J.R.B. 2003. Calcinosis–calcinogenic plants. *Toxicon* 41:1-12.

Miles, C.O., A.L. Wilkins, S.C. Munday, et al. 1992. Identification of calcium salt of epismilagenin β-D-glucuronide in the bile crystals of sheep affected by *Panicum dichotomiflorum* and *Panicum schinzii* toxicoses. *Journal of Agricultural and Food Chemistry* 40:1606-1609.

Ministerio de Agricultura y Desarrollo Rural. 2005. La cadena de la carne bovina en Colombia. Una mirada global de su estructura y dinámica 1991–2005. Observatorio Agrocadenas Colombia, Documento de Trabajo No. 273. Bogotá, Colombia.

Mora, C.R. 1943. Contribución al estudio de las plantas tóxicas en medicina. *Revista de Medicina Veterinaria* 12:5-38.

Morris, C.A., N.R. Towers, W.D. Hohenboken, et al. 2004. Inheritance of resistance to facial eczema, a review of research findings from sheep and cattle in New Zealand. *New Zealand Veterinary Journal* 52:205-215.

Ozmen, O., F. Mor, and U. Ayhan. 2003. Nitrate poisoning in cattle fed *Chenopodium album* hay. *Veterinary and Human Toxicology* 45:83-84.

Panciera, R.J, T. Martin, G.E. Burrows, et al. 1990. Acute oxalate poisoning attributable to ingestion of curly dock (*Rumex crispus*) in sheep. *Journal of the American Veterinary Medical Association* 196:1981-1984.

Panter, K.E., R.F. Keeler, and D.C. Baker. 1988. Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). *Journal of Animal Science* 66:2407-2413.

Parton, K. 2000. Onion toxicity in farmed animals. *New Zealand Veterinary Journal* 48:89.

Patnaik, G.K., and E. Köhler. 1978. Pharmacological investigation on asclepin - a new cardenolide from *Asclepias curassavica*. Part II. Comparative studies on the inotropic and toxic effects of asclepin, g-strophantin, digoxin and digitoxin. *Arzneimittel-Forschung* 28:1368-1372.

Pedraza, C., F. Villafañe, and R.D. Torrenegra. 1983. Hematuria vesical bovina y su relación con algunas especies vegetales. *Revista ACOVEZ* 7:11-19.

Peña, N.E. 1982., Contribución a la epidemiología de la cromatosis bovina en algunos municipios del Huila y Tolima (Colombia). *Revista Colombiana de Ciencias Pecuarias* 4:51-63.

Peña, N.E., L.C. Villamil, D. Parra, and C.A. Lobo. 1980. Las Enfermedades de los Animales en Colombia. Situación por Regiones Naturales. Ministerio de Agricultura, Instituto Colombiano Agropecuario - ICA. Bogotá, Colombia.

Pérez-Arbeláez, E. 1931. Plantas venenosas de Colombia. *Revista de Medicina Veterinaria* 3:189-198.

Poindexter, B. W. Feng, A. Dasgupta, and R. Bick. 2007. Oleandrin produces changes in intracellular calcium levels in isolated cardiomyocytes, a real-time fluorescence imaging study comparing adult to neonatal cardiomyocytes. *Journal of Toxicology and Environmental Health A* 70:568-574.

Porter, J.K. 1997. Endophyte alkaloids. *In* J.P.F. D'Mello, Handbook of Plant and Fungal Toxicants, pp. 51-62, CRC Press, Boca Raton, FL.

Porter, J.K., C.W. Bacon, and J.D. Robbins. 1974. Major alkaloids of a *Claviceps* isolated from toxic bermuda grass. *Journal of Agricultural and Food Chemistry* 22:838-841.

Powell, A.M., and R.M. King. 1969. Chromosome numbers in the Compositae, Colombian species. *American Journal of Botany* 56:116-121.

Prache, S. 1994. Haemolytic anaemia in ruminants fed forage brassicas, a review. *Veterinary Research* 25:497-520.

Pritchard, D.G., L.M. Markson, P.J. Brush, et al. 1983. Haemorrhagic syndrome of cattle associated with the feeding of sweet vernal (*Anthoxanthum odoratum*) hay containing dicumarol. *Veterinary Record* 113:78-84.

Puschner, B., F.D. Galey, D.M. Holstege, and M. Palazoglu. 1998. Sweet clover poisoning in dairy cattle in California. *Journal of the American Veterinary Medical Association* 212:857-859.

Quigley, C. 1956. Aboriginal fish poisons and the diffusion problem. *American Anthropologist. New Series* 58:508-525.

Rae, H.A. 1999. Onion toxicosis in a herd of beef cows. *Canadian Veterinary Journal* 40:55-57.

Rietjens, I.M.C.M., M.J. Martena, M.J. Boersma, et al. 2005. Molecular mechanisms of toxicity of important foodborne phytotoxins. *Molecular Nutrition and Food Research* 49:131-158.

Roberts, D.M., E.S. Southcott, J.M. Potter, et al. 2006. Pharmacokinetics of digoxin cross-reacting substances in patients with acute yellow oleander (*Thevetia peruviana*) poisoning, including the effect of activated charcoal. *Therapeutic Drug Monitoring* 28:784-792.

Runciman, D.J., A.M. Lee, K.F.M. Reed, and J.R. Walsh. 2002. Dicumarol toxicity in cattle associated with the ingestion of silage containing sweet vernal grass (*Anthoxanthum odoratum*). *Australian Veterinary Journal* 80:28-32.

Sarmiento, R., M. Trebert, and L. Serrano. 1982. Efectos farmacológicos de la planta *Solanum chamaecerasus* Bitter, y su posible aplicación en patología genital. *Revista de la Facultad de Medicina Veterinaria y Zootecnia* 35:51-56.

Schultes, R.E., and R. F. Raffauf. 1990. The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia, p. 108, Dioscorides Press, Portland, OR.

Seguin, P., W. Zheng, and A. Souleimanov. 2004. Alfalfa phytoestrogen content, impact of plant maturity and herbage components. *Journal of Agronomy and Crop Science* 190:211-217.

Seitz, A.L., E.M. Colodel, M. Schmitz, et al. 2005. Use of lectin histochemistry to diagnose *Sida carpinifolia* (Malvaceae) poisoning in sheep. *Veterinary Record* 156:386-388.

Sharma, O.P., and S. Sharma. 2007. A review of the hepatotoxic plant *Lantana camara*. *Critical Reviews in Toxicology* 37:313-352.

Sharma, O.P., R.K. Dawra, N.P. Kurade, and P.D. Sharma. 1998. A review of the toxicosis and biologial properties of the genus *Eupatorium*. *Natural Toxins* 6:1-14.

Sheard, C., H.D. Caylor, and C. Schlotthauer. 1928. Photosensitization of animals after the ingestion of buckwheat. *Journal of Experimental Medicine* 47:1013-1028.

Small, A.C., W.R. Kelly, A.A. Seawright, et al. 1993. Pyrrolizidine alkaloidosis in a two month old foal. *Zentralblatt für Veterinärmedizin. Reihe A* 40:213-218.

Smith, B.L. 1997. The toxicity of bracken fern (genus *Pteridium*) to animals and its relevance to man. *In* J.P.F. D'Mello, ed., Plant and Fungal Toxicants, pp. 63-76, CRC Press, Boca Raton, FL.

Stickel, F., and H.K. Seitz. 2000. The efficacy and safety of comfrey. *Public Health Nutrition* 3:501-508.

Tokarnia, C.H., J. Döbereiner, and P.V. Peixoto. 2002. Poisonous plants affecting livestock in Brazil. *Toxicon* 40:1635-1660.

Tor-Agbidye, J., L.L. Blythe, and A.M. Craig. 2001. Correlation of endophyte toxins (ergovaline and lolitrem B) with clinical disease, fescue foot and perennial ryegrass staggers. *Veterinary and Human Toxicology* 43:140-146.

Toro, G., E. Rojas, and G. Arango. 1997. Seneciosis. Enfermedad veno-ocusiva del hígado (EVOH) en Colombia. 1964-1996. Identificación, manejo y solución de un problema. *Revista de la Academia Colombiana de Ciencias* 21:35-56.

Torres, J.E. 1984a. Plantas tóxicas para el ganado. I Parte. *Carta Ganadera* 21(4):14-19.

Torres, J.E. 1984b. Plantas tóxicas para el ganado. III Parte. *Carta Ganadera* 21(5):15-17, 23-27.

Torres, L.M., P. Diaz, and G.J. Diaz. 2003. Efectos de la adición de semillas de *Senna obtusifolia* (cafelillo, bajagua) en la dieta para aves de postura y pollos de engorde. *El Cerealista* 67:35-38.

Trheebilcock, E., F. Villafañe, and A. Gil. 1978. Síndrome caída del ganado–Contribución a su estudio. *Revista ICA* 13:119-125.

Tsuzuki, J.K., T.I.E. Svidzinski, C.S. Shinobu, et al. 2007. Antifungal activity of the extracts and saponins from *Sapindus saponaria* L. *Anais da Academia Brasileira de Ciências* 79:577-583.

Tustin, R.C., C.H. Pienaar, J.M. Schmidt, et al. 1973. Enzootic calcinosis of sheep in South Africa. *Journal of the South African Veterinary Association* 44:383-395.

Vargas, O.M., L.M. Quiñonez, and J.L. Parra. 1998. Plantas tóxicas para los bovinos en la vega del río Arauca. Manual de Asistencia Técnica No. 03, CORPOICA Regional 8, Villavicencio, Meta, Colombia, 31 p.

Vermunt, J., and R. Visser. 1987. Nitrate toxicity in cattle. *New Zealand Veterinary Journal* 35:136-137.

Vetter, J. 2004. Poison hemlock (*Conium maculatum* L.) – Review. *Food and Chemical Toxicology* 42:1373-1382.

Wahab, S.M.A., and M.A. Selim. 1985. Lipids and flavonoids of *Sapindus saponaria*. *Fitoterapia* 56: 167-168.

Witte, S.T., G.D. Osweiler, H.M. Stahr, and G. Mobley. 1990. Cocklebur toxicosis in cattle associated with the consumption of mature *Xanthium strumarium*. *Journal of Veterinary and Diagnostic Investigation* 2:263-267.

Zhou, L.X., and Y. Ding. 2002. A cinnamide derivative from *Solanum verbascifolium* L. *Journal of Asian Natural Products Research* 4:185-187.

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Analysis of the Toxic Amino Acid Indospicine by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Some *Indigofera* species contain a toxic non-protein amino acid known as indospicine. Indospicine-containing plants are toxic to livestock, and cases of secondary poisoning have been documented in dogs that consume indospicine-containing meat. For the analysis of indospicine in the plant material, a method was developed based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) of the phenylisothiocyanate derivative of indospicine. Indospicine was extracted from the plant with ethanol/0.01N HCl (70:30). The sample was then derivatized with a phenylisothiocyanate solution and then analyzed by LC-MS/MS. The method was linear over the range of 1.0-25 µg/mL, recovery was 86% and the intra-day precision was 4.7% (RSD). Indospicine was confirmed in samples of *I. lespedezioides* collected in Brazil with a concentration range of 60-1400 µg/g (dry weight basis). No indospicine was detected in samples of *I. praticola* and *I. tinctoria* from Zimbabwe, Africa.

Keywords: indospicine, *Indigofera lespedezioides*, poisonous plants, LC-MS

Introduction

The *Indigofera* genus contains approximately 700 different species, many of which are agronomically important plants that are used as grazing forages and feed supplements (Aylward et al. 1987). Some *Indigofera* species, however, contain a toxic non-protein amino acid known as indospicine (2,7-diamino-7-iminoheptanoic acid; figure 1). Indospicine is thought to be hepatotoxic in cattle, sheep, mice, rats, and rabbits (Norfedlt et al. 1952, Hutton et al. 1958a,b, Hegarty and Pound 1968, 1970, Christie et al. 1969, 1975) and may be neurotoxic in horses (Hegarty and Pound 1968, Hooper et al. 1971). Dogs appear to be highly susceptible to indospicine hepatotoxicity, and there are now several reported cases of secondary poisonings occurring from dogs eating indospicine-contaminated meat (Hegarty et al. 1988, Kelly et al. 1992, FitzGerald et al. 2011). Indospicine is structurally similar to arginine and differs only in the C-6 methylene group versus the amino group that is found in arginine (figure 1). The compound was first isolated by Hegarty and Pound (1968) from *I. spicata* and its toxicity is attributed to the inhibitory action of arginine incorporation into proteins, inhibition of arginase activity, and inhibition of nitric oxide synthase (Madsen and Hegarty 1970, Madsen et al. 1970, Pass et al. 1996).

In the past, indospicine has been detected using an amino acid analyzer consisting of ion-exchange chromatography and post-column derivatization with ninhydrin (Hegarty and Pound 1970, Miller and Smith 1973, Aylward et al. 1987). Analysis times

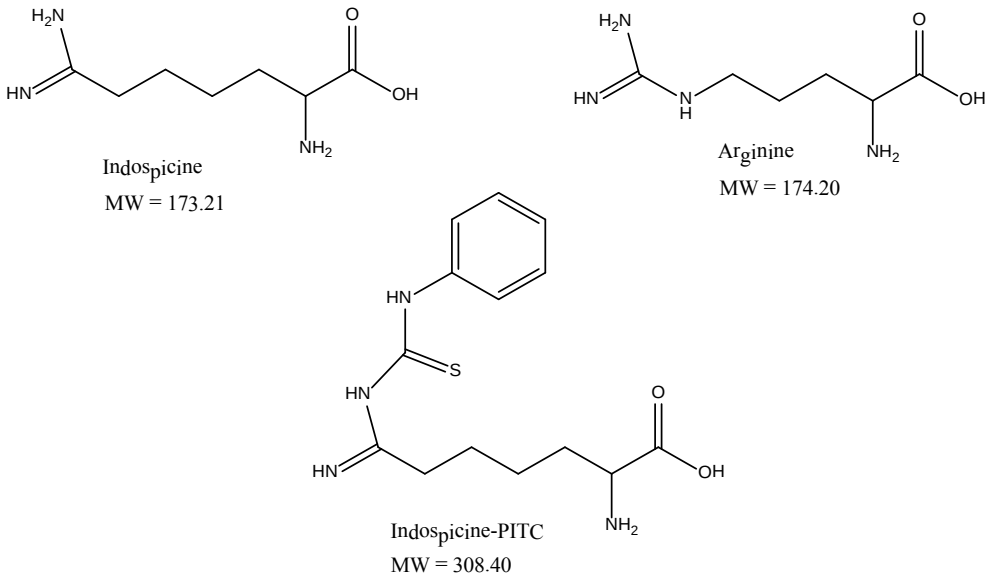

Figure 1. Chemical structures of indospicine, arginine, and the phenylisothiocyanate derivative of indospicine (indospicine-PITC).

using this methodology are lengthy (over 1 hour/sample). More recently, a reversed-phase HPLC (high performance liquid chromatography) method was reported for the detection of indospicine in horse sera and tissue samples (Pollitt et al. 1999). The method was based on pre-column derivatization with phenylisothiocyanate, HPLC separation, and detection by UV (Heinrikson and Meredith 1984). The method of Pollitt et al. (1999) appeared to work well, although to achieve the required resolution of indospicine from arginine and other amino acids in the samples, the chromatographic conditions had to be carefully selected. Concurrent with the work reported here, indospicine was reported to be analyzed by liquid chromatography-tandem mass spectrometry of the underivatized compound with detection in canine sera, liver, and muscle tissue as well as commercially prepared camel mince; however, the exact details of the analysis were not provided (FitzGerald et al. 2011).

The method presented here modifies the method of Pollitt et al. (1999) through the use of binary gradient reversed-phase chromatography and then detection by mass spectrometry. The use of tandem mass spectrometry, though not required, assured an extra level of specificity to the method. The method was used to detect indospicine in a number *Indigofera lespedezioides* plant samples originating from suspected cases of poisoning of horses in Brazil. Secondarily, samples of two different species of *Indigofera* (*I. practicola* and *I. tinctoria*) from Africa, which are associated with floppy trunk syndrome in elephants (Fowler and Mikota 2006), were analyzed for indospicine as additional plants from same genera.

Material and Methods

Plant Material and Chemicals

Astragalus lentiginosus was obtained from the general plant collections of the U.S. Department of Agriculture, Agricultural Research Service Poisonous Plant Research Laboratory (PPRL), Logan, UT, USA, and was used for the indospicine negative control, as indospicine has never been reported in *Astragalus* species. *Indigofera spicata* was a gift from Dr. Alan Seawright collected in western Queensland, Australia; it was used as the indospicine positive control sample. *Indigofera lespedezioides* associated with nervous disease in horses was collected from the state of Roraima, northern Brazil (Lima et al. 2011). *Indigofera tinctoria* and *I. praticola* were diagnostic samples (PPRL) received from Zimbabwe, Africa, and associated with floppy trunk disease in elephants. All samples had been air dried at ambient temperature and then ground to pass a 1-mm screen. A standard sample of indospicine was a gift from Dr. Steven Colegate, obtained from the collection of the Plant Toxin Research Unit of CSIRO Australia. Methanol and ethanol were reagent grade; triethylamine (99.5%) and phenylisothiocyanate (99%) were purchased from Aldrich Chemical; acetonitrile was HPLC grade (Burdick and Jackson); and water was Milli-Q-purified (Waters Millipore).

Sample Extraction and Preparation

An aliquot of plant material (0.100 g) was placed into a screw cap test tube or vial and 5.0 mL of 70% ethanol (in 0.01N HCl) (Aylward et al. 1987) added and the samples sonicated for 30 min. Samples were centrifuged for 5 min and the supernatant decanted into a screw cap test tube. The samples were then extracted two additional times with 5.0 mL of the extraction solvent (70% EtOH/0.01N HCl) in the same manner as above and the decanted extracts were combined. The final volume with no additional adjustment was measured at 15.0 mL. An aliquot (2.0 mL) of the combined extracts was added to a clean 7 mL screw cap glass vial, placed in a heat block (60°C), and the solvent removed by evaporation under a flow of nitrogen. To aid the evaporation of water in the samples, absolute ethanol (~1 mL) was added to each vial after the original volume had been reduced to approximately 0.5 mL. The samples were then evaporated to dryness. The phenylisothiocyanate (PITC) reagent was prepared from a mixture of methanol/water/ triethylamine/phenylisothiocyanate (80/10/5/5) as a slight modification to that previously reported by Pollitt et al. (1999). To each sample was added 0.250 mL of freshly prepared PITC reagent and the samples mixed by mechanical rotation for 15 min. The samples were dried under nitrogen flow on the heat block (60°C) and the dry residue was reconstituted in 1.00 mL of 50% acetonitrile/water and transferred to autosampler vials for analysis.

Indospicine Calibration Standards

A stock solution of indospicine was prepared at 1.74 mg/mL in methanol. From this was prepared a 50 µg/mL solution by dilution of 58 µL stock into 1.94 mL of methanol. Six aliquots at 0.500, 0.400, 0.300, 0.200, 0.100, and 0.020 mL were placed into 8 mL screw cap vials and the solvent removed by evaporation under nitrogen flow in a heated block (60°C). Each calibration standard was then derivatized with the PITC reagent as described above to give calibration standards in the range of 1.0-25 µg/mL.

LC-MS/MS Analysis

Analysis of indospicine was accomplished using a Finnigan Surveyor liquid chromatography system coupled to a Finnigan LCQ Advantage Max ion trap mass spectrometer and electrospray (esi) ionization source. A Thermo Betasil C18 column (100 × 2.1 mm), and guard column of equivalent phase, were used with a gradient flow of acetonitrile (MeCN) and water containing 20 mM ammonium acetate (0.300 mL/min). The programmed gradient flow was 5% MeCN (0-2 min); 5%-60% MeCN (2-10 min); 60%-95% MeCN (10-11 min); 95% MeCN (11-15 min); 95%-5% MeCN (15-16 min) and equilibration at 5% MeCN for 5 min for a total cycle time of 21 min. Flow from the column was directly coupled to the esi-source and the mass spectrometer was operated in the MS/MS mode with a selected parent mass of 309, an isolation width of *m/z* 1.5, a relative collision energy of 27%, an activation Q of 0.25, and an activation time of 30 ms. Parent ion fragments were scanned in the range of *m/z* 85-800. Indospicine detection and peak areas were made from reconstructed ion chromatograms using the selected MS/MS fragment ions at *m/z* 164 and 216. The precision of the method was measured from the repeat intra-day analyses (n = 5) of samples of *I. spicata*. Extraction efficiency was determined from a repeat single extraction of a sample that had already been previously extracted three times with 5 mL of 70% ethanol (0.01N HCl). Accuracy of the method was determined from spike recovery where % recovery = $[(C_F - C_U)/C_A] \times 100\%$: C_F = concentration of analyte measured in fortified sample (n = 3); C_U = Concentration of analyte measured in unfortified sample as measured by analysis in triplicate; and C_A = concentration of analyte added in fortified sample (50 µL of 1.74 mg/mL stock = 87 µg added to 100 mg sample). The unfortified sample was prepared by mixing the *I. spicata* control sample with approximately 50% by weight of the *Astragalus* negative control.

Results and Discussion

In the previously reported HPLC analysis of the phenylisothiocyanate (PITC) derivative of indospicine (Pollitt et al. 1999), it was critical to establish proper pH, solvent component ratios, and temperature conditions of the method to ensure proper resolution of the analyte from other amino acids in the sample. In the method reported here, and in using the mass spectrometer for the detector, such detail to resolution of the components on the chromatographic time scale was not as critical because the mass spectrometer would be used to resolve the co-eluting amino acid components based on their parent mass and the mass of their

corresponding fragment ions. Therefore, for the current method we chose chromatographic conditions that would be easy to reproduce and give a reasonable analysis time with acceptable chromatographic qualities. The resulting method used a simple binary gradient of water and acetonitrile with a standard reversed-phase (C18) column. Under the chromatographic conditions established, indospicine-PITC was found to elute at approximately 5.4 min (figure 2). Arginine-PITC was found to essentially co-elute with indospicine-PITC at 5.44 min and thus analysis of indospicine using a UV detector is not possible under these chromatographic conditions.

The esi-MS spectra of indospicine and arginine produce the expected MH^+ ions at 309 and 310, respectively, for their PITC derivatives (figure 2). It was possible to resolve amino acid derivatives based solely on the MH^+ ions in the analysis of the standard compounds. The analysis of an indospicine negative sample (*Astragalus lentiginosus*) presented no false positive as evident from the RIC at MH^+ = 309, even in the presence of arginine (figure 3A). Indospicine was easily detected in the positive control sample (*I. spicata*) under the same analytical conditions (figure 3B). However, the use of their MS/MS fragment ions could provide an additional level of specificity to ensure no possible contamination from extraneous 309 ions. The MS/MS data for indospicine-PITC and arginine-PITC are presented in figure 4. Fragment losses were similar for the two compounds with major losses of 18, 34, 51, 93, and 135 Da with the exception of an extra M-35 ion for indospicine-PITC. The two most abundant MS/MS fragment ions at *m/z* 174 and 216 were therefore used for detection and quantitation of indospicine (figure 5) with no false positive peaks resulting from the co-eluting arginine-PITC component.

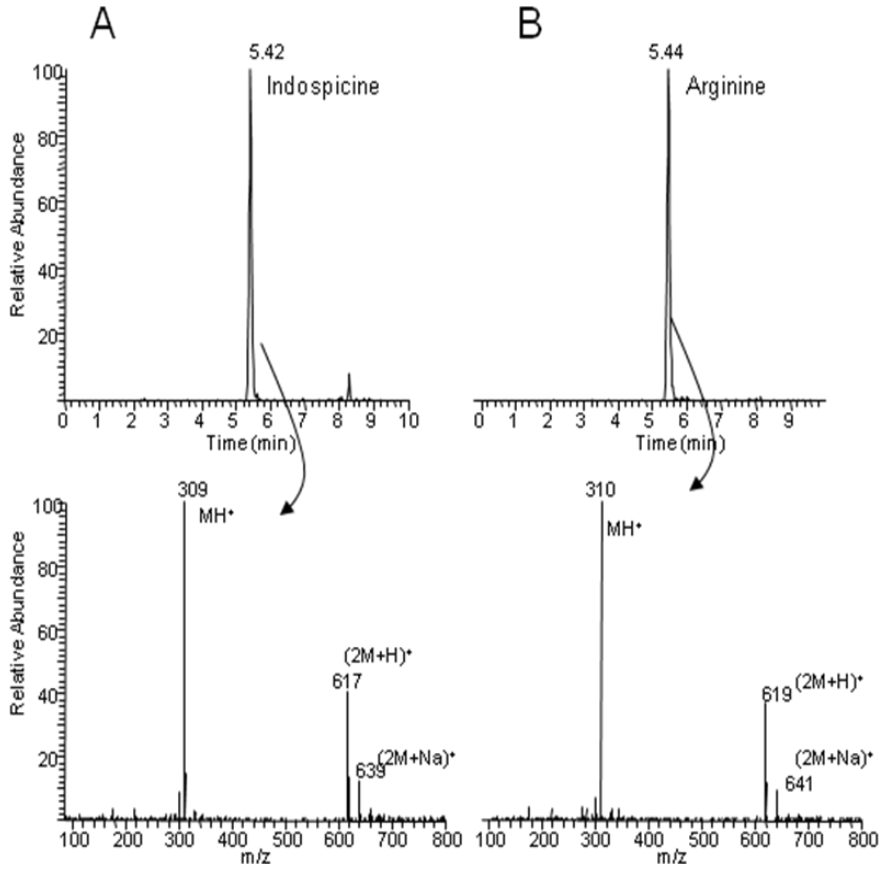

Figure 2. LC-esiMS analysis of standard solutions of indospicine (A) and arginine (B) and their corresponding esiMS spectra.

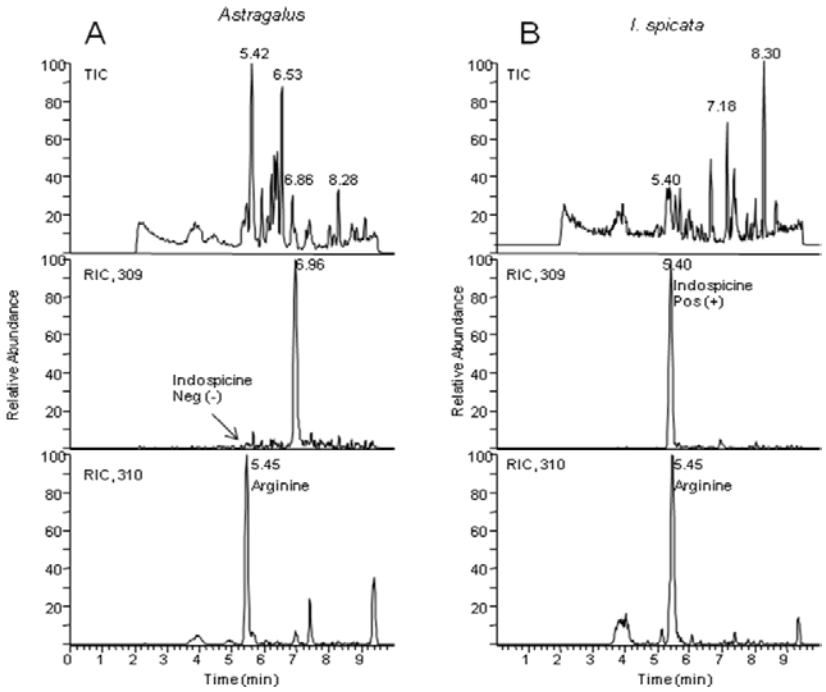

Figure 3. LC-esiMS analysis of *Astragalus* plant material (A) used for indospicine negative control and *Indigofera spicata* (B) used for indospicine positive control. Each sample includes the total ion chromatogram (TIC) and their reconstructed ion chromatograms for *m/z* 309 (indospicine) and *m/z* 310 (arginine).

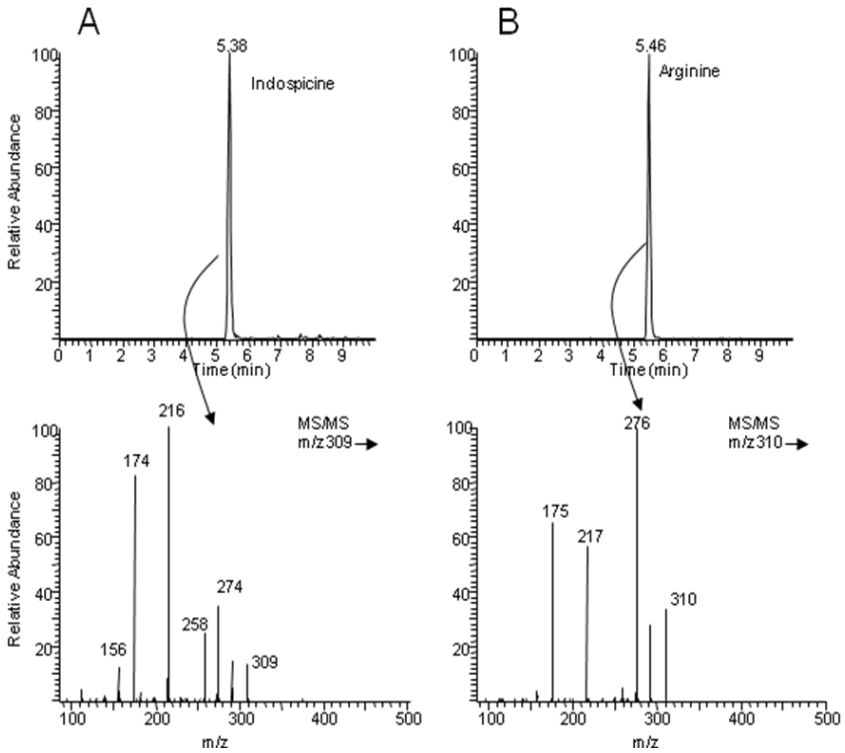

Figure 4. LC-esiMS/MS analysis of standard solutions of indospicine (A) and arginine (B) and their corresponding MS/MS spectra resulting from fragmentation of the parent ions for indospicine ($MH^+ = 309$) and arginine ($MH^+ = 310$).

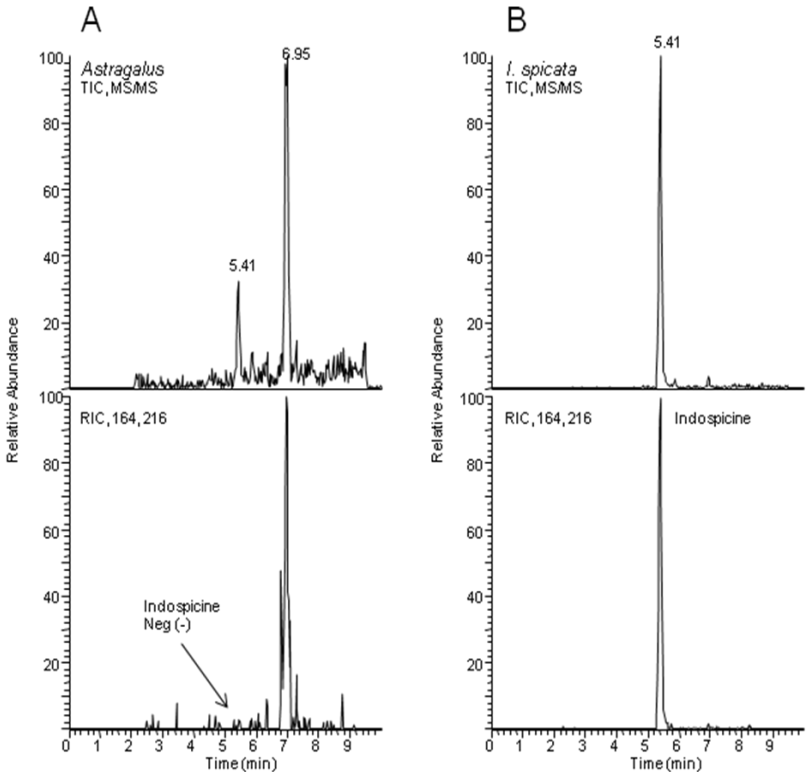

Figure 5. LC-esiMS/MS analysis of *Astragalus* plant material (A) used for indospicine negative control and *Indigofera spicata* (B) used for indospicine positive control. Each sample includes the total ion chromatogram (TIC) and the reconstructed ion chromatograms for *m/z* 309 -›› 174, 216 the selected ions for indospicine.

Indospicine was extracted as previously reported by Aylward et al. (1987) with an acidic aqueous ethanol (ethanol/water/0.1N HCl, 70:30:1) solvent mixture. This extraction solution extracted at least three times more indospicine in comparison to a simple methanolic extract of the sample (data not presented). The extraction was estimated to be 98% complete after three extractions and intra-day precision was 4.7%. The calibration was linear over the range of 1-25 µg/mL ($r^2 = 0.9962$). Recovery of the method was found to be 86% from samples spiked with a standard solution of indospicine. The recovery was slightly lower than expected given the simplicity of the extraction procedure and the apparent completeness of the extraction. However, the low recovery may indicate that some binding of indospicine with the plant matrix occurs and that a different extraction solvent or method may be required to increase the extraction recovery. Alternate extraction solvents were not explored other than a simple methanol extraction.

The developed method was applied to a small number of *Indigofera* samples received by the laboratory from Brazil and Africa from areas of possible livestock poisonings. All samples of *I. lespedezioides* were positive for indospicine, ranging from 60-1178 µg/g (dry weight basis) (table 1). We found one previous analysis of *I. lespedezioides* that reported a concentration of indospicine at 0.02% (200 µg/g) (Aylward et al. 1987). The two samples from the state of Roraima (Bom Fim and Amajari), where the disease occurs, had significantly higher concentrations of indospicine than the sample collected from Manaus. Interestingly, the sample from Manaus was harvested from plants originally collected in the state of Roraima (where the disease occurs) and planted one year before in Manaus (state of Amazonas) in a place where the disease does not occur. There are three reported toxic *Indigofera* species for horses (*I. spicata* in Florida, *I. linnaei* (= *I. domini, I. enneaphyla*) in Australia, and *I. lespedezioides* in Brazil. It has not been proven that indospicine is the toxic compound causing nervous disease in horses, and there is some suspicion that a nitrotoxin is responsible (Majak et al. 1992). Nevertheless, indospicine is found in the three species that cause nervous signs in different parts of the world (Australia, United States, and Brazil), and at least *I. lespedezioides* collected in Brazil does not contain nitro-compounds (data not shown).

Table 1. Analysis of *Indigofera* samples for indospicine by LC-MS/MS for method validation and submitted samples from possible livestock intoxications

Sample	Indospicine (µg/g) ± s.d.	Validation parameter
I. spicata/A. lentiginosus (unfortified) n=3	733 ± 31.6	
I. spicata/A. lentiginosus (fortified w/870 µg/g) n=3	1491 ± 54.9	Recovery 87%
I. spicata (positive control) n=5	1136 ± 46.0	Intra-day RSD 4.1%
I. spicata (extraction #4)	21	Extraction~ 98%
I. lespedezioides		
Bom Fim[1]	263	
Manaus[2]	63	
Amajaru[1]	1178	
I. lespedeziodes[3]	488	
I. practicola[4]	n.d.	
I. tinctoria[4]	n.d.	
I. practicola[4]	n.d.	
A. lentiginosus (negative control)	n.d.	

[1] Collected in the state of Roraima, Brazil, February 2010.
[2]Collected in the state of Amazonas, Brazil, February 2010, from plants collected in the state of Roraima (where the disease occurs) and planted 1 year before in Manaus (state of Amazonas) in a place where the disease does not occur.
[3] Collected in the state of Roraima, Brazil, April 2008.
[4] Collected from Fothergill Island, Zimbabwe, Africa, May 2008.

In addition, in Australia the disease in horses was treated and prevented with arginine or arginine-containing substances, and it has been suggested that indospicine may competitively interfere with the utilization of arginine in protein metabolism (Hooper et al. 1971).

Indospicine was not detected in *I. praticola* and *I. tinctoria* samples collected from Zimbabwe, Africa. This was not an unexpected result because indospicine was not suspected to be associated with the floppy trunk syndrome in elephants (Fowler and Mikota 2006).

References

Aylward, J.H., R.D. Court, K.P. Haydock, et al. 1987. *Indigofera* species with agronomic potential in the tropics. Rat toxicity studies. *Australian Journal of Agricultural Research* 38:177-186.

Christie, G.S., N.P. Madsen, and M.P. Hegarty MP. 1969. Acute biochemical changes in rat liver induced by the naturally occurring amino acid indospicine. *Biochemical Pharmacology* 18:693-700.

Christie, G.S., M. Wilson, and M.P. Hegarty. 1975. Effects on the liver in the rat of ingestion of *Indigofera spicata*, a legume containing an inhibitor of arginine metabolism. *Journal of Pathology* 117:195-205.

FitzGerald, L.M., M.T. Fletcher, A.E.H. Paul, et al. 2011. Hepatotoxicosis in dogs consuming a diet of camel meat contaminated with indospicine. *Australian Veterinary Journal* 89:95-100.

Fowler, M., and S. Mikota. 2006. Veterinary problems of geographical concern. *In* M. Fowler and S. Mikota, eds., Biology, Medicine, and Surgery of Elephants, p. 441. Blackwell Publishing, Ames, IA.

Hegarty, M.P., and A.W. Pound. 1968. Indospicine, a new hepatotoxic amino acid from *Indigofera* spicata. *Nature* 217:354-355.

Hegarty, M.P., and A.W. Pound. 1970. Indospicine, a hepatotoxic amino acid from *Indigofera* spicata: isolation, structure, and biological studies. *Australian Journal of Biological Sciences* 23:831-842.

Hegarty, M.P., W.R. Kelly, D. McEwan, et al. 1988. Hepatotoxicity to dogs of horsemeat contaminated with indospicine. *Australian Veterinary Journal* 65:337-340.

Heinrikson, R.L., and S.C. Meredith. 1984. Amino acids analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Analytical Biochemistry* 136:65-74.

Hooper, P.T., B . Hart, and G.W.Smith. 1971. The prevention and treatment of Birdsville disease of horses. *Australian Veterinary Journal* 47:326-329.

Hutton, E.M., G.M. Windrum, and C.C. Kratzing. 1958a. Studies on the toxicity of *Indigofera endecaphylla* I. Toxicity for rabbits. *Journal of Nutrition* 64:321-337.

Hutton, E.M., G.M. Windrum, and C.C. Kratzing. 1958b. Studies on the toxicity of *Indigofera endecaphylla* II. Toxicity for mice. *Journal of Nutrition* 65:429-440.

Kelly, W.R., M.P. Young, M.P. Hegarty, and G.D. Simpson. 1992. The hepatotoxicity of indospicine in dogs. *In* L.F. James, R.F. Keeler, E.M. Bailey, P.R. Cheeke, and M.P. Hegarty, eds., Poisonous Plants, pp. 126-130. Iowa State University Press, Ames, IA.

Lima, E.F., B. Riet-Correa, F. Riet-Correa, et al. 2011. Poisonous plants affecting the nervous system of horses in Brazil. *In* F. Riet-Correa, J. Pfister, A.L. Schild, and T. Wierenga, eds., Poisoning by Plants, Mycotoxins, and Related Toxins, pp. 290-294. CAB International, Wallingford, U.K.

Madsen, N.P., G.S. Christie, and M.P. Hegarty. 1970. Effect of indospicine on incorporation of L-arginine-^{14}C into protein and transfer ribonucleic acid by cell-free systems from rat liver. *Biochemical Pharmacology* 19:853-857.

Madsen, N.P., and M.P. Hegarty. 1970. Inhibition of rat liver homogenate arginase activity *in vitro* by the hepatotoxic amino acid indospicine. *Biochemical Pharmacology* 19:2391-2393.

Majak, W., M. Benn, D. McEwan, and M.A. Pass. 1992. Three nitropropanoyl esters of glucose from *Indigofera linnaei*. *Phytochemistry* 31:2393-2395.

Miller, R.W., and C.R. Smith, Jr. 1973. Seeds of *Indigofera* species: Their content of amino acids that may be deleterious. *Journal of Agriculture and Food Chemistry* 21:909-912.

Nordfeldt, S., L.A. Henke, K. Morita, et al. 1952. Feeding tests with *Indigofera endecaphylla* Jacq. (creeping indigo) and some observations on it poisonous effects on domestic animals. Technical Bulletin 15. University of Hawaii, Agricultural Experiment Station, pp. 5-23.

Pass, M.A., A. Hossein, S. Pollitt, and M.P. Hegarty. 1996. Effects of the naturally occurring arginine analogues indospicine and canavanine on nitric oxide mediated functions in aortic endothelium and peritoneal macrophages. *Natural Toxins* 4:135-140.

Pollitt, S., M.P. Hegarty, and M.A. Pass. 1999. Analysis of the amino acid indospicine in biological samples by high performance liquid chromatography. *Natural Toxins* 7:233-240.

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Clinical and Pathological Aspects and Cerebellar Lectin Binding in Cattle Poisoned With *Solanum fastigiatum* var. *fastigiatum* and *Solanum bonariense*

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Abstract

Microscopic and lectin histochemical studies were performed using the cerebella of 33 natural cases of *Solanum fastigiatum* var. *fastigiatum* intoxication in cattle from southern Brazil and 2 natural and 4 experimental cases of *Solanum bonariense* from Uruguay. The following biotinylated lectins were used in both cases: WGA, sWGA, BS-I, Con-A, RCA-I, DBA, and UEA-I, with the addition of LCA in *S. fastigiatum* poisoning cases. Histologically, the lesions consisted of fine vacuolization, distention of portions of the Purkinje cells, axonal spheroids measuring 14-50 µm in the granular cell layer and adjacent white matter and, proliferation of the Bergmann's glia. Lectin histochemistry revealed strong reactivity of stored material in Purkinje neurons with the lectins sWGA, Con-A, and LCA in *S. fastigiatum* cases. A similar pattern was found in *S. bonariense* cases with a most intense reaction to WGA, and less intense reaction to Con-A, whereas BS-I and RCA-I binding was absent to poor in these neurons in all the cases studied. Lectin reactivity in Purkinje cells between cases was independent of cell damage (from mild to severe loss of neurons). Both *S. fastigiatum* and *S. bonariense* have similar lectin binding, suggesting a similar pathogenesis. Since comparable binding patterns have been described in animals poisoned with swainsonine-containing plants, perhaps the toxins in these plants contain related glycosidase-inhibiting toxins or inhibit glycoprotein and lysosomal metabolism through some related mechanism. The results of this study showed that in spontaneous poisoning by *S. fastigiatum* and *S. bonariense* in cattle, the pattern of lectin binding is similar to those observed in *S. fastigiatum* experimental conditions.

Keywords: Cattle, cerebellar degeneration, lectin histochemistry, neurotoxicity, poisoning, *Solanum fastigiatum, S. bonariense*

Introduction

Solanum fastigiatum var. *fastigiatum* and *S. bonariense* intoxication is an important cerebellar disorder in cattle in southern Brazil and Uruguay (Riet-Correa et al. 1983, Rech et al. 2006, Verdes et al. 2006). *S. fastigiatum* is one of the major plant poisonings in cattle in Rio Grande do Sul, a state in

southern Brazil (Rissi et al. 2007). Similar disorders induced by other species of *Solanum* have been reported in cattle grazing *S. dimidiatum* and in goats grazing *S. viarum* in the United States (Menzies et al. 1979, Porter et al. 2003) and cattle eating *S. kwebense* (*S. tettense*) in South Africa (Pienaar et al. 1976, van der Lugt et al. 2010) and *S. cinereum* in Australia (Bourke 1997). *S. fastigiatum* poisoning was reproduced in sheep that developed similar lesions to those observed in cattle (Zambrano et al. 1985). Poisoning by *S. paniculatum* in cattle in northeastern Brazil exhibited similar clinical signs and pathological lesions as those caused by *S. fastigiatum* (Medeiros et al. 2004).

The neurological disease of *S. fastigiatum* and *S. bonariense* poisoning is characterized by periodic episodes of seizures, loss of balance, nystagmus, opisthotonus, tremors, hypermetria, extension of the neck, head tilt, and ataxia (Riet-Correa et al. 2009). The age of affected cattle ranges from 6 months to 10 years. In some animals a loss in body condition is observed. Death is uncommon and some animals have to be euthanized because of severe injuries caused by repeated falls.

Grossly, the brain appears normal or cerebellar atrophy is observed (Rech et al. 2006). Histologic lesions include fine vacuolization and diffuse loss of Purkinje cells, axonal spheroids in the granular layer and white matter, Bergman gliosis in the molecular layer, and atrophy of molecular and granular layers. Ultrastructurally, there are numerous lipid inclusions and membranous bodies in the cytoplasm of Purkinje cells (Riet-Correa et al. 1983, Barros et al. 1987). A lectin histochemical study of the cerebella of two cattle experimentally poisoned with *S. fastigiatum* showed accumulation of specific oligosaccharides and other terminal sugars in the degenerated cells, suggesting a glycolipid storage disease (Paulovich et al. 2002). However, the lectin-binding patterns in cerebella of cattle naturally poisoned with *S. fastigiatum* var. *fastigiatum* or naturally and experimentally poisoned with *S. bonariense* have not been documented. Lectin histochemistry using paraffin-embedded sections may be useful in the identification of specific sugars and hence aid in diagnosing glycoprotein and glycolipid storage diseases (Alroy et al. 1984, Alroy et al. 1986, Driemeier et al. 2000, Paulovich et al. 2002). The objectives of this retrospective study were to describe the clinical signs and compare the morphological and lectin histochemical findings of 33 natural cases of *S. fastigiatum* var. *fastigiatum* intoxication in cattle from southern Brazil and 2 natural and 4 experimental cases of *S. bonariense* poisoning from Uruguay.

Material and Methods

Thirty-three cases of spontaneous *S. fastigiatum* var. *fastigiatum* poisoning in cattle occurring from 2006 to 2009 were evaluated. The cases originated from three municipalities of central Rio Grande do Sul State, southern Brazil. Additionally, two heifers naturally poisoned with *S. bonariense* on farms in Soriano, western Uruguay, and four cattle experimentally intoxicated with the same plant and one control were used. Details of the experimental study were previously reported (Verdes et al. 2006).

The history and clinical signs were obtained from the owners or the practicing veterinarians in all cases. At necropsy, the brains were collected and fixed in 10% buffered neutral formalin for 5 to 7 days. The following sections of the brain were evaluated histologically: medulla at the level of the obex, pons, cerebellum, midbrain at the level of the rostral colliculus, thalamus, basal nuclei, hippocampus, and frontal, parietal, and occipital lobes. The samples were routinely processed and stained with hematoxylin and eosin (HE).

For the lectin histochemical study, after deparaffination with xylene, additional samples of cerebellum were immersed in 0.3% hydrogen

Table 1. Lectins used in the histochemical study and their major specificities

Acronym	Source	Major specificity[a]
WGA	*Triticum vulgaris,* Wheatgerm	D-N-acetyl chitobiose, N-acetyl lactosamine and some sialyl residues
sWGA	Succinyl-WGA	β-(1-4)-D-N-acetyl-glucosamine
BS-I	*Bandeirea simplicifolia*-I	α-D-Galactose
Con-A	*Concanavalina ensiformis*	α-D-glucose and α-D-mannose
RCA-I	*Ricinus communis*	β-D-galactose
DBA	*Dolichos biflorus*	α-D-N-acetyl-galactosamine
UEA-I	*Ulex europaeus*-I, Gorse	α1,2-linked fucosyl residues
LCA	*Lens culinaris*	D-Mannose and D-Glucose

[a]Goldstein and Hayes (1978)

peroxide in methanol for 30 min at room temperature, rinsed several times in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and submerged in PBS containing 0.1% bovine serum albumin for 15 min. They were then incubated with the eight biotinylated lectins (Vector Laboratories Inc., Burlingame, CA, USA) shown in Table 1. The optimal concentration for each lectin, which allowed maximum staining with minimum background, was at a dilution of 30 µg/mL in PBS for 1 h followed by incubation with avidin-biotin-peroxidase complex (ABC) (Vector Laboratories Inc.) for 45 min. The horseradish peroxidase was activated by incubation for 4 to 10 min with buffered0.05 M Tris-HCl solution, pH 7.6, containing 0.02% diaminobenzidine (DAB) and 0.05% H_2O_2.

All sections were counterstained with Mayer's hematoxylin. The following negative controls were performed: the lectins were omitted or blocked by incubating them with their blocking sugars (0.1-0.2 M in PBS) for 1 h at room temperature before application to the sections. In the protocol for *S.fastigiatum*, cerebellum of one 3-year-old bovine without neurological signs and morphological lesions in the brain was used as a control. In the *S. bonariense* experiment, unaffected cerebellar samples were obtained from a 1-year-old bovine on the same farm which was also used as a control during the experimental reproduction (Verdes et al. 2006). The lectin binding was analyzed using the following semiquantitative scale of stained structures and subjectively scored as follows: (0) none, (1) weakly positive, (2) moderately positive, and (3) strongly positive. Two pathologists, blinded to previous procedures, evaluated the sections.

Results

Neurological clinical signs observed in all affected cattle included proprioceptive and cerebellar deficits such as incoordination, hypermetria, tremors, frequent falls, and transitory seizures when moved or stimulated. Seizures were sporadic and limited to a few seconds at a time. After these episodes, numerous animals assumed a wide base stance or appeared apparently normal. Progressive weight loss was observed in many animals.

Gross change was noted only in two cattle naturally poisoned with *S. fastigiatum* that included moderate atrophy of the cerebellum. Microscopically, the lesions were restricted to the cerebellum and consisted of fine vacuolization and distention of portions of the Purkinje cells; some degenerated cells had swollen and eosinophilic cytoplasm and others had peripherally placed nuclei. In some cases, the vacuoles were confluent and occupied one pole or the most of the pericarya of the neurons. Axonal spheroids measuring 14-50 µm were visualized frequently in the granular layer and adjacent white matter. In chronic cases, there were severe loss of Purkinje cells in many folia and proliferation of the Bergmann's glia. Occasionally, focal gliosis and mild histiocytic perivascular cuffs were visualized in the white matter of the cerebellum of three cases. The control animals did not have cerebellar lesions. No alterations were observed in other organs.

There were different lectin binding patterns between the affected and control cerebella. Results are summarized in tables 2 and 3. In the *S. fastigiatum* study, there was a clear binding affinity of the cytoplasmic vacuoles in the Purkinje cells for sWGA (figure 1), LCA (figure 2), and Con-A (figure 3). Similar results were achieved for sWGA in natural and experimental *S. bonariense* cases and notable, although less intense reaction, was observed for Con-A. Histochemical marcation was also different for WGA lectin with a moderate reaction in *S. fastigiatum* cases (figure 4) and more accentuated in the *S. bonariense* cerebellum; BS-I and RCA-I binding was absent to poor in all the cases studied. DBA and UEA-I binding was absent in all the cases. Lectin reactivity in Purkinje cells between cases was independent of cell damage stage (from mild to severe loss of neurons). Furthermore, BS-I lectin presented marked binding in the endothelium of blood vessels in all affected and normal cerebella. Purkinje cells of the control bovines showed null to weak reactivity for sWGA, WGA, and Con-A.

Discussion

The clinical signs and gross and microscopic findings observed in cattle in the current study are similar to those previously described in *S. fastigiatum* and *S. bonariense* poisoning in cattle (Riet-Correa et al. 1983, Zambrano et al. 1985, Rech et al. 2006, Verdes et al. 2006). A fine vacuolar degeneration of neuronal perikaria with progressive axonal degeneration resulting in the death of these cells and eventual atrophy of the cerebellum are the main pathological features of this disease. Other species such as *S. dimidiatum*, *S. kwebense* (*tettense*), *S. viarum*, and *S. cinereum* have been associated with neurological disorders in ruminants

Table 2. Intensity of lectin binding in Purkinje cells of *S. fastigiatum* var. *fastigiatum*-poisoned and control cattle

WGA	sWGA	BS-I	Con-A	RCA-I	DBA	UEA-I	LCA
0-2[a] (0-1)	2-3 (1)	0-1 (0)	3 (0-1)	0-1(0)	0 (0)	0 (0)	2-3 (0)

[a]Numbers indicate intensity of binding on a subjective scale of 0 (no reactive) up to 3 (maximum reactivity). Control results from normal cattle are provided in the parentheses.

Table 3. Intensity of lectin binding in Purkinje cells of spontaneous and experimental *S. bonariense*-poisoned and control cattle

	WGA	sWGA	BS-I	Con-A	RCA-I	DBA	UEA-I
Spontaneous	2-3[a] (1)	2 (0-1)	0 (0)	2 (0-1)	0 (0)	0-1 (0-1)	0-1 (0-1)
Experimental	2 (1)	2-3 (0-1)	0 (0)	1-2 (0-1)	0-1(0)	0-1 (0-1)	0-1 (0-1)

[a]Numbers indicate intensity of binding on a subjective scale of 0 (no reactive) up to 3 (maximum reactivity). Control results from normal cattle are provided in the parentheses.

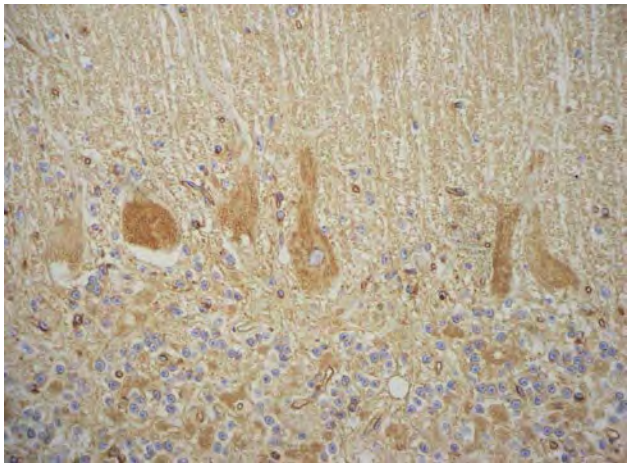

Figure 1. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Strong binding to sWGA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

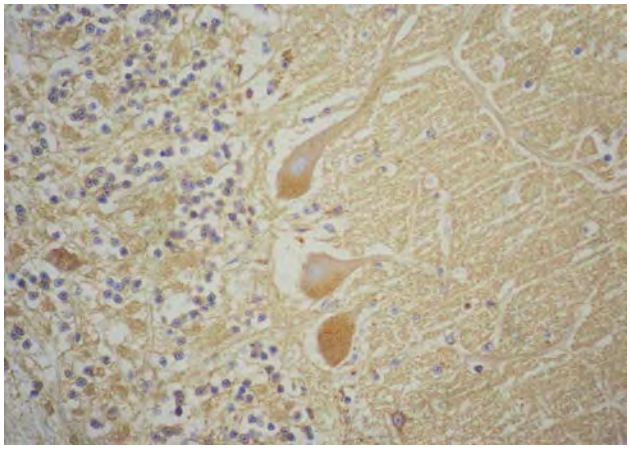

Figure 2. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate to strong binding to LCA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

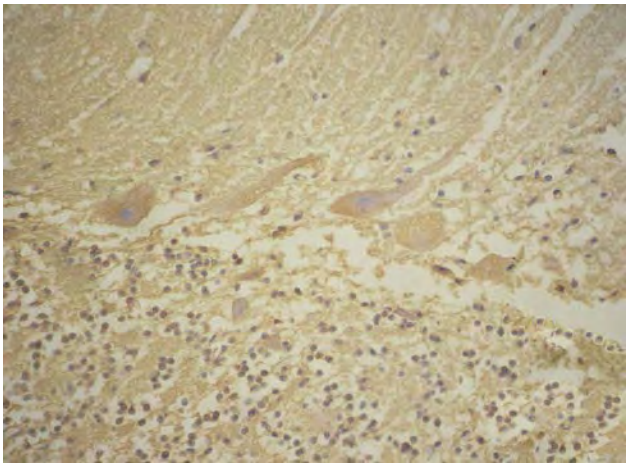

Figure 3. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate to strong binding to Con-A in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

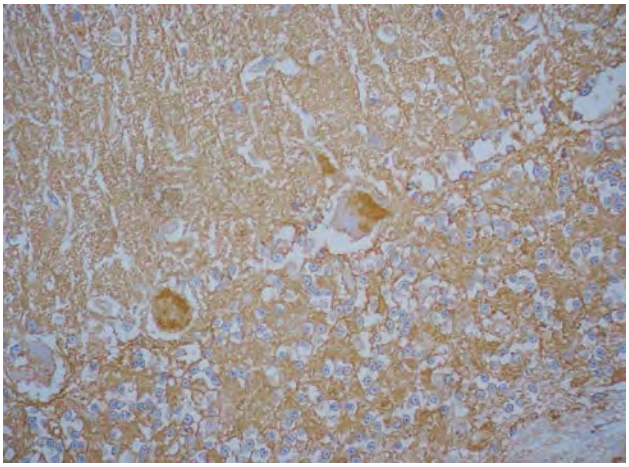

Figure 4. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate binding to WGA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

in other countries where similar clinical manifestations and pathological lesions were observed (Pienaar et al. 1976, Menzies et al. 1979, Bourke 1997, Verdes et al. 2006, van der Lugt et al. 2010). In South Africa, the poisoning by *S. kwebense* (*tettense*) is known as maldronksiekte, which literally means mad- or crazy-drunk-disease (Pienaar et al. 1976).

Electron microscopy studies revealed lipidic inclusions and cytoplasmic membranous and lamellar bodies accompanied by ribosome disaggregation in cerebellar Purkinje cells of cattle intoxicated with *S. fastigiatum* and *S. bonariense* (Riet-Correa et al. 1983, Barros et al. 1987, Verdes et al. 2006). Another typical degenerative histopathologic and ultrastructural change observed is the presence of axonal spheroids that represent swollen myelinated axons (probably by cytoskeletal distortion) filled with electron-dense residual bodies, swollen mitochondria, and an increase in the ratio of axoplasm/myelin (Riet-Correa et al. 1983, Barros et al. 1987, Verdes et al. 2006). It is possible that the neurotoxin(s) contained by these *Solanum* spp. form(s) a complex with lipid material that cannot readily be metabolized by Purkinje cells (Barros et al. 1987) but a direct or indirect role of the neurotoxin(s) on neuronal protein synthesis and/or axonal transport was not discarded from playing a role in the pathogenesis of these cerebellar cortical degeneration (Verdes et al. 2006).

In an experimental reproduction of the disease, specific lectins reacted strongly with stored material in affected Purkinje neurons (Paulovich et al. 2002). Although the toxic principle responsible for the intoxication by *S. fastigiatum* is unknown (Riet-Correa et al. 2009), these previous results have suggested that the poisoning induced by *S. fastigiatum* is classified as a glycolipid storage disease. In *S. dimidiatum* and *S. kwebense* (*tettense*), calystegine B_2 appears to be the toxin responsible for the development of the disease (Nash et al. 1993, Burrows and Tyrl 2001). However, swainsonine and/or calystegines were not detected in *S. bonariense* samples (R.J. Molyneux, 2006, personal communication). Additional studies are needed to better characterize the toxic substances present in *S. fastigiatum* and *S. bonariense*.

Induced storage diseases in domestic herbivores are typically related to ingestion of plants. These conditions include swainsonine toxicosis, *Trachyandra* poisoning, *Phalaris* poisoning, Gomen disease, and *Solanum* poisoning (Maxie and Youssef 2007) and can be classified as glycolipid or glycoprotein storage diseases (Alroy et al. 1984, Alroy et al. 1986). Previous studies have demonstrated that lectin histochemical and ultrastructural analyses allow the identification and characterization of the glycoproteins and glycolipids implicated in these disorders (Alroy et al. 1984, Alroy et al. 1986, Driemeier et al. 2000, Cholich et al. 2009, van der Lugt et al. 2010). Lectins are carbohydrate-binding proteins and glycoproteins of non-immune basis that agglutinate cells and/or precipitate glycoconjugates having saccharides of appropriate complementarity (Goldstein and Hayes 1978).

In the present study, accumulated material in the perikarya of Purkinje cells had marked binding affinities for sWGA, Con-A, and LCA, indicating β-(1-4)-D-N-acetyl-glucosamine, α-D-mannose and α-D-glucose, and D-mannose and D-glucose residues, respectively, and impairment of the function of lysosomal enzymes. Furthermore, D-N-acetyl chitobiose and N-acetyl lactosamine residues, indicated by WGA binding, were detected with appreciable intensity. These findings partially conform to those observed in the cerebellum of cattle poisoned experimentally by *S. fastigiatum* (Paulovich et al. 2002) and spontaneously by *S. kwebense* (*tettense*) (van der Lugt et al. 2010). However, β-D-galactose residues were detected only in the experimental cases previously reported (Paulovich et al. 2002). This finding differs from the current study. It is surmised that this difference is associated with the period of ingestion of the plant since many naturally poisoned cattle included in the present study grazed *S. fastigiatum* or *S. bonariense* over extended and undetermined periods.

Lectin-binding patterns observed in affected Purkinje cells of the present investigation are similar to those detected in plant-induced α-mannosidosis (including poisonings by plants of the genera *Swainsona*, *Oxytropis*, *Astragalus*, *Sida*, and *Ipomoea*), but in the last condition there is additional vacuolization in pancreatic, liver, and kidney epithelial cells (Alroy et al. 1984, Alroy et al. 1985, Driemeier et al. 2000, Armién et al. 2007, Cholich et al. 2009). In addition, these degenerative lesions also occur in neurons of the medulla oblongata and pons (Cholich et al. 2009).

To our knowledge, this is the first time the lectin histochemical aspects of spontaneous cases of poisoning by *S. fastigiatum* var. *fastigiatum* and spontaneous and experimental cases of poisoning by *S. bonariense* in cattle have been characterized and compared. The results of this study show that in all

the cases evaluated in cattle, the pattern of lectin binding is similar to that observed in naturally occurring or experimental reproduction of cerebellar cortical degeneration induced by Solanaceous species in cattle. Further studies are needed to define the specific material stored in accumulated lysosomes in Purkinje cells and the potential role of a distorted cytoskeleton in axonal transport alteration suggested by this cerebellar cortical degeneration in cattle (Verdes et al. 2006).

Acknowledgments

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References

Alroy, J., U. Orgad, A.A. Ucci, and M.E.A. Pereira. 1984. Identification of glycoprotein storage diseases by lectins: a new diagnostic method. *Journal of Histochemistry and Cytochemistry* 32:1280-1284.

Alroy, J., U. Orgad, A.A. Ucci, and V.E. Gavris. 1985. Swainsonine toxicosis mimics lectin histochemistry of mannosidosis. *Veterinary Pathology* 22:311-316.

Alroy J., A.A. Ucci, V. Goyal, and W. Woods. 1986. Lectin histochemistry of glycolipid storage diseases on frozen and paraffin-embedded tissue sections. *Journal of Histochemistry and Cytochemistry* 34:501-505.

Armién, A.G., C.H. Tokarnia, P.V. Peixoto, and K. Frese. 2007. Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoea carnea* subsp. *fistulosa* (Convolvulaceae). *Veterinary Pathology* 44:170-184.

Barros, S.S., F. Riet-Correa, M.B. Andujar, et al. 1987. *Solanum fastigiatum* var. *fastigiatum* and *Solanum* sp. poisoning in cattle: ultrastructural changes in the cerebellum. *Pesquisa Veterinária Brasileira* 7:1-5.

Bourke, C.A. 1997. Cerebellar degeneration in goats grazing *Solanum cinereum* (Narrawa burr). *Australian Veterinary Journal* 75:363-365.

Burrows, G.E., and R.J. Tyrl. 2001. *Toxic plants of North America*. Iowa State University Press, Ames, Iowa. 1342 p.

Cholich, L.A., E.J. Gimeno, P.G. Teibler, et al. 2009. The guinea pig as an animal model for *Ipomoea carnea* induced α-mannosidosis. *Toxicon* 54:276-282.

Driemeier, D., E.M. Colodel, E.J. Gimeno, and S.S. Barros. 2000. Lysosomal storage disease caused by *Sida carpinifolia* poisoning in goats. *Veterinary Pathology* 37:153-159.

Goldstein, I.J., and C.E. Hayes. 1978. The lectins: carbohydrate binding proteins of plants and animals. *Advances in Carbohydrate Chemistry Biochemistry* 35:127-340.

Maxie, M.G., and S. Youssef. 2007. Nervous system. *In* M.G. Maxie, ed., *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*, 5th ed., vol. 1, pp. 281-457. Saunders Elsevier, Edinburg, Scotland, U.K.

Medeiros, R.M.T., R.F. Guilherme, F. Riet-Correa, et al. 2004. Intoxicação experimental por *Solanum paniculatum* (jurubeba) em bovinos. *Pesquisa Veterinária Brasileira* 24(suppl.):41.

Menzies, J.S., C.H. Bridges, and E.M. Bailey. 1979. A neurological disease of cattle associated with *Solanum dimidiatum*. *Southwestern Veterinarian* 32:45-49.

Nash, R.J., M. Rothschild, E.A. Porter, et al. 1993. Calystegines in *Solanum* and *Datura* species and the deaths-heads hawk moth (*Acherontia atropus*). *Phytochemistry* 34:1281-1283.

Paulovich, F.B., E.L. Portiansky, E.J. Gimeno, et al. 2002. Lectin histochemical study of lipopigments present in the cerebellum of *Solanum fastigiatum* var. *fastigiatum* intoxicated cattle. *Journal of Veterinary Medicine A* 49:473-477.

Pienaar, J.G., T.S. Kellerman, P.A. Basson, et al. 1976. Maldronksiekte in cattle: a neuropathy caused by *Solanum* kwebense N.E. Br. *Onderstepoort Journal of Veterinary Research* 43:67-74.

Porter, M.B., R.J. Mackay, E. Uhl, et al. 2003. Neurologic disease putatively associated with ingestion of *Solanum viarum* in goats. *Journal of American Veterinary Medical Association* 223:501-504.

Rech, R.R., D.R. Rissi, A. Rodrigues, et al. 2006. Intoxicação por *Solanum fastigiatum* (Solanaceae) em bovinos: epidemiologia, sinais clínicos e morfometria das lesões cerebelares. *Pesquisa Veterinária Brasileira* 26:183-189.

Rissi, D.R, R.R. Rech, F. Pierezan, et al. 2007. Intoxicações por plantas e micotoxinas associadas a plantas em bovinos no Rio Grande do Sul: 461 casos. *Pesquisa Veterinária Brasileira* 27:261-268.

Riet-Correa, F., M.C. Méndez, A.L. Schild, et al. 1983. Intoxication by *Solanum fastigiatum* var. *fastigiatum* as a cause of cerebellar degeneration in cattle. *Cornell Veterinarian* 73:240-256.

Riet-Correa, F., R.M.T. Medeiros, J. Pfister, et al. 2009. *Poisoning by Plants, Mycotoxins and Related Substances in Brazilian Livestock*. Editora Pallotti, Santa Maria, Brazil. 246 p.

van der Lugt, J.J., S.S. Bastianello, A.M. van Ederen, and E. van Wilpe 2010. Cerebellar cortical degeneration in cattle caused by *Solanum kwebense*. *The Veterinary Journal* 185:225-227.

Verdes, J.M., A. Moraña, F. Gutiérrez, et al. 2006. Cerebellar degeneration in cattle grazing *Solanum bonariense* ("Naranjillo") in Western Uruguay. *Journal of Veterinary Diagnostic Investigation* 18:299-303.

Zambrano, M.S., F. Riet-Correa, A.L. Schild, and M.C. Méndez. 1985. Intoxicação por *Solanum fastigiatum* var. *fastigiatum*: evolução e reversibilidade das lesões em bovinos, e suscetibilidade de ovinos, coelhos, cobaios e ratos. *Pesquisa Veterinária Brasileira* 5:133-141.

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Fetotoxicity of *Astragalus lentiginosus* (Locoweed) in Spanish Goats

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Keywords: *Astragalus*, locoweed, fetal movement, embryotoxic, radioultrasound

Introduction

Locoweed species—*Astragalus* and *Oxytropis* spp. that contain the indolizidine alkaloid swainsonine—are widely distributed on rangelands in North and South America, Mexico, China, and other rangelands throughout the world. Other range plant species including *Sida* spp. (Seitz et al. 2005), *Ipomoea* spp. (Damir et al. 1987), and *Swainsona* spp. (Laws and Anson 1968) from Brazil, Africa, and Australia, respectively, also contain swainsonine and other metabolic toxins that poison livestock and have been problematic for ranchers. Swainsonine poisons livestock by inhibiting the metabolic enzymes lysosomal α-mannosidase and mannosidase II (Dorling et al. 1980). Inhibition of these enzymes results in abnormal oligosaccharide accumulation in cellular lysosomes with accompanying characteristic neurovisceral vacuolation in multiple organ systems (Stegelmeier et al. 1995).

Clinically, locoweeds cause intention tremors, generalized depression, nervousness, proprioceptive deficits, aberrant behavior, reproductive dysfunction, emaciation, and death (James et al. 1970). Poisonings with similar etiologies have been reported in Brazil and Mozambique from *Ipomoea carnea* (Damir et al. 1987, De Balogh et al. 1999) and in Australia from Darling Pea *(Swainsona galegiofolia)* (Hartley 1978). Swainsonine is found in these plant species and is believed to be partially responsible for the reported toxicoses. The manifestation of clinical effects varies somewhat, depending on the animal species involved. Additionally, locoweeds cause embryo and fetal death, abortions, generalized reproductive dysfunction, and occasional birth defects. There has been a substantial amount of research in sheep fed locoweeds at various stages of gestation (James 1971, Panter et al. 1999); however, there has been relatively no research done on goats, particularly pregnant Spanish goats. Thus, the purpose of this study was to describe the clinical effects of locoweed ingestion on pregnant goats, focusing with ultrasound on the embryo/fetotoxic effects during the late first trimester and early-second trimester of pregnancy using Spanish goats.

Materials and Methods

Nine female Spanish goats were hand-mated to like bucks and divided into two groups. Five pregnant does were dosed twice daily with finely ground *Astragalus lentiginosus* via oral gavage beginning on day 30 of gestation. The plant dosage was calculated to deliver 8 mg swainsonine/kg body weight (BW)/day (140-230 g dry ground *Astragalus lentiginosus*). The *Astragalus lentiginosus* (USDA-ARS Poisonous Plant Research Lab accession #1998-02) was collected just south of St. Johns, AZ, and contained 0.17% swainsonine, dry weight. Four similarly bred goats were dosed with equivalent amounts of ground alfalfa hay as negative controls and treated identical to the locoweed-treated goats. Gestation day 30 was considered day 0 of treatment. Blood samples were collected from each goat in a vacutainer vial via the jugular vein on days 1-7, and every 5 days thereafter or until fetal death was confirmed. Blood samples were maintained at room temperature for 30-45 minutes, after which serum was separated by centrifugation at 2,300 rpm. Serum was collected and frozen at –20ºC and later analyzed for swainsonine and progesterone. Serum

swainsonine is an indicator of locoweed exposure and serum progesterone is an indicator of fetal viability.

Fetal movement was observed by ultrasound every 5 days beginning on day 30 of gestation, and fetal activity was assessed using visual evaluation. An Aloka Model 900 ultrasound with a 5 mhz abdominal transducer was used for ultrasonography observations. Briefly, an echogenic gel was applied to the abdomen of each goat on either side of the udder and 2-5 cm anterior. Once the fetus was observed, a 5- min scan was performed and recorded to videotape for future analysis. Later, fetal activity was evaluated from the video tapes and each voluntary movement was counted and recorded. The fetal heartbeat was noted to confirm fetal viability, but no attempt was made to determine the fetal heart rate.

Swainsonine Analysis

Serum swainsonine concentrations were determined using a method previously published with some modification (Stegelmeier et al. 1995). Briefly, 0.6 mL of serum was combined with 0.3 mL sodium acetate (0.25 M, pH 4.0), vortexed, and boiled for 10 min. The supernatant was removed and 75 µL was put into a multiwell plate in duplicate with 15 µL of 0.0008 U/well Jack Bean α-mannosidase (Sigma Chemical Co., St. Louis, MO) and 10 µL of 10 mM p-nitrophenyl-α-D-mannosidase (Sigma Chemical Co., St. Louis, MO). The mixture was incubated for 30 min at 37ºC, after which 100 µL glycine (2.5 M, pH 10.3) was added to stop the reaction. The amount of swainsonine (α-mannosidase inhibition) in the test samples was then determined photometrically at 405 nm using a microplate reader and microplate manager software (Bio-Rad 3550 UV, Bio-Rad laboratories, Melville, NY). An analysis of swainsonine standards with concentrations ranging from 1,000 to 1.2 ng/mL was performed in a similar manner using blood serum from normal, unexposed animals spiked with known amounts of swainsonine. The control swainsonine concentrations were verified by capillary gas chromatography using previously described methods (Molyneux et al. 1989). Samples higher than the linear range of the standard curve (>800 ng/mL) were diluted with sodium acetate (0.25 M, pH 4.0). The standards for these assays were similarly modified by diluting the normal serum. Serum α-mannosidase activity is inversely related to swainsonine concentration as shown in figure 1.

Progesterone Analysis

Serum progesterone levels were determined by radioimmunoassay using a non-extraction, solid phase I-125 radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). The Coat-A-Count progesterone kit is highly specific with low cross-reactivity to other steroids. Specific cross-reactivity is listed in the procedure manual (Diagnostic Products Corporation). Inter- and intra-assay coefficients of variation were 6.15% and 4.25%, respectively. Progesterone kits were validated at 0.5, 2.5, 5, 10, 20, and 40 ng/mL.

Results

Locoweed-fed goats developed mild proprioceptive deficits, as manifested by difficulty stepping over a small wooden barrier, within 9 to 10 days of treatment. These clinical signs became more severe over time, and treated goats became lethargic, were hesitant to move, exhibited pronounced intention tremors, and displayed severe proprioceptive deficits. Several animals developed severe rear limb weakness and partial paresis. These animals were euthanized and necropsied.

Serum swainsonine was rapidly elevated in treated goats, reaching concentrations of > 400 ng/mL after the first day of treatment and peaking above 600 ng/mL (figure 1). Serum α-mannosidase levels were not measured in this study but have been shown to be inversely related to serum swainsonine levels. No swainsonine was detected in sera from control goats.

Serum progesterone significantly ($P<0.05$) declined in locoweed-fed goats after 5 days on locoweed, beginning on gestation day 35 and continuing throughout the treatment period (figure 2). This was indicative of the negative effect of locoweed on the fetus, placenta, ovary, and other structures important in the maintenance of pregnancy in goats.

All pregnant goats, including treatment and controls, were evaluated with ultrasound and had viable embryos (embryonic vesicles appeared normal) on day 30 when the treatments began. Embryonic/fetal movement was detected in most animals between days 36 and 38, which is typical when movement first begins in sheep and goats (Panter et al. 1990). Fetal movement was similar between control and locoweed-fed goats until after day 40 of gestation ($P>0.05$; figure 3).

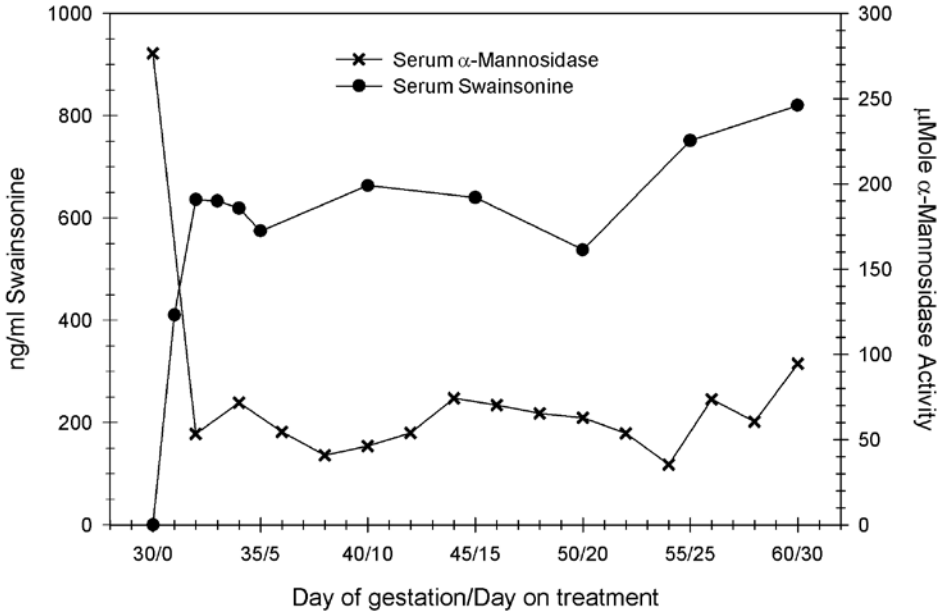

Figure 1. Serum swainsonine concentrations over time in goats dosed with 8 mg swainsonine/kg BW/day as ground *Astragalus lentiginosus*. Swainsonine was determined using a competitive binding assay against Jack Bean α-mannosidase (Stegelmeier et al. 1995). Locoweed-induced decline in α-mannosidase is adapted from Panter et al. (1999).

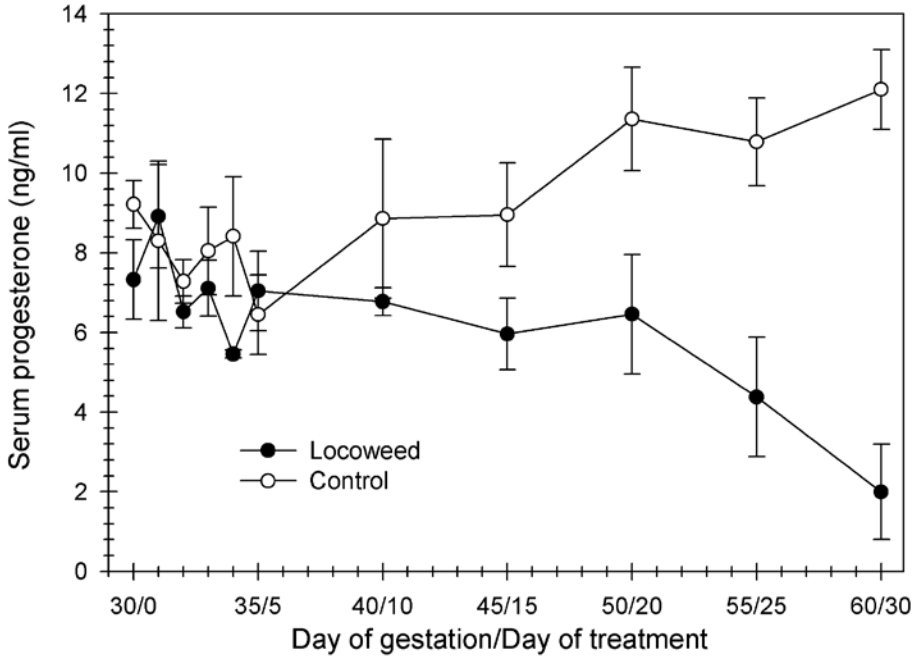

Figure 2. Serum progesterone concentrations (ng/ml) in goats treated with locoweed compared with controls. Declining serum progesterone levels are consistent with the fetal loss shown in figure 3.

Fetal death was observed by ultrasound in one goat on day 40 of gestation (10 days after treatment began). Fetal movement in the surviving fetuses was reduced (P<0.05) compared with controls, and fetal death in 2 more locoweed-treated goats was observed on gestation day 45 and the fourth on gestation day 55 (figure 3). Fetal movement remained normal in control goats throughout pregnancy, and goat kids were within normal parameters at birth.

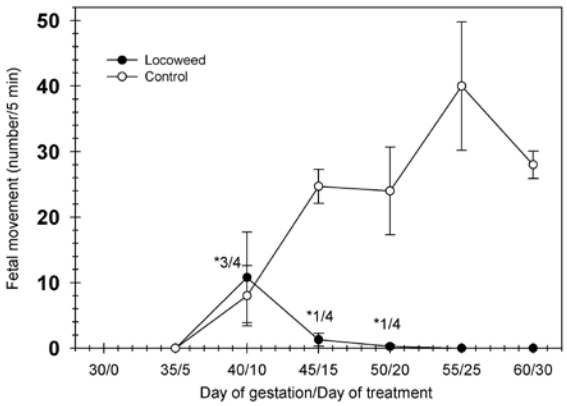

Figure 3. Comparison of fetal activity between locoweed-treated and control goats. Fetal viability and number of movements in real time were visually determined from video-recorded ultrasound images. Fetal movement first begins between 35 and 38 days of gestation in goats. The values shown in the figure represent the number of viable fetuses/total fetuses detected at a given ultrasound time

All fetuses from goats fed locoweed were negatively impacted, and all fetuses died between 10 and 25 days after the beginning of locoweed treatment (figure 3). As early as treatment day 10 in one goat (day 40 of gestation), no fetal heart beat was evident by ultrasound and the fetus was dead. Fetal death was observed in the other 4 goats on treatment days 15 (2 animals) and 20. Ultrasound evaluation revealed dead fetuses in various stages of resorption, and follow-up necropsy confirmed that fetuses were in advanced stages of autolysis. Histologically, all of the treated does had neurovisceral vacuolation in tissues characteristic of locoweed poisoning. The fetuses did not show the typical neurovisceral vacuolation; however, their tissues were in advanced stages of decomposition and were of little histological value.

Discussion

In this study, Spanish goats and their fetuses were determined to be very sensitive to locoweed poisoning at the doses fed during the first trimester of pregnancy. This is important as many animals, including sheep, cattle, and goats, are exposed to locoweed during early gestation, and field reports often indicate that many females recycle during the breeding season or are not pregnant at the end of the breeding season. Pregnant Spanish goats fed locoweed exhibited clinical signs similar to those described in horses, i.e., ataxia, severe proprioceptive deficits, excitement, muscular tremors, lumbar paresis, and aberrant behavior. These clinical signs had a relatively rapid onset, indicating the severity of the intoxication, and the rear limb paralysis was typically seen when animals were excited or stressed. Rear limb paralysis has not been a commonly reported clinical effect from intoxication by swainsonine-containing locoweeds but has been reported for nitro-bearing species (Mathews 1940, James et al. 1980). Detailed histopathology from this study will be reported elsewhere.

Locoweed exposure in utero also had severe effects on fetal goats. Fetal movement was depressed, indicative of fetotoxicty. Fetal death occurred as early as 10 days after treatment began and all fetuses were dead by 25 days. The fetuses were autolytic and the ultrasound observations superimposed on the progesterone levels suggested that fetal death may be multifaceted, i.e. directly affecting the fetus (fetal death) or affecting the placenta. Locoweed given to pregnant sheep and goats has been shown to affect fetal and placental development (Van Kampen and James 1971, Panter et al. 1987, Hafez et al. 2007). Gross histological findings in sheep include fluid accumulation in the placenta (hydrops allantois, hydrops amnii), altered cotyledonary development, with fetal death followed by abortions. Panter et al. (1987) also showed that fetal heart rate was reduced and fetal heart contractions were irregular and weak. Fetal cardiac insufficiency and right heart failure may contribute to the fluid accumulation in the fetus and placenta (Panter et al. 1999) and cause fetal death and abortion (Panter et al. 1987). James (1971, 1976) fed ewes locoweed during specific gestational time periods and studied lamb fetal development. Sheep fetuses exposed to locoweed in utero before 100 days of gestation did not have any lesions associated with locoweed intoxication (James 1971, 1976). Cytoplasmic vacuolization occurred in lambs whose dams were fed locoweed from 100 to 120 days of gestation (James 1971).

Conclusions

This research suggests that Spanish goats are very sensitive to the negative effects of locoweed on reproduction. The early reduction in fetal movement and fetal death is indicative of this sensitivity. Also, the clinical signs of poisoning were pronounced relatively early but slightly different from what has been reported, i.e. the rear-end paresis and severe propioceptive deficits. Additional studies of the hormonal, placental, and early fetal lesions in poisoning are needed to better understand the mechanism of locoweed-induced fetal death. This work demonstrates that dosage of swainsonine ingested is an important aspect in predicting risk of poisoning. We know that low doses of swainsonine will also induce reproductive dysfunction; however, ingestion must occur over a longer period of time as expected. This threshold dosage is understood in many species but has not been fully described in goats and will require further research.

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References

Damir, A.H., S.E.I. Adam, and G. Tartour. 1987. The effects of *Ipomoea carnea* on goats and sheep. *Veterinary and Human Toxicology* 29(4):316-319.

De Balogh, K.I.M., A.P. Dimande, J.J. Van Der Lugt, et al. 1999. Lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. *Journal of Veterinary Diagnostic Investigation* 11: 266-273.

Dorling, P.R., C.R. Huxtable, and S.M. Colegate. 1980. Inhibition of lysosomal a-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *The Biochemical Journal* 191:649-665.

Hafez, S.A., T. Caceci, L.E. Freeman, and K.E. Panter. 2007. Angiogenesis in the caprine caruncles in non-pregnant and pregnant normal and swainsonine-treated does. *The Anatomical Record* 290:761-769.

Hartley, W.J. 1978. A comparative study of Darling pea (*Swainsona* spp.) poisoning in Australia with locoweed (*Astragalus* and *Oxytropis* spp.) poisoning in North America. *In* R.F. Keeler, K.R. Van Kampen, and L.F. James, eds., Effects of Poisonous Plants on Livestock, pp. 363-369, Academic Press, New York, NY.

James, L.F. 1971. Lesions in neonatal lambs resulting from maternal ingestion of locoweed. *The Cornell Veterinarian* 61:667-670.

James, L.F. 1976. Effect of locoweed (*Astragalus lentiginosus*) feeding on fetal lamb development. *Canadian Journal of Comparative Medicine* 40:380-384.

James, L.F., K.R. Van Kampen, and A.E. Johnson. 1970. Physiopathologic changes in locoweed poisoning in livestock. *American Journal of Veterinary Research* 31:663-672.

James, L.F., W.J. Hartley, M.C. Williams, and K.R. Van Kampen. 1980. Field and experimental studies in cattle and sheep poisoned by nitro-bearing *Astragalus* or their toxins. *American Journal of Veterinary Research* 41:377-382.

Laws, L., and R.B. Anson. 1968. Neuropathy in sheep fed *Swainsona luteola* and *S. galegifolia*. *Australian Veterinary Journal* 44(10):447-452.

Mathews, F.P. 1940. The toxicity of red-stemmed peavine (*Astragalus emoryanus*) for cattle, sheep, and goats. *Journal of the American Veterinary Medical Association* 47:125-134.

Molyneux, R.J., L.F. James, K.E. Panter, and M.H. Ralphs. 1989. The occurrence and detection of swainsonine in locoweeds. *In* L.F. James, A. Elbein, R.J. Molyneux, and C.D. Warren, eds., Swainsonine and Related Glycosidase Inhibitors, pp. 100-117, Iowa State University Press, Ames, IA.

Panter, K.E., T.D. Bunch, L.F. James, and D.V. Sisson. 1987. Ultrasonographic imaging to monitor fetal and placental developments in ewes fed locoweed (*Astragalus lentiginosus*). *American Journal of Veterinary Research* 48:686-690.

Panter, K.E., T.D. Bunch, R.F. Keeler, et al. 1990. Multiple congenital contractures (MCC) and cleft palate induced in goats by ingestion of piperidine alkaloid-containing plants: Reduction in fetal movement as the probable cause. *Clinical Toxicology* 28(1):69-83.

Panter, K.E., L.F. James, B.L. Stegelmeier, et al. 1999. Locoweeds: effects on reproduction in livestock. *Journal of Natural Toxins* 8:53-62.

Panter, K.E., M.H. Ralphs, L.F. James, and B.L. Stegelmeier. 1999. Effects of locoweed (*Oxytropis sericea*) on reproduction in cows with a history of locoweed consumption. *Veterinary and Human Toxicology* 41(5):282-286.

Seitz, A.L., E.M. Colodel, M. Schmitz, et al. 2005. Use of lectin histochemistry to diagnose *Sida carpinifolia* toxidosis: an induced mannosidosis in horses. *Equine Veterinary Journal* 35(5):434-438.

Stegelmeier, B.L., L.F. James, K.E. Panter, and R.J. Molyneux. 1995. Serum swainsonine concentration and alpha-mannosidase activity in cattle and sheep ingesting *Oxytropis sericea* and *Astragalus lentiginosus* (locoweeds). *American Journal of Veterinary Research* 56:149-154.

Van Kampen, K.R., and L.F. James. 1971. Ovarian and placental lesions in sheep from ingesting locoweed (*Astragalus lentiginosus*). *Veterinary Pathology* 8:193-199.

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Locoweed Poisoning in the Native Grasslands of China

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Abstract

Locoweeds, including certain species of *Oxytropis* and *Astragalus* that contain swainsonine, are important poisonous plants throughout the world and especially in the vast native grasslands of China. Livestock sustainability and health on the Chinese rangelands are seriously threatened by desertification and locoweed invasion. Enormous economic losses occur every year threatening the local advancement of animal husbandry and endangering the sustainability of the livestock producers in these regions. The purpose of this paper is to review and describe the distribution of locoweed species in China, review information where toxicoses are prevalent, and report current research data on plant–endophyte relationships with locoweed species, poisonings, and relevant management practices, where they exist.

Keywords: Poisonous plants, China, grasslands, locoweed, *Oxytropis*, *Astragalus*, swainsonine, endophyte, *Embellisia oxytropis*, *Undifilum oxytropis*

Introduction

The native grasslands of China are the second largest in the world, comprising more than 400 million hectares (about 40 percent of the land mass of China). The grassland ecosystem in China is massive and ecologically and environmentally important, and it has supported the livestock-based livelihood and survivability of ethnic minorities for centuries. It is currently estimated that about 90 percent of the native grasslands in China has been degraded, with 30 percent in very poor condition (Zhao et al. 2005). Associated with the degradation of grasslands are a loss in productivity; reduced ability to support livestock; a concomitant increase in poisonous plants, rodent, and other pest infestations; and accelerated desertification. The spread of poisonous plants is considered the second-most serious problem after desertification for China's northern grassland region (Zhao et al. 2005). According to previous surveys, the area of native grasslands in northern China where toxic weeds occur at a harmful level is 333 million hectares, with the major threat being locoweed, accounting for about 33 percent of the affected area (figure 1) (Zhao et al. 2005). More recently, the locoweed-infested areas have been expanding (Zhao et al. 2005), and sustainability of livestock production in these critical grassland regions is in peril.

The primary toxic compound in locoweed is the indolizidine alkaloid swainsonine (Molyneux et al. 1989), which is now believed to be produced by the endophyte *Undifilum oxytropis* (Pryor et al. 2009) and which was previously referred to as *Embellisia oxytropis* (Braun et al. 2003, Ralphs et al. 2008, Lu et al. 2009). Interestingly, Broquist et al. (1985) reported production of swainsonine and slaframine (both indolizidine alkaloids with similar biosynthetic

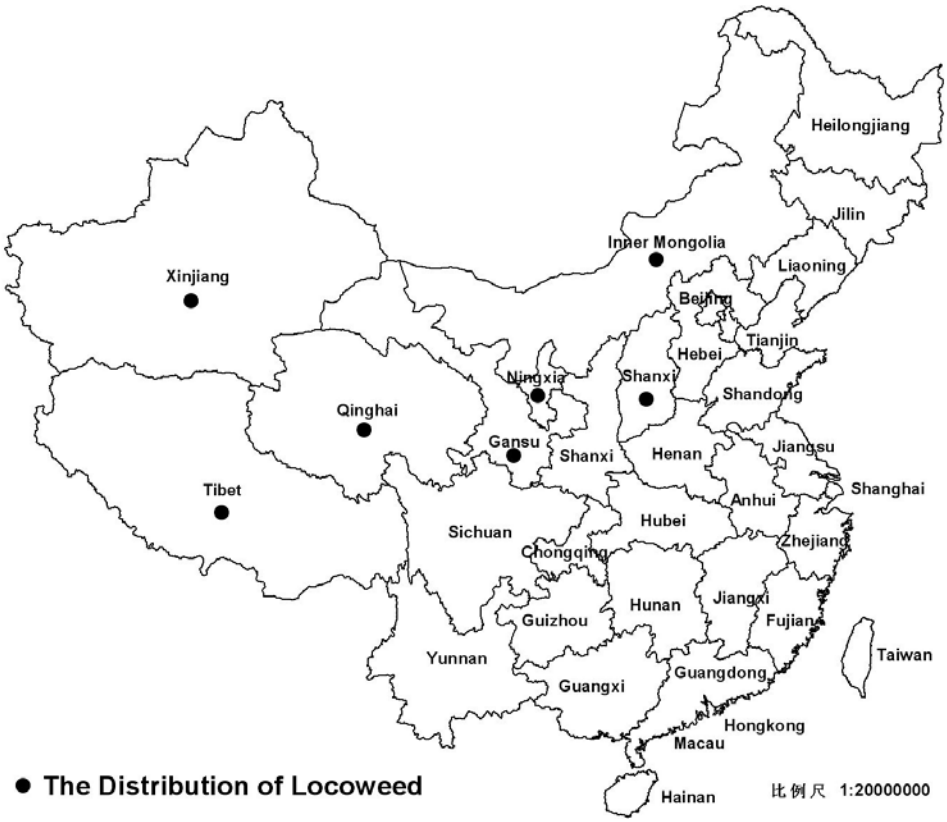

Figure 1. Geographical distribution of locoweeds in China

pathways) production by the non-endophytic fungus *Rhizoctonia leguminicola*, a mold that colonizes certain legumes. In cell culture, *R. leguminicola* has been used to produce swainsonine and slaframine for research applications.

While much research has been conducted in China on locoweeds, endophytes, and swainsonine, more research is required to fully understand the plant–endophyte relationship, determine ways to control the expanding infestation by locoweeds, and develop management guidelines to reduce livestock losses and improve sustainability of this important grassland resource.

Species and Distribution

In China, the locoweeds are mainly distributed in the northwestern and northern regions including Inner Mongolia, Gansu, Ningxia, Qinghai, Xinjiang, Tibet, and Shanxi. According to preliminary surveys (Liu et al. 2010), the area of locoweed infestation in China exceeds 110 million hectares, which accounts for about 2.8 percent of the total grassland area in this northern region. It is reported that 45 species of locoweeds (22 species of *Oxytropis* and 23 species of *Astragalus*) occur in China (Huang et al. 2003). Of these, eight species of *Oxytropis* and three species of *Astragalus* contain swainsonine (table 1).

Livestock Losses Caused by Locoweeds

Clinical signs of locoweed poisoning (swainsonine toxicosis) in livestock in China are the same as those reported in other parts of the world, which include body-weight loss, proprioreceptive deficits, nervousness under stress, cardiovascular disease, reproductive losses, and death (Yang 2002). Substantial livestock losses caused by locoweed have been recorded in China. Over 100,000 sheep were poisoned in Minle County in Gansu from 1953 to 1973; more than 10,000 sheep deaths were caused by *O. ochrocephala* in Haiyuan County in Ningxia from 1976 to 1987; 55,000 animal deaths occurred from locoweed poisoning in the Qinghai Province from 1957 to 1985; and 11,500 animals died as a result of eating *O. sericopetala* near Lhasa in Tibet from 1977 to 1979 (Wang 1999). In horses, reports of reproductive losses are common: there were 16 abortions out of 26 pregnant mares which consumed locoweed, and 75 percent of the mares in another herd in Gonghe County in Qinghai failed to conceive in 1980. Locoweed was believed to be the cause (Zhang and Liu 1994). As the native grasslands of China continue to be over-utilized and degraded, the locoweed areas will expand and livestock poisoning caused by locoweeds will continue to increase.

Table 1. Locoweed species in China, geographical location, and swainsonine content

Species	Geographical location	Swainsonine content (%)
Astragalus hamiensis	Inner Mongolia (Zhong et al. 2007)	0.005 (Liu et al. 2009)
Astragalus strictus	Tibet (Wang et al. 2007)	0.004 (Liu et al. 2009)
Astragalus variabilis	Inner Mongolia, Gansu, Ningxia (Lu et al. 2006, Zhao et al. 2003)	0.010 (Liu et al. 2009)
Oxytropis deflexa	Qinghai (Zhao et al. 2003)	0.013 (Liu et al. 2009)
Oxytropis falcata	Qinghai (Zhao et al. 2003)	not quantified (Huo et al. 2008)
Oxytropis glabra	Inner Mongolia, Gansu, Xinjiang, Tibet, Shanxi (Lu et al. 2006, Wang et al. 2007, Zhao et al. 2003)	0.008 (Liu et al. 2009)
Oxytropis glacialis	Tibet (Wang et al. 2007)	0.018 (Liu et al. 2009)
Oxytropis kansuensis	Gansu, Qinghai, Tibet (Wang et al. 2007, Zhao et al. 2003)	0.015 (Liu et al. 2009)
Oxytropis latibracteata	Qinghai (Zhao et al. 2003)	0.010 (Liu et al. 2009)
Oxytropisochrocephala	Inner Mongolia, Gansu, Ningxia, Qinghai, Tibet (Lu et al. 2006, Wang et al. 2007, Zhao et al. 2003)	0.015 (Liu et al. 2009)
Oxytropis sericopetala	Tibet (Wang et al. 2007)	not quantified (Yu et al. 2006)

Management of Locoweeds and Intoxication

Locoweeds can be temporarily controlled by physically pulling or burning the plants in situ; however, because it has a large tap root, burning does not completely kill the plant (Fan et al. 2006). Herbicides such as 2,4-D can be used over larger areas to control locoweeds; however, this is costly and it will kill other desirable forage plants. There are no herbicides that specifically target *Oxytropis* or *Astragalus* species (Wu et al. 2001). Herbicides may also contaminate the air and water, thereby causing environmental concerns, or plants may develop resistance. Moreover, some herbicides may kill only the top growth, allowing regeneration from the roots as well as germination of seed reserves in the soil (Fan et al. 2006). Seed reserves in the soil are abundant and seedlings will re-establish stands of locoweed when environmental conditions permit. Long-term control must be multidimensional and should include range practices to manage competitive grasses to prevent reinvasion once locoweeds are suppressed.

In China, various methods of treatment have been utilized by livestock producers (Zhao et al. 2005). Livestock should first be removed from locoweed-infested sites. Furthermore, Zhao et al. (1999) reported that the mixture of vinegar residue and coarse flour could detoxify the sheep poisoned with *O. glabra* (figure 2). Chang et al. (2007) reported that "Fengcaolin bolus" could relieve suffering in sheep poisoned with *O. ochrocephala*. Toxicity and the timing when symptoms of toxicity became evident appeared to be delayed, but it did not prevent injury of the tissues and organs (liver, kidney, and muscle) by the toxins; thus it simply delayed the onset of symptoms in sheep and did not lead to complete detoxification.

Figure 2. *Oxytropis glabra*, one of the locoweed species infesting large areas of the grasslands of northern China.

The concept of vaccination against locoweed poisoning is attractive; however, practical immunization against small-molecular-weight plant toxins, although successful, has been limited (Than et al. 1998, Lee et al. 2003). Development of swainsonine-protein conjugates for immunologic response has been reported (Tong et al. 2001a).

A bacterium strain that can degrade swainsonine has been isolated and cultured (Zhao 2008). Efforts are under way to develop a method for colonizing

Figure 3. Grasslands of northern China extensively infested with the locoweed *Oxytropis glabra*.

this strain in the rumen to precondition livestock to reduce locoweed poisoning.

Management for the control of locoweeds should consider protecting the native grassland ecosystems and conserving ecological balance to reduce locoweed infestation (figure 3). Management strategies also should include methods to restore degraded grasslands to support a sustainable development of animal husbandry. Management to achieve these goals requires a long treatment cycle. In one strategy, highly competitive forage plants were selected and sown in the locoweed-infested areas to reduce locoweed density and thus reduce risks of livestock poisoning (Zhao et al. 2008); however, this strategy has not yet been applied on a large scale in locoweed-infested areas of China.

Swainsonine in Locoweeds

In China, swainsonine was first isolated from *O. ochrocephala* by Cao et al. (1989), who also confirmed that swainsonine inhibits α-mannosidase in livestock, resulting in locoism. Subsequently, swainsonine was also isolated from *A. variabilis* by Huang et al. (1992), *A. strictus* by Zhao et al. (1993), *O. kansuensis* by Tong et al. (2001b), *O. glacialis* in Tibet by Tan et al. (2002), *O. glabra* by Ge et al. (2003), and *O.falcata* by Huo et al. (2008). The swainsonine content of *O. kansuensis*, *A. variabilis*, *A. strictus*, *O. sericopetala*, and *O. glacialis* was analyzed and semi-quantified by thin-layer chromatography (TLC) (Tong et al. 2003). Most recently, swainsonine content of *A. variabilis*, *A. strictus*, *A. hamiensis*, *O. glabra*, *O. kansuensis*, *O. ochrocephala*, *O. glacialis*, *O. deflexa*, and *O.latibracteata* was determined by Liu et al. (2009). These results showed that the mean swainsonine content of *O. kansuensis* at 0.148 mg/g was the highest of the Chinese species and that of *A. hamiensis* at 0.044 mg/g was the lowest among these species. The average swainsonine content at flowering was 0.139 mg/g, which was the highest among all phenological stages, while the average content in flowers was 0.162 mg/g, which was the highest among all plant parts. This research also demonstrated that swainsonine content of locoweeds increased with elevation.

Swainsonine in Endophytes

Thus far, 10 strains of endophyte have been isolated from *O. glabra* in Inner Mongolia by Lu et al. (2009). Forty-two strains of endophyte have been isolated from *A. variabilis, A. strictus, O. glabra, O. kansuensis, O. ochrocephala, O. glacialis*, *and O. sericopetala.* Of these, 11 strains produce swainsonine and have been classified as *Undifilum oxytropis* according to both morphological characteristic and the 5.8SrDNA/ITS sequence analysis (Yu 2009). PCR-RFLP analysis of intergenic spacer (IGS) region for those 11 endophyte strains showed that the interspecific or intraspecific variations were

present among the endophytes from different locoweed species (Yu et al. 2011).

Prospects for Locoweed Utilization

Despite its toxic properties, *Oxytropis* and *Astragalus* have potential as forage resources, being palatable and rich in crude protein. A deep-rooted legume, locoweed is drought tolerant and cold resistant, and it has a low nutritional requirement that enables it to grow under harsh environmental conditions. These characteristics make it very useful for stabilizing moving sands and conserving soil and water. More research is needed to understand how locoweeds might be managed as forage in arid and semi-arid grasslands in China. The relationship between the endophyte, swainsonine, and the plant must be well understood before much progress can be made to utilize locoweeds safely for livestock forage.

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References

Broquist, H.P., P.S. Mason, B. Wickwire, et al. 1985. Swainsonine production in the mold *Rhizoctonia leguminicola*. *In* A.A. Seawright, M.P. Hegarty, L.F. James, and R.F. Keeler, eds., Plant Toxicology, pp. 301-308. Queensland Poisonous Plants Committee, Yeerongpilly, Australia.

Braun, K., J. Romero, D. Liddell, and R. Creamer. 2003. Production of swainsonine by fungal endophytes of locoweed. *Mycology Research* 107:980-988.

Cao, G.R., S.J. Li, D.X. Duan, et al. 1989. The isolation and identification of toxic components from *Oxytropis ochrocephala. Journal of the Northwest Sci-Tech University of Agriculture and Forestry* 17:1-6.

Chang, J.J., C.H. Mo, B.Y. Zhao, et al. 2007. Trial of Fengcaoling bolus in preventing sheep suffering from *Oxytropis ochrantha* toxicity. *Pratacultural Science* 24:76-78.

Fan, Z.F., Y.Y. Fan, B.Y. Zhao, et al. 2006. Progress in research on prevention of poisoning from locoweed. *Heilongjiang Animal Science and Veterinary Medicine* 1:24-25.

Ge, P.B., B.Y. Zhao, D.W. Tong, et al. 2003. Extraction and fractionation and identification of swainsonine on structure from *Oxytropis glabra. Chinese Agricultural Science Bulletin* 19:1-4.

Huang, Y.D., Z.G. Xiao, J.C. Meng, and F.K. Kong. 1992. The separation and analysis of toxic components from *Astragalus variabilis*. *Journal of Traditional Chinese Veterinary Medicine* 4:3-6.

Huang, Y.Q., E.Y. Zhang, and W.F. Pan. 2003. Current status of locoweed toxicity. *Shandong Science* 16:34-39.

Huo, X.H., B.Y. Zhao, J.J. Wang, et al. 2008. Preliminary chemical test of *Oxytropis falcata* Bunge and alkaloids analysis by TLC. *Acta Agriculture Boreali-Occidentalis Sinica* 17:24-28.

Lee, S.T., B.L. Stegelmeier, K.E. Panter, et al. 2003. Evaluation of vaccination against methyllycaconitine toxicity in mice. *Journal of Animal Science* 81:232-238.

Liu, Z.Y., Z.X. Wang, B.Y. Zhao, et al. 2009. The study on dynamic variation law of swainsonine from major locoweeds in China. Proceedings of the Chinese Association of Veternary Internal Medicine (CAVIM), pp. 556-564. Qingdao, China.

Liu, L., Y. Feng, Z.H. Jia, and B.Y. Zhao. 2010. Research progress on development and utilization of locoweed in China. *Acta Ecology and Animal Domestication* 31:102-105.

Lu, P., M.L. Zhao, G.D. Han, et al. 2006. The locoweed in Inner Mongolia and research progress. *Chinese Journal of Grassland* 28:63-68.

Lu, P., D. Child, M.L. Zhao, et al. 2009. Culture and identification of endophytic fungi from *Oxytropis grabra* DC. *Acta Ecology Sinica* 29:53-58.

Molyneux, R.J., L.F. James, K.E. Panter, and M.H. Ralphs. 1989. The occurrence and detection of swainsonine in locoweeds. *In* L.F. James, A. Elbein, R.J. Molyneux, and C.D. Warren, eds., Swainsonine and Related Glycosidate Inhibitors, pp. 100-117. Iowa State University Press, Ames, IA.

Pryor, B.M., R. Creamer, and R.A. Shoemaker. 2009. *Undifilum*, a new genus for endophytic *Embellisia oxytropis* and parasitic *Helminth osporium bornmuelleri* on legumes. *Botany* 87:178-194.

Ralphs, M.H., R. Creamer, D. Baucom, et al. 2008. Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). *Journal of Chemical Ecology* 34:32-38.

Tan, Y.Y., J.H. Wang, Q.F. Li, et al. 2002. Extraction and separation of alkaloids from glacial crazyweed (*Oxytropis glacialis*). *Chinese Journal of Animal and Veterinary Sciences* 33:352-355.

Than, K.A., Y. Cao, A. Michalewicz, and J.A. Edgar. 1998. Development of a vaccine against annual ryegrass toxicity. *In* T. Garland and C.A. Barr, eds., Toxic Plants and Other Natural Toxicants, pp. 165-168. CABI Publishing, Wallingford, U.K.

Tong, D.W., G.R. Cao, and D.L. Cheng. 2001a. Study on synthesis of swainsonine-BSA. *Journal of the Northwest Sci-Tech University of Agriculture and Forestry* 29:9-12.

Tong, D.W., G.R. Cao, and X.J. Zhao. 2001b. Isolation and identification of swainsonine from *Oxytropis kansuensis* Bunge. *Acta Agriculture Boreali-occidentalis Sinica* 10:6-8.

Tong, D.W., G.R. Cao, G.X. Geng, et al. 2003. Determination on content of swainsonine in five locoweeds. *Chinese Journal of Veterinary Science* 23:183-184.

Wang, J.H. 1999. Harmfulness and treatment of locoweed in China. Proceedings of Science and Technology for Social and Economic Development: Toward the 21st Century, p. 216. 1999 CAST Annual Meeting, Hangzhou, China.

Wang, J.J., B.Y. Zhao, Z.F. Fan, and Silang Yuzhen. 2007. Distrbution, harmfulness and treatment of locoweed in Tibet. *Prataculture & Animal Husbandry* 6:36-40.

Wu, D., J.H. Wang., and Y.H. Wang. 2001. Studies on the prevention or killing off and utilization of China locoweed. *Chinese Qinghai Journal of Animal and Veterinary Sciences* 31:29-30.

Yang, X.P. 2002. Clinical symptoms of toxicosis in animals by crazyweed. *Journal of the Wuhang Institute of Science and Technology* 15:25-28.

Yu, Y.T. 2009. Isolation, identification and genetic polymorphism of swainsonine-producing fungal endophytes from locoweeds in China. Northwest Agriculture and Forestry University, Yangling, Shaanxi, China. Ph.D. thesis.

Yu, Y.T., Z.B. Liu, X.H. Zhao, et al. 2006. Purification and identification of swainsonine in the *Oxytropis serioopetala*. *Animal Husbandry and Veterinary Medicine* 38:1-3.

Yu, Y.T., Q.M. Zhao, J.N. Wang, et al. 2011. Swainsonine-producing fungal endophytes from major locoweed species in China. *Toxicon* 56:330-338.

Zhang, J., and X.C. Liu. 1994. Survey and progress of studies on poisoning from locoweed. *Journal of Traditional Chinese Veterinary Medicine* 1:46-48.

Zhao, B.Y., G.R. Cao, and S.J. Li. 1993. The separation and analysis of toxic components from *Astragalus strictus*. *Chinese Journal of Veterinary Science and Technology* 23:20-21.

Zhao, B.Y., D.W. Tong, P.B. Ge, et al. 2003. Locoweed harm investigation in the west grassland of China. *Chinese Journal of Grassland* 25:65-68.

Zhao, B.Y., Y.Y. Fan, Z.F. Fan, et al. 2005. Harmfulness of locoweed and controlling of animal poisoning in Chinese western grassland. *Journal of Health Toxicology* 19:310-311.

Zhao, B.Y., Z.Y. Liu, X.P. Wang, et al. 2008. Damage and control of poisonous-weeds in Chinese western grassland. *Scientia Agriculture Sinica* 41:3094-3103.

Zhao, Q.E., Y.G. Liu, and T.Y. Liu. 1999. The first exploration for treating sheep poisoning from *Oxytropis glabra*. *Animal Toxicology* 14:14.

Zhao, X.H. 2008. Isolation and identification of swainsonine degrading bacteria and degradation character. Northwest Agriculture and Forestry University, Yangling, Shaanxi, China. Ph.D. thesis.

Zhong, Y.S., B.G. Song, J.G. Yu, et al. 2007. Survey of major poisonous plants and livestocks poisoning in Ejin Banner. *Progress in Veterinary Medicine* 28:113-116.

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Locoweed Toxicity, Ecology, Control, and Management

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Abstract

Locoweed is the most widespread poisonous plant problem in the Western United States. Some species of *Astragalus* and *Oxytropis* contain the indolizidine alkaloid swainsonine that causes the poisoning syndrome known as locoism. Swainsonine is produced by the endophyte *Undifilum oxytropis*. Swainsonine inhibits several key mannosidase enzymes of lysosomal and glycoprotein metabolism, resulting in buildup of partially metabolized sugars, and disrupted protein synthesis and function including altered hormones, enzymes, and receptor binding. Nearly all body systems are adversely affected. Populations of almost all locoweed species cycle as they typically increase during wet years and die back during drought. Locoweeds are relatively palatable during some seasons of the year. The most effective management strategy to prevent poisoning is to deny livestock access to locoweeds during critical periods when they are more palatable than associated forage. However, horses are uniquely sensitive to poisoning and may eat toxic doses even when other forages are available. Reserving locoweed-free pastures or controlling existing locoweed populations with herbicides can provide "safe" pastures for critical periods. Good range management and wise grazing strategies can provide adequate forage for livestock and prevent them from grazing locoweed during critical periods when it is palatable.

Keywords: locoweed, *Astragalus*, *Oxytropis*, range management, ecology, pathology, poisoning

Introduction

Locoweed poisoning of livestock is the most widespread poisonous plant problem on Western U.S. rangelands (Kingsbury 1964). Species of *Astragalus* and *Oxytropis* occur in every major plant community. There are 401 species and 207 varieties of *Astragalus* and *Oxytropis* (Welsh 2007), and of those, 22 species and 35 varieties are indigenous to North America (Welsh 2001). However, not all of these plants are toxic. Only 21 species have been associated with locoism or shown to contain the toxic alkaloid swainsonine (table 1). *Astragalus* spp. may also contain other toxins including nitrotoxins and selenium (James et al. 1981, Welsh et al. 2007). Swainsonine has also been reported in some of the selenium accumulator plants (*A. bisulcatus*, *A. drummondii*, *A. praelongus*) (Fox et al. 1998), but if those plants are eaten, the high selenium concentrations would cause acute selenium poisoning before swainsonine intoxication could develop. Additionally, these selenium indicator plants are generally unpalatable, thus there is little chance of livestock consuming them at doses and duration required to develop locoism.

Locoweeds also cause serious poisoning problems in other arid and semi-arid regions of the world. In China, there are 270 species of *Astragalus* and 120 species of *Oxytropis*, of which 10 contain swainsonine and are considered locoweeds (Shi

Table 1. Locoweed (*Astragalus* and *Oxytropis*) species, habitat, and distribution

Species	Common name	Habitat	Distribution
A. allochrous	Rattleweed	Desert grassland	AZ, NM
A. asymmertricus	Horse loco	Annual grasslands	CA
A. didymocarpus		Creosote deserts	CA, AZ, NV
A. emoryanus[1]	Red stem peavine	Creosote, Mesquite, P/J	NM, TX
A. humistratus	Ground cover milkvetch	P/J woodlands	AZ, NM
A. lentiginosus[2]	Spotted locoweed	Salt-desert shrub, sage, P/J	AZ, UT, NV, ID
A. lonchocarpus	Great rushy milkvetch	P/J woodlands	CO, UT, AZ, NV
A. missouriensis	Missouri milkvetch	Shortgrass prairies	Canada to TX
A. mollissimus[2]	Woolly loco	Shortgrass prairies	CO, KA, OK, TX, NM
A. nothoxys	Beaked milkvetch	Oakbrush, P/J woodlands	AZ
A. oxyphysus	Diablo loco	Desert grasslands	CA
A. pubentissimus	Green river milkvetch	Salt-desert shrub	CO, WY, UT
A. purshii	Pursh loco	Sagebrush, P/J woodlands	WY, MT, ID, NV
A. pycnostachyus	Brine milkvetch	Salt marshes and beaches	CA
A. tephrodes	Ashen milkvetch	Oakbrush, P/J woodlands	AZ, NM
A. thurberi	Thurber milkvetch	Creosote, Oak, P/J woodlands	AZ, NM
A. wootoni	Garbancillo	Creosote desert	AZ, NM, TX
O. besseyi	Red loco	Gravely hill tops	MT, WY
O. campestris	Yellow loco	Prairies, Mt. meadows	MT, Canada
O. lambertii	Lambert locoweed	Short and mid-grass prairies	MT, ND, SD, WY, CO, NM
O. sericea	White locoweed	rocky soils, foothills and Mt.	MT. SD, WY, CO, NM, UT

[1] Primarily contains nitro toxins, but swainsonine is also present.
[2]There are many varieties of *A. lentiginosus* and *A. mollissimus* that have been referred to as separate species in the past. Species taken from Marsh (1909), Molyneux et al. (1991), Smith et al. (1992), and Fox et al. (1998).

1997). Zhao et al. (2003) reported that three *Oxytropis* locoweed species caused significant poisoning problems in their respective regions: *O. glacialis* in the alpine areas of the Qinghai and Tibetan plateaus; *O. kansuensis* in the loess plateaus of the Sichuan basin and Gansu Province; and *O. glabra* of the arid deserts and semi-arid grasslands of Inner Mongolia. In Western Australia, the closely related genus *Swainsona* causes a disease called "pea struck" in cattle that is very similar to locoism. The toxic alkaloid swainsonine was originally discovered in *Swainsona canescens* (Colegate et al. 1979). Other plants that contain swainsonine include the *Ipomoea* species (Molyneux et al. 1995, Haraguchi et al. 2003, Hueza et al. 2005), *Turbina cordata* (Dantas et al. 2007), and *Sida carpinifolia* (Loretii et al. 2003, Pedrosa et al. 2009). Most of these plants are found in Brazil and though they contain swainsonine, the poisoning is somewhat different and more work is needed to definitively characterize these significant poisoning problems.

The *Astragalus* and *Oxytropis* genera are members of the Leguminosae family, having irregular, papilianaceous (butterfly-wing-like) flowers, with a larger banner petal, flanked by two wing petals and a keel petal. The major distinguishing feature between the two genera is that the keel petal in *Oxytropis* is prolonged into a distinct porrect beak (hence the name point loco), while the keel petal is blunt in *Astragalus*. Another distinguishing feature in most North America *Oxytropis* species is that they are acaulescent (without a stem). The flowering heads extend from a reproductive scape, but the leaves arise from the crown (caudex). In *Astragalus,* the stems are multi-branched and leaves and flowering heads arise from all stems. Leaves from both species are alternate pinnately compound. *Astragalus* species are technically called milkvetches, and *Oxytropis* species are called poison vetch or point loco.

Locoweed poisoning is a significant impediment to livestock production on Western rangelands. Early livestock losses were so severe that Western senators demanded that the USDA establish a research station in Hugo, CO, to study locoweed poisoning (Marsh 1909). C.D. Marsh, one of the original researchers who studied locoweed poisoning, reported that poisoning was often confused with starvation because the incidence of poisoning increased during seasons of feed shortage on overgrazed rangelands. The researchers astutely observed that the animals started eating locoweed in the late winter and early spring before new grass started growing. Poisoned livestock seemed to thrive at first, then rapidly fell off in body condition as poisoning progressed. Marsh (1909) concluded that

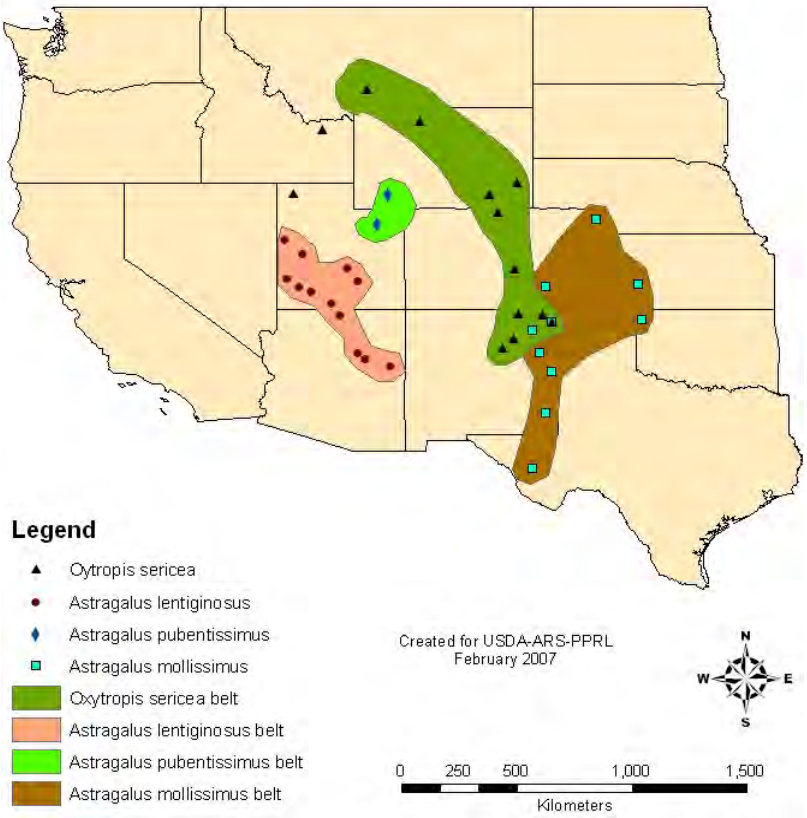

Figure 1. Incidence of poisoning by the major locoweed species investigated by the USDA-ARS Poisonous Plant Research Laboratory, Logan, UT.

an abundance of good feed resulting from improved range conditions would greatly reduce and perhaps eliminate the locoweed problem. Range conditions have improved greatly over the last 100 years, yet locoweed poisoning continues to be a significant problem. Figure 1 shows the recent incidences of poisoning by locoweed species investigated by the U.S. Department of Agriculture, Agricultural Research Service Poisonous Plant Research Laboratory, Logan, UT. Figure 2 shows the distribution of the major locoweed species.

Chemistry

The quinolizidine alkaloid swainsonine was first discovered in *Swainsona canescens* in Australia, and shown to inhibit essential enzymes α-mannosidase and mannosidases II (Colegate et al. 1979). Later, swainsonine was found in locoweeds in the Western United States and shown to cause the pathological disease locoism (Molyneux and James 1982). Initial methodology to detect swainsonine was lengthy and difficult; however, Gardner et al. (2001) developed an LC-MS (liquid chromatography-mass spectrometry) assay to reliably quantify swainsonine in plant and animal tissue.

Astragalus species are generally more toxic than *Oxytropis* (table 2). Swainsonine concentration in garboncillo (*A. wootoni*) was 0.37% compared with 0.16-0.18% in *A. lentiginosus* and *A. mollissimus,* and was lowest (0.04%) in white locoweed (*O. sericea*) (Ralphs et al. 2008). Early studies found that swainsonine and a closely related compound, slaframine, are produced from the fungus *Rhizoctonia leguminicola* (Broquist 1985, Wickwire and Broquist 1989). Recently, a similar swainsonine-producing endophytic fungus (*Embellisia oxytropis*) was discovered in locoweeds (Braun et al. 2003) and was shown to synthesize swainsonine (Romero et al. 2004). It has subsequently been renamed *Undifilum oxytropis* (Pryor et al. 2009). The endophyte grows mostly in the aboveground plant parts (Cook et al. 2009a) and can be detected and quantified by PCR (polymerase chain reaction) (Cook et al. 2009b). The endophyte is found in all of the major locoweeds in the Western United States and produces varying amounts of swainsonine (Ralphs et al. 2008). However, the endophyte was suppressed in some individual plants and some populations, in which it did not produce measurable amounts of swainsonine.

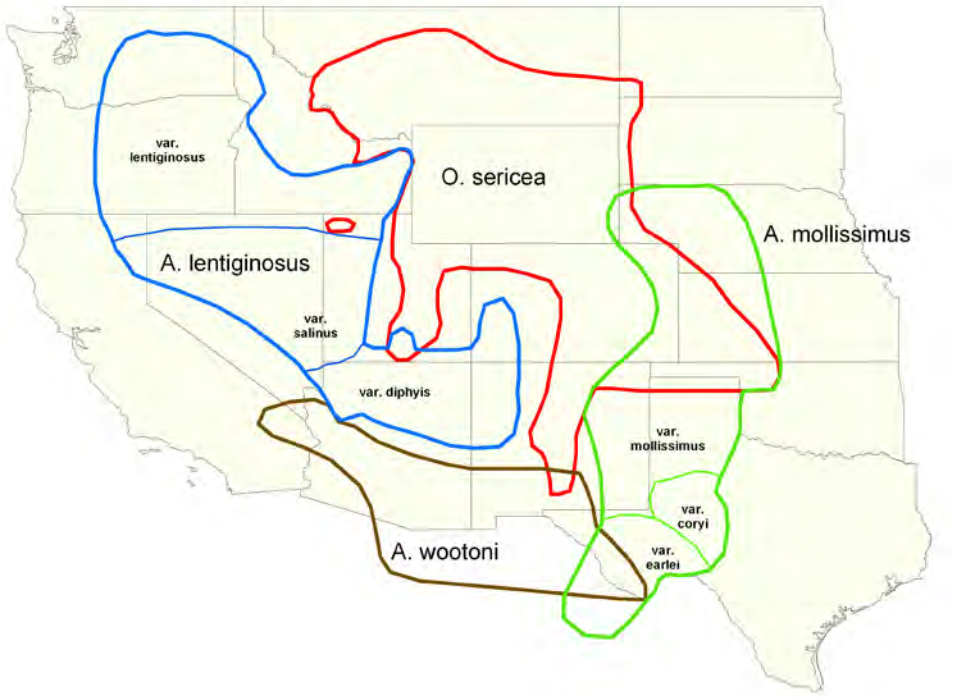

Figure 2. Distribution of major locoweeds in the Western United States.

This apparent suppression is passed on to succeeding generations (Ralphs et al. 2011). If the endophyte can be inhibited, or its synthesis of swainsonine interrupted, perhaps locoweeds could be rendered non-toxic. Currently, research is under way to elucidate the factors that influence the growth of the endophyte and its synthesis of swainsonine.

Poison Syndrome

Swainsonine inhibits essential mannosidase enzymes in lysosomal and glycoprotein metabolism, and extended inhibition disrupts many physiologic systems including hormone and enzyme synthesis and receptor binding (Stegelmeier et al. 1999a). Clinical signs of poisoning only develop after several weeks of continued locoweed ingestion. When livestock begin to eat locoweed, some animals may temporarily do well. However, within 14 days poisoned animals become reluctant to move, lose their appetite, and subtle tremors are visible when they move. With continued poisoning, animals deteriorate—developing severe weight loss and wasting, proprioceptive deficits, nervousness when stressed, cardiovascular disease, water belly (hydrops amnii)—and die. Other locoweed-related effects include altered micronutrient metabolism and markedly decreased feed conversion efficiency, abortions, reduced fertility of both sexes, neurological disturbances ranging from extreme depression to aggression, compromised immune system resulting in increased disease, and impaired ability to eat or drink leading to weight loss and eventual starvation. At high elevation, poisoned cattle often develop congestive heart failure (brisket disease or high mountain disease; figure 3) (James et al. 1986).

Reproductive loss is the greatest economic cost associated with locoweed poisoning (Panter et al. 1999). Abortions are common throughout the gestation period. Offspring that go full term may be born small and weak and death rates are high. Neonates from poisoned livestock are often behaviorally retarded and lack the instinct to nurse and form maternal bonds (Pfister et al. 2006). Young animals that survive, and even healthy offspring, may continue to be poisoned as swainsonine is passed through their mothers' milk (James and Hartley 1977). Such neonates quickly become lethargic, depressed, and have lower weight gains (Ralphs et al. 1994c).

Wasting and lack of weight gains constitute other significant losses. Stocker cattle lose weight while grazing locoweed and do not begin to gain for several weeks after they stop grazing locoweed (Ralphs et al. 2000). Torell et al. (2000) estimated that moderately poisoned steers lost $75 per head, and severely poisoned steers lost $282 per head.

Figure 3. Steer with congestive heart failure (brisket disease). Notice the extensive subcutaneous dependent edema. This steer was fed locoweed (*Oxytropis sericea)* mixed with ground alfalfa hay for 47 days.

Locoed steers going on to the feedlot were slower to start gaining weight and finished approximately 66 lbs less than healthy steers from the same lot (G. Duff, 2000, unpublished data), thus increasing the time and expense of finishing to the desired market condition. In addition, the compromised immune system and poor immunologic response to vaccines may lead to increased incidence, severity, and mortality from other infectious diseases (Stegelmeier et al. 1998a).

Locoism is a chronic poisoning. Animals must eat locoweed over extended periods to become poisoned. Most studies suggest that the toxic dose is species specific and most likely related to the amount of swainsonine required to inhibit cellular mannosidases. Higher doses may slightly shorten the development of lesions in some tissues as the tissue specific toxicokinetics are altered, but they do not appear to be directly tissue toxic (Stegelmeier et al. 1999b). Generally, poisoned animals progress in a duration-dependent rather than a dose-dependent fashion (Stegelmeier et al. 1999b). In grazing trials, signs of poisoning appeared after 30 to 45 days grazing on locoweed (Ralphs et al. 1993). In a pen feeding trial where spotted locoweed was 25 percent of the ration, the first lesions detected were swelling and vacuolation of the uroepthelium of the renal pelvis and urinary bladder after only 3 to 4 days of poisoning. Similarly, subtle neuronal swelling and vacuolation develop in just 8 days of poisoning. Obvious clinical signs of poisoning were easily apparent after 21 days (Van Kampen and James 1970). In a dose response study, sheep fed white locoweed for 30 days at doses as low as 0.2 mg swainsonine/kg bw/day developed characteristic locoweed-associated biochemical and histologic lesions and decreased weight gains (Stegelmeier et al. 1999b). Locoweed-induced lesions develop in species-specific tissues and locations. For example, sheep and cattle develop severe cellular swelling and vacuolation in certain neurons (figure 4), thyroid epithelium, exocrine pancreatic epithelium, and renal tubular epithelium and reticuloendothelial cells in many other tissues. Horses develop similar neuronal lesions, but the lesions in the thyroid and pancreas are minimal. Rodents and deer develop severe vacuolation in the mesenchymal organs (figure 5) but are relatively resistant to many of the neuronal lesions. Deer and rodents develop neurologic clinical disease only after extended, high-dose locoweed exposures (Stegelmeier et al. 1994, 2005, 2007).

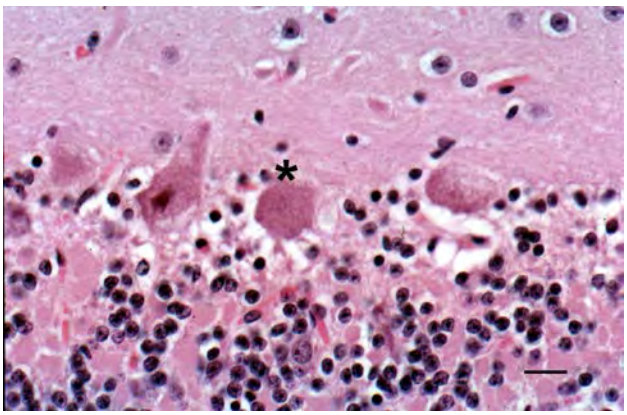

Figure 4. Photomicrograph of the cerebellum from a sheep that was treated with ground locoweed (*Oxytropis sericea)* to obtain doses of 1.8 mg swainsonine/kg BW/day for 30 days. Notice the swelling and fine vacuolation of Purkinje cells (*). H&E Bar = 30 um.

At high doses (8 mg swainsonine/kg bw/day), pregnant goats showed clinical symptoms of intoxication within 9 days, including rear limb paresis and severe proprioceptive deficits (Furlani et al. 2007), indicating that goats also may be highly sensitive to swainsonine toxicity. Most of these pregnant animals aborted or suffered fetal death before the conclusion of the study. Locoweed associated lesions were present in both the goats and their fetuses. Locoweed poisoning also damages male reproduction. Panter et al. (1989) have shown that poisoned bucks have decreased spermatogenesis (figure 6) with production of abnormal, often vesiculated spermatozoa and altered seminal secretions. Spermatogenesis recovered when poisoning was discontinued, but if neurologic damage is severe, previously poisoned animals may never develop normal libido or mating behavior (Panter 1989).

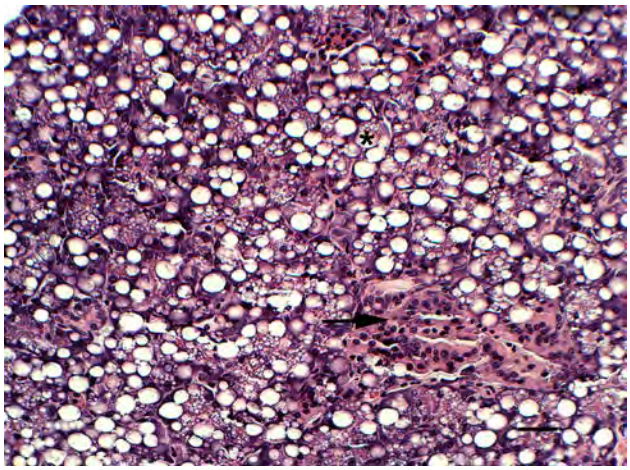

Figure 5. Photomicrograph of pancreas of a deer that was treated with 15% locoweed (*Astragalus lentiginosus*)/ alfalfa/grain pellets for 60 days. Notice the extensive swelling and vacuolation of the exocrine epithelial cells (*). The endocrine islet cells are relatively normal (arrow). H&E Bar = 30 um.

As suggested previously, many locoweed lesions are reversible. Sustained poisoning and swelling ultimately results in cellular death or necrosis. If tissues cannot regenerate, the resulting lesions and subsequent clinical signs are likely to be permanent. For example, neuronal death is a permanent change and the subsequent histologic changes relating to neuronal loss are likely to be found in previously poisoned animals (figure 7). If there is extensive neuronal damage, the clinical neurologic changes also are likely to be permanent. Previously locoed horses have permanent neurologic damage, and though they might be used as reproductive animals, they should never be used for work animals. Similarly, if neurologic damage is severe enough, previously poisoned animals are not likely to be able to breed, successfully complete pregnancy, or adequately care for neonates. These residual changes were shown in a behavioral study where poisoned sheep were allowed to recover for 5 weeks. These animals had minimal histologic neuronal lesions, but behavioral signs persisted months after exposure (Pfister et al. 1996). Though these behavioral changes are subtle and were composed of poisoned animals' inability to complete a learned operant task, such changes should be evaluated on an individual basis, considering the animal's function before developing a prognosis. Most of the reversible clinical signs of locoweed poisoning resolve within 2 weeks (Stegelmeier et al. 2007).

Although some of the signs and effects of poisoning linger and many of the histologic lesions may take months to resolve, swainsonine is rapidly cleared from blood and other body tissues (clearance time half life 20-60 h; Stegelmeier et al. 1998b). A conservative withdrawal period of 25 days will ensure that swainsonine has cleared animal tissues and products.

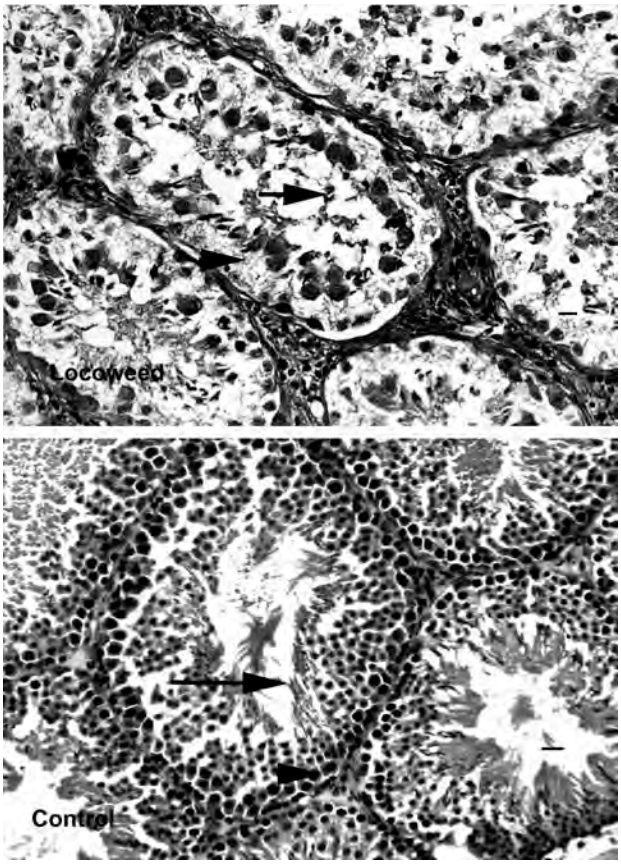

Figure 6. Photomicrograph of seminiferous tubules from a locoweed poisoned ram (top) and a normal ram (bottom). The poisoned ram was fed 10% locoweed pellets (*Astragalus lentiginosus*) for 45 days. Notice the vacuolation and distended sertoli cells (arrowhead) and lack of spermatogenesis (arrow) in the locoweed-treated ram. The normal ram has normal sertoli cells (arrowhead) and numerous spermatocytes in various stages of maturation (arrow). There are also normal spermatozoa in the lumen. H&E Bar = 30um.

Horses are highly susceptible to locoweed poisoning (Stegelmeier et al. 2007). Poisoned horses are generally depressed, lack appetite, and lose weight (figure 8). With continued exposure, horses develop tremors and proprioceptive deficits that are most apparent when they are moved. They may also develop behavioral changes. When poisoned horses are stressed or stimulated, they may become anxious and develop aggression, maniacal fits, and uncontrollable trembling and seizure-like fits. Horses also may start eating locoweed before cattle

do and, under some conditions, they may consume more locoweed than do cattle (Pfister et al. 2003). This also may increase their risk of poisoning. Cattle and sheep are moderately susceptible to poisoning.

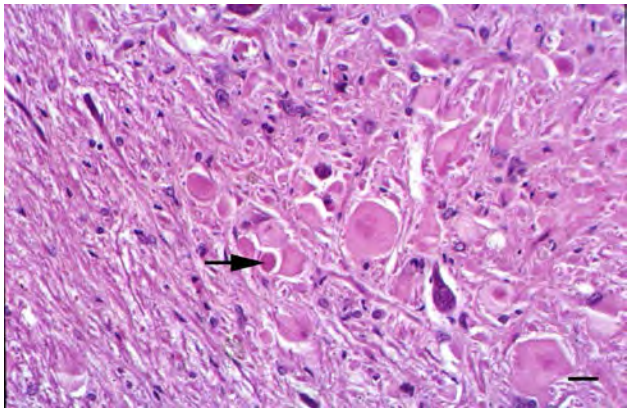

Figure 7. Photomicrograph of the cuneate nucleus in the proximal spinal cord of a deer that was treated with 15% locoweed (*Oxytropis sericea*)/alfalfa/grain pellets for 60 days. Notice the extensive axonal dysplasia with numerous axonal spheroids (arrow). There is also gliosis and minimal lymphocytic inflammation. H&E Bar = 30 um.

Figure 8. Locoweed-poisoned horse that was grazing locoweed (*Astragalus lentiginosus*) for 45 days. Notice the lack of body condition and the rough, dull appearance of the coat.

They quickly develop depression as they become lethargic and reluctant to move. Proprioceptive deficits become evident at about 30 days. Poisoned animals may shy away from common objects, have difficulty drinking, and have problems finding, prehending, and chewing feed. Poisoned cattle often can be identified at a distance as their coat is dull and their eyes appear glassy.

Deer and rodents are relatively resistant to locoweed poisoning. However, at high doses over long periods, deer also develop a dull, shaggy coat and lose weight (figure 9). This lack of obvious neurologic disease can make it difficult to distinguish locoweed poisoning from other wasting diseases in deer (Stegelmeier et al. 2005).

Figure 9. Mule deer (*Odocoileius homionus)* that was dosed with 15% locoweed (*Oxytropis ericea)*/alfalfa/grain pellets for 60 days. Notice the lack of condition with rough fur, much of which is partially shed.

Ecology and Population Cycles

Locoweeds have different survival strategies that perpetuate the species through long-term climatic cycles and short-term weather conditions (Ralphs et al. 2003), as follows:

1. Annual plants avoid drought by seed-dormancy through dry cycles and germinate in years when sufficient moisture is available.
2. Biennial or short-lived perennial plants rely on both timely and adequate moisture for germination, growth, flowering, and to set seed.
3. Long-lived perennial plants grow where moisture is more abundant and more regularly available. The plants flower and produce seed for many years following initial establishment though they, too, may die out during extended droughts.

The seed bank in the soil supports these cycles. Seeds in soil often exceed 1,000/m^2 and may range up to 2,800/m^2 (Ralphs and Cronin 1987). Almost all of these seeds are viable. The hard seed coat allows the seed to remain viable for many years and provides it with the ecological advantage to exploit environmental conditions and maintain the “boom and bust” population cycles. Livestock poisoning

follows these cycles, often in catastrophic proportions.

Winter Annuals

Garbancillo (*A. wootonii* Sheldon; figure 10) is a winter annual in the Southwest deserts ranging from southern California, eastward through Arizona and New Mexico into the Trans-Pecos areas of Texas, and northern Mexico. In wet winters, it can be a major component of the creosote bush and saguaro cactus desert, mesquite savannahs, and desert grasslands. It germinates following autumn rains and continues to grow through the winter, becoming a large, robust plant with white to pinkish-purple flowers, followed by large, inflated, single-compartment pods. Once seeds are set, it dies back. Since Garbancillo remains green and actively growing over winter, it is relatively palatable and poses a significant risk of poisoning. Swainsonine concentration was highest of the locoweed species sampled (0.37%, table 2).

Figure 10. Garbancillo (*A. wootonii* Sheldon).

Emory milkvetch (*A. emoryanus* Rydb. Cory; figure 11) is a winter annual found throughout much of the Rio Grande Valley of Texas and the southern half of New Mexico. It is a short-statured plant with prostrate stems (4-40 cm long) radiating from a caudex, with pink to purple flowers appearing from February through June. It contains both the locoweed toxin swainsonine (Davis et al. 1984) and nitro toxins (Williams et al. 1979). In dry years, plants are small and scattered. When precipitation is timely and abundant, seeds germinate and plants grow profusely, often forming a veritable carpet on large areas of rangeland. Abundant precipitation fell in eastern New Mexico during autumn 1974, causing unusually high rates of germination. There was adequate winter and spring moisture to continue growth, and densities were high throughout the region surrounding Roswell in the spring 1975. Williams et al. (1979) reported that death loss averaged 2 to 3 percent, and almost all the cows in the region were poisoned to some degree. Only sporadic incidences of poisoning problems have occurred since.

Figure 11. Emory milkvetch (*A. emoryanus* Rydb. Cory).

Biannual or Short-lived Perennial Semi-desert Species

Generally, these plants germinate in autumn, following storms of late summer and early fall. They persist through the winter, and some of them will flower as annuals the next spring. Many do not flower in the first year but continue active growth as long as water is available, become dormant in the hot, dry portions of summer, and often grow again in autumn. In the spring of the second year, they become large and robust and are sufficiently mature to produce flowers and fruit. If the spring of the second year is dry and moisture is inadequate, many of the potentially perennial plants die, having been functionally biennial. When conditions of moisture are adequate, however, they may survive and flower the third year. Seldom is precipitation adequate for continued growth and seed production for more than a few years (Welsh 1989).

Spotted loco, or freckled milkvetch (*A. lentiginosus* Dougl. ex Hook; figure 12), is a complex species with 42 varieties (Welsh 2007), mainly denoted by their geographical distribution. The species is characterized by the red spots or splotches on the pods. Stems are prostrate ranging from 10 to 100 cm long, and often form a large, bush-like plant. Flowers are pink-purple to lavender and appear from April to June.

Table 2. Swainsonine concentration (% of dry weight and standard error) in *Astragalus* and *Oxytropis* species and varieties (Ralphs et al. 2002, 2008)

Species	Variety	N	Swainsonine	
			%	SE
A. wootoni		11	0.37	0.06
A. pubentissimus		10	0.21	0.012
A. mollissimus	*earleii*	30	0.22	0.012
	mollissimus	15	0.14	0.007
	thompsonii	25	0.001	0.0004
A. lentiginosus	*diphysus*	10	0.23	0.018
	lentiginosus	5	0.15	0.016
	wahweapensis	10	0.15	0.019
	araneosus	15	0.11	0.011
O. sericea	*sericea*	26	0.04	0.005
O. lambertii	*bigelovii*	50	0.04	
	lambertii	30	nd[1]	
	articulate	30	nd	

[1] nd=not detected. The limit of quantitation is typically 0.001% swainsonine.

Figure 12. Spotted loco, also known as freckled milkvetch (*A. lentiginosus* Dougl. ex Hook).

Double bladder freckled milkvetch (*A. lentiginosus* var. *diphysus* Gray Jones) is locally abundant through northern Arizona, southern Utah, and northwestern New Mexico in the yucca grassland and pinyon/juniper forests. Swainsonine concentration is moderate (0.23%, table 2). Poisoning problems are erratic and seem to be tied to its population cycles. Population outbreaks occurred in 1983-85, 1991-93, and again in 1998. Populations appear to require two successive wet years to establish. The first year, seeds germinate and establish but are not very apparent. If sufficient moisture is available the second year, they grow rapidly into large recumbent clumps and appear to dominate the plant community (S. Welsh, 2001, personal observation).

Wahweap milkvetch (*A. lentiginosus* var. *wahweapensis* Welsh) occurs on plateaus and drainages of Lake Powell in southern Utah in mixed desert shrub up into the sagebrush and pinyon/juniper plant communities. Ralphs and Bagley (1988) reported that population outbreaks occurred every 6 to 8 years between 1946 and 1986 on gravely benches surrounding the Henry Mountains in southeast Utah. These outbreaks were associated with above-average fall and spring precipitation. Catastrophic losses occurred in those years when Wahweap milkvetch was abundant. Swainsonine concentration is moderate (0.15%, table 2).

Woolly locoweed (*A. mollissimus* var. *mollissimus* Torr.; figure 13) is characterized by dense, curly pubescence and short-caulescent stems 10-30 cm long. Flowers are dull, pinkish-lavender to purple. It, too, is a large complex, consisting of eight geographical varieties occurring in the prairie States from Nebraska southward to Mexico. Woolly locoweed is the principal poisonous plant in the short-grass prairie of western Kansas, the panhandle of Oklahoma, eastern New Mexico, and west Texas. In 1893, 25,000 cattle were poisoned on woolly locoweed in western Kansas. Swainsonine concentration is moderate (0.14%, table 2). Woolly locoweed germinates in wet years and establishes thick stands. However, it rarely persists more than 2 to 3 years because of recurring droughts and damage inflicted by the larvae of the four-lined locoweed weevil (Pomerinke et al. 1995). Ralphs et al. (1993)

reported that the standing crop of woolly locoweed averaged 170 lb/ac in a grazing trial at Gladstone, NM, in 1991, but totally died out 2 years later.

Figure 13. Woolly locoweed (*A. mollissimus* Torr.).

Big Bend or **Earle's locoweed** (*A. mollissimus* var. *earleii* Rydb. Tidestrom) has caused significant poisoning problems in the Trans Pecos and Big Bend region of west Texas. Swainsonine concentration is moderate (0.22%, table 2). It flourishes on the volcanic soils of the Davis Mountains as well as limestone soils of the surrounding areas. Outbreaks and resulting losses occurred in 1976, 1980-81, 1984, 1992-93, and 2005.

Some varieties of woolly locoweed are not highly toxic. Ralphs et al. (2008) reported only traces of swainsonine in *A. mollissimus* var. *thompsonii* (Wats.) Barneby, which grows in sandy soils and shale in the Four Corners region of the Colorado Plateau. Vallotton and Sterling (2002) also reported only trace amounts of swainsonine in varieties of *thompsonii, mogollonicus,* and *matthewsii.* These trace amounts of swainsonine are not likely to cause poisoning.

Green River milkvetch (*A. pubentissimus* T. & G; figure 14) is a short-lived perennial forb that is occasionally abundant along the Green River corridor in southwestern Wyoming and eastern Utah. Stems are erect, 9 to 45 cm long, and flowers are pink-purple. It grows on lightly alkaline soils derived from shales or sandstone and is a component of the salt-desert shrub, mixed desert shrub, and pinyon/juniper plant communities. In years of abundance, it is the principal forb in these desert communities, occurring as very dense stands. James et al. (1968) reported that outbreaks of Green River milkvetch occurred in 1917-1918, 1957-58, and 1965-66, resulting in epidemics of poisoning. In the last outbreak in the Uinta Basin of Utah, 55 percent of a band of 1,900 ewes died and most of the reminder of the band aborted. In another band, 45 percent aborted.

Figure 14. Green River milkvetch (*A. pubentissimus* T. & G.).

Long-lived Species

White locoweed (*Oxytropis sericea* Nutt. ex T. & G; figure 15) is acaulescent (without stems) with white flowering heads arising from reproductive scapes 7 to 32 cm tall. It is more persistent and less cyclic than the *Astragalus* locoweeds. It is also the most widespread locoweed on Western rangelands. It grows on short grass prairies and eastern foothills of the Rocky Mountains from Montana to New Mexico, and on mountain grasslands in the Rocky Mountains and Great Basin. Payne (1957) reported that its preferred habitat is rocky soils, and Ralphs et al. (1989a) suggested that it exhibits a stress-tolerant survival strategy: its long tap root can access deep percolated water allowing it to survive drought, temperature, and wind stress.

In spite of its stress-tolerant survival strategy, its populations appear to be affected by precipitation patterns. Marsh (1909) observed that white locoweed was particularly abundant in wet years, but it nearly disappeared in dry seasons. Following the great drought of the early 1950s, white locoweed poisoning was severe in northeastern New Mexico in the wet years from 1954-1962. There was a short population outbreak from 1977 to 1979, and then a major extended outbreak from 1987 to 1996. Purvines and Graham (1999) reported a positive correlation between white locoweed density and above-average spring precipitation during this period.


Figure 15. White locoweed (*Oxytropis sericea* Nutt. ex T. & G.).

Ralphs et al. (2002b) documented the decline of white locoweed populations in New Mexico, Colorado, and Utah during the droughts between 1996 and 2001. In New Mexico, vigor of white locoweed plants declined during the severe winter and early-spring drought in 1996, and most of the plants died during the successive dry years in 1997 and 1998. In Colorado, a large number of white locoweed plants died in the moderate drought in 1998 and early 1999. Almost all of the plants in the region died in the 2000 drought. The mountain site in northwest Utah had above-average precipitation in 1997 and 1998, but mortality increased as total precipitation declined during the drought of 1999 and 2000. There has been no establishment of new white locoweed plants to date at any of the three locations.

Because of its widespread distribution and more persistent populations, white locoweed has been responsible for the majority of locoweed poisoning problems in the Western United States. Substantial research has been conducted to reduce the incidence of poisoning (Graham et al. 2009). Despite the magnitude of its poisoning problems, its swainsonine concentration is the lowest of the locoweeds sampled (0.04%, table 2).

Lambert's locoweed (*O. lambertii* Pursh; figure 16) is also acaulescent and is somewhat shorter in stature; its flowers are lavender in color. One of its distinguishing features is its unique pubescence, described as malpighian (pick-shaped) hairs. It has three varieties. However, only *O. lambertii* var. *bigelovii* was toxic (swainsonine concentration 0.04%, table 2) in southern Utah, northern Arizona, and southwestern New Mexico. Varieties *articulata* in Oklahoma and Kansas and *lambertii* in Colorado, Utah, and Wyoming did not contain substantial levels of swainsonine (Ralphs et al. 2002d).


Figure 16. Lambert's locoweed (*O. lambertii* Pursh).

Conditions of Grazing and Poisoning

The early literature suggested that locoweeds were distasteful and animals were forced to start eating them because of hunger (Kingsbury 1964). However, once they started, animals seemed to become addicted to locoweeds. Research showed that locoweeds are not addicting, but are relatively more palatable than associated forages during some seasons of the year (Ralphs et al. 1989b). Both sheep (Ralphs et al. 1991) and cattle (Ralphs et al. 1993) that were severely poisoned ceased grazing locoweed when green grass became plentiful.

Preference for locoweed is relative to the availability and palatability of other forage. Many

locoweeds are cool-season species that green up and start growth early in the spring, go dormant during the summer, then resume active growth in fall. Livestock will readily graze these green-growing locoweeds in spring and fall when associated warm-season grasses are dormant and dry. Sheep preferred the regrowth foliage of Green River milkvetch to dormant grasses during late fall and early winter on desert range in eastern Utah (James et al. 1968). Cattle readily grazed Wahweap milkvetch in proportion to its availability on desert winter range in southeastern Utah (Ralphs et al. 1988b). In a series of grazing studies on short-grass prairies in Northeast New Mexico (Ralphs et al. 1993, 1994a,b,c, 1997a,b, 2000, 2001a,b, 2002a,b,c), cattle readily grazed white locoweed in March, April, and May, but stopped grazing it in June as warm-season grasses became abundant while white locoweed matured and became coarse and rank. On mixed-grass prairies on the eastern foothills of the Rocky Mountains in north central Colorado, cattle ceased grazing white locoweed when it matured following flowering in mid-June and became rank and unpalatable in 1998. However, they continued to graze it throughout the summer in 1999 when abundant summer precipitation caused locoweed leaves to remain succulent (Ralphs et al. 2001a). Horses readily grazed green, actively growing spotted locoweed in the spring in preference to dormant blue grama on pinyon/juniper ranges in northern Arizona. In contrast, cows selected spotted loco only after they had depleted other green forbs, and ceased grazing spotted loco in late May when the warm-season grasses began rapid growth (Pfister et al. 2003).

Management to Reduce Risk of Poisoning

Supplements

Many minerals and feed additives have been investigated to prevent locoweed poisoning but none have been proven to be effective. Mineral supplements neither prevented poisoning nor delayed symptoms in sheep fed Garbancillo (James and Van Kampen 1974). They also failed to prevent cattle from grazing white locoweed (Allison and Graham 1999). Electrical charges on clay minerals may bind to swainsonine, but a variety of clays and minerals did not prevent locoweed poisoning in a series of clinical feeding trials (Bachman et al. 1992, Pulsipher et al. 1994); nor did vitamin E/selenium injections hasten recovery of poisoned animals (Richards et al. 1999).

On the other hand, there was concern that growth implants may enhance locoweed poisoning. Estradiol implants did not cause steers to select more locoweed in a grazing trial and did not affect the degree of poisoning or rate of recovery in a locoweed feeding trial (Mikus et al. 2001).

Anecdotal evidence suggested that cattle on a higher plane of nutrition such as alfalfa hay or grazed on winter wheat, which is often the first plant to green up in spring, may be more inclined to graze the highly nutritious locoweed in the spring. However, these practices did not increase locoweed consumption (Ralphs et al. 1997 b, 2002c).

Native Cattle and Breeds

The poisonous plant literature is filled with statements that native livestock are less likely to be poisoned than new, inexperienced livestock. Locoweed poisoning does not follow this general trend. Cattle that are familiar with locoweed will likely select it first (Ralphs et al. 1987).

Early observations by Marsh (1909) suggested that black cattle and black-faced sheep were more inclined to be poisoned by locoweed than white-faced cattle and sheep. In a recent grazing study comparing breeds, Brangus steers consumed more locoweed than Hereford and Charolais steers (Duff et al. 2001). It was speculated that the gregarious nature of Brangus cattle may have facilitated the social acceptance of locoweed among the steers.

Grazing Management Recommendations

Livestock should be denied access to locoweeds during critical periods when they are relatively more palatable than associated forages (Ralphs et al. 2002a). On short-grass prairies of northeastern New Mexico, stocker cattle should not be turned onto locoweed-infested rangelands until warm-season grasses start growth in late May or early June (Ralphs et al. 1993, 1994a). Cattle on rangeland year-round should be removed from locoweed-infested sites in the spring when locoweed is green and growing, and warm-season grasses remain dormant. They can be returned to locoweed-infested pastures in summer when warm-season grasses are growing and abundant.

Most locoweed species are endemic, growing only in certain habitats or on specific soils. Fences could be constructed on soil or vegetation boundaries to provide seasonal control of grazing. Reserving locoweed-free pastures for grazing during critical periods in spring and fall can prevent locoweed poisoning.

Locoweed-free areas can be created by strategic herbicide use (McDaniel 1999, Ralphs and Ueckert 1988). However, natural population cycles should be considered to determine the practicality of spraying large areas and the potential lifetime of control. With the abundant seed bank in the soil, locoweeds are sure to germinate and reestablish when environmental conditions are favorable.

Animals that start eating locoweed may influence others to start. Social facilitation or peer pressure is a very strong influence inducing others to start eating locoweed (Ralphs et al. 1994b). Graham developed the "eat and pull" management strategy, whereby livestock should be watched closely and removed if they start eating locoweed to prevent poisoning and prevent them from influencing others to start (Torrell et al. 2000).

Grazing pressure can also force cattle to begin grazing locoweed when they run short of desirable forage (Ralphs 1987, Ralphs et al. 1994a). Ranchers should not overstock locoweed-infested ranges but rather, they should ensure that adequate forage is always available. Improper use of some grazing systems can cause livestock to graze locoweed. Rest-rotation grazing systems are designed to force livestock to uniformly graze all forage in a pasture. This caused cattle and horses to start grazing spotted locoweed in western Utah (James et al. 1969). Changing to a three-herd, four-pasture deferred rotation grazing system stopped locoweed poisoning by reducing the grazing pressure and allowing the cattle to select alternative forages in preference to white locoweed (Ralphs et al. 1984). The heavy grazing pressure associated with short-duration grazing systems may also induce poisoning problems.

Conditioned food aversion can be used as a management tool to train animals to avoid grazing locoweed (Ralphs et al. 1997a). In the conditioning protocol, animals are brought into a pen and fed fresh-picked locoweed, then lithium chloride (an emetic that causes gastrointestinal distress) is administered by stomach tube. The animals associate the induced illness with the taste of the plant and subsequently avoid eating it. Naive animals that are unfamiliar with the target plant form strong and lasting (> 3 years) aversions following a single dose (Ralphs 1997, Ralphs and Provenza 1999, Ralphs et al. 2001b). Averted animals must be kept separate from non-averted animals on locoweed areas to prevent social facilitation from extinguishing the aversions. Aversion conditioning may be feasible where losses are heavy and persist year after year.

Dead locoweed stalks present a risk of toxicity (Ralphs et al. 1988b). Swainsonine in dead plant material is stable and does not leach out. In fact, its concentration may increase as soluble cell contents desiccate and the plant dries. In desert and semi-desert regions, these dead stalks may remain for 1 or 2 years. They retain their nutrient content and digestibility, much like dried alfalfa, and may be relatively more palatable than dry senescent grasses during the winter (Ralphs et al. 1988b). Although a population may die back, the site may not be safe to graze until the old stalks have decomposed.

Control

Locoweeds can be controlled through the use of common rangeland herbicides. Most research has been conducted on white and woolly locoweed (Ralphs et al. 1988a, McDaniel et al. 2007). White locoweed is most sensitive to clopyralid, requiring as little as 0.12-0.25 lb ae/ac. Picloram at 0.25-0.5 lb ae/ac is most reliable in controlling both species. It can also be applied as Grazon P + D (1:4 mixture with 2,4-D). Metsulfuron is effective at very low rates (0.375-0.5 oz ai/ac). Herbicides should be applied while locoweed is actively growing. The greatest success has been when herbicides are applied during early bloom or in fall during late vegetative growth. Under ideal conditions (relative humidity > 50%, moderate temperatures 60-75 F, soil temperatures > 55F, and moist soil), the lower rates can give good control. Other species have been reported to be controlled by these herbicides at similar rates: *O. lambertii* (Alley 1976); *A. mollissimus* var. *earleii* (Freeman et al. 1982); *A. wootonii* (Ueckert 1985); and *A. miser* (Williams and Ralphs 1988).

Biological Control

Most varieties of woolly locoweed are naturally controlled by the four-lined locoweed weevil (*Cleonidius trivittatus*) (Pomerinke et al. 1995). Its c-shaped larvae bore into the tap root, and as few as two larvae per plant will kill most plants (Thompson et al. 1999). As these insects infect a stand, the entire population will die out. Populations of woolly locoweed seldom last more than 2 to 3 years because of the weevil in combination with drought. Although weevils can be reared in the lab or collected from the field, neither is practical for control due to labor costs. Conservation of existing weevil populations offers the best method for biocontrol of woolly locoweed. This can be accomplished with judicious use of insecticides for grasshoppers and range

caterpillars (Gardner and Thompson 1999). Controlling woolly locoweed with herbicides may limit the usefulness of the weevils by killing their food source during development. The best management practice may be simply to fence the woolly locoweed patches or prevent livestock access until woolly locoweed dies out naturally (Gardner and Thompson 1999). The four-lined locoweed weevil also has been observed in some northern populations of white locoweed, but it apparently does not control its populations (Parker 2008).

Summary

Locoweed is the most widespread poisonous plant problem in the Western United States. Knowledge of sites where locoweeds grow and of environmental conditions that cause their populations to increase can be used to predict the risk of poisoning. Locoweeds are relatively palatable during some seasons. Many locoweeds are the first plants to start growing in the spring, and they may also resume growth in the fall. Cattle and sheep generally prefer the green-growing locoweeds to other forage that is dormant in the fall, winter, and spring. The most effective management strategy is to deny livestock access to locoweeds during critical periods when locoweeds are more palatable than associated forage. Reserving locoweed-free pastures or controlling existing locoweed populations with herbicides can provide "safe" pastures for critical periods. Watching animals closely and removing those that begin eating locoweed can prevent further intoxication and also prevent animals from influencing others to start. Good range management and wise grazing strategies can provide adequate forage for livestock and avoid critical periods of the year when locoweed is relatively more palatable than associated forages.

References

Alley, H.P. 1976. Research in weed science. *Wyoming Agricullture Experiment Station Research Journal* 91R, pp. 79-82.

Allison, C., and J.D. Graham. 1999. Reducing locoism with management decisions. *In* T.M. Sterling and D.C. Thompson, eds., Locoweed Research Updates and Highlights, pp. 64-66. New Mexico Agriculture Experiment Station Research Report 730.

Bachman, S.E., M.L. Galyean, G.S. Smith, et al. 1992. Early aspects of locoweed toxicosis and evaluation of a mineral supplement or clinoptilolite as dietary treatments. *Journal of Animal Science* 70:3125-3132.

Braun, K., J. Romero, C. Liddell, and R. Creamer. 2003. Production of swainsonine by fungal endophytes of locoweed. *Mycological Research* 107:980-988.

Broquist, H.P. 1985. The indolizidine alkaloids, slaframine and swainsonine: contaminants in animal forages. *Annual Review of Nutrition* 5:391-409.

Colegate, S.M., P.R. Dorling, and C.R. Huxtable. 1979. A spectroscopic investigation of swainsonine: an alpha-mannosidase inhibitor isolated from *Swainsona canescens* (a plant poisonous to livestock). *Australia Journal of Chemistry* 32:2257-2264.

Cook, D., D.R. Gardner, M.H. Ralphs, et al. 2009a. Swainsonine concentrations and endophyte amounts of *Undifilum oxytropis* in different plant parts of *Oxytropis sericea*. *Journal of Chemical Ecology* 35:1272-1278.

Cook, D., D.R. Gardner, K.D. Welch, et al. 2009b. Quantative PCR method to measure the fungal endophyte in locoweeds. *Journal of Agriculture and Food Chemistry* 57:6050-6054.

Dantas, A.F.M., F. Riet-Correa, D.R. Gardner, et al. 2007. Swainsonine-induced lysosomal storage disease in goats caused by the ingestion of *Turbina cordata* in northeastern Brazil. *Toxicon* 49: 111-116

Davis, D., P. Schwarz, T. Hernandez, et al. 1984. Isolation and characterization of swainsonine from a Texas locoweed (*Astragalus emoryanus*). *Plant Physiology* 76:972-975.

Duff, G.C., M.H. Ralphs, D. Walker, et al. 2001. Influence of beef breeds (Hereford, Charolais, Brangus) on locoweed consumption. *Professional Animal Scientist* 18:33-37.

Freeman, M.R., D.N. Ueckert, and J.T. Nelson. 1982. Woolly locoweed and forage response to herbicides in west Texas. Texas Agriculture Experiment Station Bulletin 1398.

Fox, W.E., K.W. Allred, and E.H. Roalson. 1998. A guide to the common locoweeds and milkvetches of New Mexico. New Mexico Agriculture Experiment Station Circular 557.

Furlani, S., K.E. Panter, J.A. Pfister, and B.L. Stegelmeier. 2007. Fetotoxic effects of locoweed (*Astragalus lentiginosus*) in pregnant goats. *In* K.E. Panter, T.L. Wierenga, and J.A. Pfister, eds., Poisonous Plants: Global Research and Solutions, pp. 130-135. CAB International, Wallingford, U.K.

Gardner, D.R., R.J. Molyneux, and M.H. Ralphs. 2001. Analysis of swainsonine: extraction methods, detection and measurement in populations of locoweeds (*Oxytropis* spp.). *Journal of Agriculture and Food Chemistry* 49:4573-4580.

Gardner, K.T., and D.C. Thompson. 1999. Are rangeland insect spray programs enhancing weed problems? *In* T.M. Sterling and D.C. Thompson, eds., Locoweed Research: Updates and Highlights, pp. 50-51. New Mexico Agriculture Experiment Station Research Report 730.

Graham, D., R. Creamer, D. Cook, et al. 2009. Solutions to locoweed poisoning in New Mexico and the western United States. *Rangelands* 31(6):3-8.

Haraguchi, M., S.L. Gorniak, K. Ikeda, et al. 2003. Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). *Journal of Agriculture and Food Chemistry* 51:4995-5000.

Hueza, I.M., J.L. Guerra, M. Haraguchi, et al. 2005. The role of alkaloids in *Ipomoea carnea* toxicosis: a study in rats. *Experimental and Toxicological Pathology* 57(1):53-58.

James, L.F., and W.J. Hartley. 1977. Effects of milk from animals fed locoweed on kittens, calves, and lambs. *Journal of American Veterinary Research* 38:1263-1265.

James, L.F., and K.R. Van Kampen. 1974. Effect of protein and mineral supplementation on potential locoweed (*Astragalus* spp.) poisoning in sheep. *Journal of the American Veterinary Medical Association* 164:1042-1043.

James, L.F., K.L. Bennett, K.G. Parker, et al. 1968. Loco plant poisoning in sheep. *Journal of Range Management* 21:360-365.

James, L.F., W.J. Hartley, and K.R. Van Kampen. 1981. Syndromes of *Astragalus* poisoning in livestock. *Journal of the American Veterinary Medical Association* 178:146-150.

James, L.F., W.J. Hartley, D. Nielsen, et al. 1986. Locoweed (*Oxytropis sericea*) poisoning and congestive heart failure in cattle. *Journal of the American Veterinary Medical Association* 189:1549-1556.

James, L.F., K. R. Van Kampen, and J.R. Staker. 1969. Locoweed (*Astragalus lentiginosus*) poisoning in cattle and horses. *Journal of the American Veterinary Medical Association* 155:525-530.

Kingsbury, J.M. 1964. Poisonous Plants of the United States and Canada. Prentice-Hall, Englewood Cliffs, NJ.

Loretti, A.P., E.M. Colodel, E.J. Gimeno, and L. Driemeier. 2003. Lysosomal storage disease in *Sida carpinifolia* toxicosis: an induced mannosidosis in horses. *Equine Veterinary Journal* 35:434-488.

Marsh, C.D. 1909. The Loco-weed Disease of the Plains. USDA Bureau of Animal Industry Bulletin 112.

McDaniel, K.C. 1999. Controlling locoweed with herbicides. *In* T.M. Sterling and D.C. Thompson, eds., Locoweed Research Updates and Highlights, pp. 52-53. New Mexico Agriculture Experiment Station Research Report 730.

McDaniel, K.C., T.M. Sterling, and S. Ivey. 2007. Herbicide control of locoweeds. *In* K.E. Panter, T.L. Wierenga, and J.A. Pfister, eds., Poisonous Plants: Global Research and Solutions, pp. 353-358. CAB International, Wallingford, U.K.

Mikus, J.H., G.C. Duff , C.R. Krehbiel, et al. 2001. Effects of an estradiol implant on locoweed consumption, toxicity, and recovery in growing beef steers. *Professional Animal Scientist* 17:109-114.

Molyneux, R.J., and L.F. James. 1982. Loco intoxication: indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Science* 216:190-191.

Molyneux, R.J., R.A. McKenzie, B.M. O'Sullivan, and A.D. Elbein. 1995. Identification of the glycosidase inhibitors swainsonine and calystegine B2 in Weir Vine (*Ipomoea* sp. Q6 [1]) and correlation with toxicity. *Journal of Natural Products* 58(6):878-886.

Panter, K.E., L.F. James, and W.J. Hartley. 1989. Transient testicular degeneration in rams fed locoweed (*Astragalus lentiginosus*). *Veterinary and Human Toxicology* 31(1):42-46.

Panter, K.E., L.F. James, B.L. Stegelmeier, et al. 1999. Locoweeds: effects on reproduction in livestock. *Journal of Natural Toxins* 8:53-62.

Parker, J.E. 2008. Effects of insect herbivory by the four-lined locoweed weevil, *Cleonidius trivittatus* Say (Coleoptera: Curculionidae), on locoweeds *Astragalus mollissimus* and *Oxytropis sericea.* New Mexico State University, Las Cruces, NM. Master's thesis.

Payne, G.F. 1957. Ecology and life history of the poisonous plant, white locoweed (*Oxytropis sericea* Nutt.). Texas A&M University, College Station, TX. PhD dissertation.

Pedroso, P.M., R. Von Hohendorf , L.G. de Olivera, et al. 2009. *Sida carpinifolia* (Malvaceae) poisoning in fallow deer (*Dama dama*). *Journal of Zoological and Wildlife Medicine* 40:583-585.

Pfister, J.A., B.L. Stegelmeier, C.D. Cheney, et al. 1996. Operant analysis of chronic locoweed intoxication in sheep. *Journal of Animal Science* 74:2622-2632.

Pfister, J.A., B.L. Stegelmeier, D.R. Gardner, and L.F. James. 2003. Grazing of spotted locoweed (*Astragalus lentiginosus*) by cattle and horses in Arizona. *Journal of Animal Science* 81:2285-2293.

Pfister, J.A., T. Davidson, K.E. Panter, et al. 2006. Maternal ingestion of locoweed. III. Effects on lamb behaviour at birth. *Small Ruminant Research* 65:70-78.

Pomerinke, M.A., D.C. Thompson, and D.L. Clason. 1995. Bionomics of *Cleonidius trivittatus* (Coleoptera: Curculionidae): native biological control of purple locoweed (Rosales: Fabaceae). *Environmental Entomology* 24:1696-1702.

Pryor, B.M., R. Creamer, R.A. Shoemaker, et al. 2009. *Undifilum*, a new genus for endophytic *Embellisia oxytropis* and parasitic *Helmintosporiuim bornmuelleri* on legumes. *Botany* 87:178-194.

Pulsipher, G.D., M.L. Galyean, D.M. Hallford, et al. 1994. Effects of graded levels of bentonite on serum clinical profiles, metabolic hormones, and serum swainsonine concentrations in lambs fed locoweed (*Oxytropis sericea*). *Journal of Animal Science* 72:1561-1569.

Purvines, J., and D. Graham. 1999. When rain falls may affect locoweed density. *In* T.M. Sterling and D.C. Thompson, eds., Locoweed Research Updates and Highlights, pp. 32-33. New Mexico Agriculture Experiment Station Research Report 730.

Ralphs, M.H. 1987. Cattle grazing white locoweed: influence of grazing pressure and palatability associated with phenological growth stage. *Journal of Range Management* 40:330-332.

Ralphs, M.H. 1997. Persistence of aversions to larkspur in naive and native cattle. *Journal of Range Management* 50:367-370.

Ralphs, M.H., and E.H. Cronin. 1987. Locoweed seed in soil: density, longevity, germination, and viability. *Weed Science* 35:792-795.

Ralphs, M.H., and V.L. Bagley. 1988. Population cycles of Wahweap milkvetch on the Henry Mountains and seed reserve in the soil. *Great Basin Naturalist* 48:541-547.

Ralphs, M.H., and D.N. Ueckert. 1988. Herbicide control of locoweeds: a review. *Weed Technology* 2:460-465.

Ralphs, M.H., and F.D. Provenza. 1999. Conditioned food aversions: principles and practices, with special reference to social facilitation. *Proceedings of the Nutrition Society* 58:813-820.

Ralphs, M.H., L.F. James, D.B. Nielsen, and K.E. Panter. 1984. Management practices reduce cattle loss to locoweed on high mountain range. *Rangelands* 6:175-177.

Ralphs, M.H., L.V. Mickelsen, and D.L. Turner. 1987. Cattle grazing white locoweed: diet selection patterns of native and introduced cattle. *Journal of Range Management* 40:333-335.

Ralphs, M.H., L.V. Mickelsen, D.L. Turner, and D.B. Nielsen. 1988a. Control of white locoweed (*Oxytropis sericea* Nutt.). *Weed Science* 36:353-358.

Ralphs, M.H., L.F. James, D.B. Nielsen, et al. 1988b. Cattle grazing Wahweap milkvetch in southeastern Utah. *Journal of Animal Science* 66:3124-3130.

Ralphs, M.H., B. Benson, and J.C. Loerch. 1989a. Soil-site relationships of white locoweed on the Raft River Mountains. *Great Basin Naturalist* 49:419-424.

Ralphs, M.H., K.E. Panter, and L.F. James. 1989b. Feed preferences and habituation of sheep poisoned by locoweed. *Journal of Animal Science* 68:1354-1362.

Ralphs, M.H., K.E. Panter, and L.F. James. 1991. Grazing behavior and forage preference of sheep with chronic locoweed toxicosis suggest no addiction. *Journal of Range Management* 44:208-209.

Ralphs, M.H., D. Graham, R.J. Molyneux, and L.F. James. 1993. Seasonal grazing of locoweeds by cattle in northeastern New Mexico. *Journal of Range Management* 46:416-420.

Ralphs, M.H., D. Graham, and L.F. James. 1994a. Cattle grazing white locoweed in New Mexico: influence of grazing pressure and phenological growth stage. *Journal of Range Management* 47:270-274.

Ralphs, M.H., D. Graham, and L.F. James. 1994b. Social facilitation influences cattle to graze locoweed. *Journal of Range Management* 47:123-126.

Ralphs, M.H., D. Graham, L.F. James, and K.E. Panter. 1994c. Locoweed effects on a calf crop. *Rangelands* 16:35-37.

Ralphs, M.H., D. Graham, M.L. Galyean, and L.F. James. 1997a. Creating aversions to locoweed in naive and familiar cattle. *Journal of Range Management* 50:361-366.

Ralphs, M.H., D. Graham, M.L. Galyean, and L.F. James. 1997b. Influence of over-wintering feed regimen on consumption of locoweed by steers. *Journal of Range Management* 50:250-252.

Ralphs, M.H., D. Graham, G. Duff, et al. 2000. Impact of locoweed poisoning on grazing steer weight gains. *Journal of Range Management* 53:86-90.

Ralphs, M.H., G. Greathouse, A.P. Knight, and L.F. James. 2001a. Cattle preference for Lambert locoweed over white locoweed throughout their phenological stages. *Journal of Range Management* 54:265-268.

Ralphs, M.H., F.D. Provenza, J.A. Pfister, et al. 2001b. Conditioned food aversion: from theory to practice. *Rangelands* 23:14-18.

Ralphs, M.H., J.D. Graham, and L.F. James. 2002a. Locoweed poisoning on shortgrass prairies: management recommendations to reduce risk of poisoning. *Rangelands* 24:30-34.

Ralphs, M.H., D.R. Gardner, J.D. Graham, et al. 2002b. Clipping and precipitation influences on locoweed vigor, longevity and toxicity. *Journal of Range Management* 55:394-399.

Ralphs, M.H., G. Greathouse, A.P. Knight, et al. 2002c. Prior feeding practices do not influence locoweed consumption. *Journal of Range Management* 55:390-393.

Ralphs, M.H., S.L. Welsh, and D.R. Gardner. 2002d. Distribution of the locoweed toxin swainsonine in populations of *Oxytropis lambertii*. *Journal of Chemical Ecology* 28:701-707.

Ralphs, M.H., J.A. Pfister, S.L. Welsh, et al. 2003. Locoweed population cycles. *Rangelands* 25(5):14-18.

Ralphs, M.H., R. Creamer, D. Baucom, et al. 2008. Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). *Journal of Chemical Ecology* 34:32-38.

Ralphs, M.H., D. Cook, D.R. Gardner, and D.S. Grum. 2011. Transmission of the locoweed endophyte to successive generations. *Journal of Fungal Ecology*. In press.

Richards, J.B., D.M. Hallford, and G.C. Duff. 1999. Serum luteinizing hormone, testosterone, and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. *Theriogenology* 52:1055-1066.

Romero J, Creamer R, Zepeda H, et al. 2004. Toxicosis of *Embellisia* fungi from locoweed (*Oxytropis lambertii*) is similar to locoweed (*Oxytropis lambertii*) toxicosis in rat. *Journal of Animal Science* 82:2169-2174.

Shi, Z.C. 1997. Major Poisonous Plants of China Grasslands. China Agriculture Press, Beijing, China.

Smith, G.S., K.W. Allred, and D.E. Kiehl. 1992. Swainsonine content of New Mexican locoweeds. *Proceedings Western Section American Society of Animal Science* 43:405-407.

Stegelmeier, B.L., R.J. Molyneux, and L.F. James. 1994. The pathology of swainsonine and locoweed (*Astragalus mollissimus*) in rodents. *Veterinary Pathology* 31:620.

Stegelmeier, B.L., P.D. Snyder, L.F. James, et al. 1998a. The immunologic and toxic effects of locoweed (*Astragalus lentiginosus*) intoxication in cattle. *In* T. Garland and A.C. Barr, eds. Toxic Plants and Other Natural Toxicants, pp. 285-290. CAB International, Wallingford, U.K.

Stegelmeier, B.L., L.F. James, K.E. Panter, et al. 1998b. Tissue swainsonine clearance in sheep chronically poisoned with locoweed. *Journal of Animal Science* 76:1140-1144.

Stegelmeier, B.L., L.F. James, K.E. Panter, et al. 1999a. The pathogenesis and toxicokinetics of locoweed (*Astragalus* and *Oxytropis*) poisoning in livestock. *Journal of Natural Toxins* 8:35-45.

Stegelmeier, B.L., L.F. James, K.E. Panter, et al. 1999b. Dose response of sheep poisoned with locoweed (*Oxytropis sericea*). *Journal of Veterinary Diagnostic Investigation* 11:448-456.

Stegelmeier, B.L., L.F. James, D.R. Gardner, et al. 2005. Locoweed (*Oxytropis sericea*)-induced lesions in mule deer (*Odocoileius hemionus*). *Veterinary Pathology* 42(5):566-578.

Stegelmeier, B.L., S.T. Lee, L.F. James, et al. 2007. The comparative pathology of locoweed poisoning in livestock, wildlife and rodents. *In* K.E. Panter, T.L. Wierenga, and J.A. Pfister, eds., Poisonous Plants: Global Research and Solutions , pp. 359-365. CAB International, Wallingford, U.K.

Thompson, D.C., J.L. Knight, T.M. Sterling, and K.T. Gardner. 1999. Locoweed weevils prefer certain varieties of locoweed. *In* T.M. Sterling and D.C. Thompson, eds., Locoweed Research: Updates and Highlights, pp. 42-49. New Mexico Agriculture Experiment Station Research Report 730.

Torrell, L.A., L.P. Owen, K.C. McDaniel, and D. Graham. 2000. Perceptions and economic losses from locoweed in northeastern New Mexico. *Journal of Range Management* 53:376-383.

Ueckert, D.N. 1985. Management of selected poisonous plants on semiarid rangelands in west Texas with herbicides. *In* A.A. Seawright, M.P. Hegarty, L.F. James, and R.F. Keeler, eds., Plant Toxicology, pp. 32-41. Queensland Poisonous Plant Committee, Queensland, Australia.

Van Kampen, K.R., and L.F. James. 1970. Pathology of locoweed poisoning in sheep: sequential development of cytoplasmic vacuolation in tissues. *Pathological Veterinarian* 7:503-508.

Vallotton, A.D., and T.M. Sterling. 2002. Variation in swainsonine content among extraction methods and between locoweed genera. *Proceedings of the Western Society of Weed Science* 55:18.

Welsh, S.L. 1989. *Astragalus* L. and *Oxytropis* DC: definitions, distributions, and ecological parameters. *In* L.F. James, A.D. Elbein, R.J. Molyneux, and C.D. Warren, eds., Swainsonine and Related Glycosidase Inhibitors, pp. 3-13. Iowa State University Press, Ames, IA.

Welsh, S.L. 2001. Revision of North American species of *Oxytropis* de Candolle (Leguminosae). E.P.S. Inc., Orem, UT.

Welsh, S.L. 2007. North American species of *Astragalus* Linnaeus (Leguminosae), a taxonomic revision. M.L. Bean Museum, Brigham Young University, Provo, UT.

Welsh, S.L, M.H. Ralphs, K.E. Panter, J.A. Pfister, and L.F. James. 2007. Locoweeds of North America: taxonomy and toxicity. *In* K.E. Panter, T.L. Wierenga, and J.A. Pfister, eds., Poisonous Plants: Global Research and Solutions, pp. 20-29. CAB International, Wallingford, U.K.

Wickwire, B.M., and H.P. Broquist. 1989. Early steps of slafamine and swainsonine biosynthesis in *Rhizoctonia leguminicola*. *In* L.F. James, A.D. Elbein, R.J. Molyneux, and C.D. Warren, eds., Swainsonine and Related Glycosidase Inhibitors, pp. 125-137. Iowa State University Press, Ames, IA.

Williams, M.C., and M.H. Ralphs. 1988. Control of Wasatch milkvetch (*Astragalus miser* var. *oblongifolius*) on mountain range. *Weed Technology* 3:110-113.

Williams, M.C., L.F. James, and B.O. Bond. 1979. Emory milkvetch (*Astragalus emoryanus* var *emoryanus*) poisoning in chicks, sheep and cattle. *American Journal of Veterinary Research* 40:403-406.

Zhao, B.Y., D.W. Tong, P.B. Ge, et al. 2003. Locoweed harm investigation in the west grasslands of China. *Grassland of China* 25:65-68.

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Pathological Effects of Short-Term *Crotalaria retusa* Ingestion by Guinea Fowl (*Numida meleagris*)

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Abstract

Crotalaria retusa and its seeds contain toxic pyrrolizidine alkaloids that can contaminate feeds and food, thus poisoning livestock and humans. The objective of this work was to determine the toxic effects of the ingestion of *Crotalaria retusa* seeds by guinea fowl (*Numida meleagris*). Eighteen young guinea fowl were randomly assigned to 3 groups and treated with 0 (control), 1.0 mg/kg or 5.0 mg/kg of crushed *Crotalaria retusa* seeds mixed into the daily ration for 7 consecutive days. After 7 days, blood samples were collected to determine serum glucose, cholesterol, total proteins, urea and creatinine concentrations, and ALT and AST activities. After sampling, the guinea fowl were euthanized and necropsied, and samples of liver, kidneys, lungs, and heart were collected and prepared for histologic studies. At necropsy all animals treated with 5.0 mg/kg of *C. retusa* presented mild ascites (clear fluid), the liver were uniformly pale, icteric, and soft, and the gallbladders were distended and filled with bile, but the bile aspect and the gall bladder wall were normal. The histopathological examination of the livers from *C. retusa*-treated animals revealed centrilobular swollen and vacuolated hepatocytes, moderate in guinea fowl treated with 5.0 mg/kg and mild in those treated with 1.0 mg/kg. One animal treated with 5.0 mg/kg presented centrilobular necrosis and hepatomegalocytosis with prominent nucleolus. The histological examination of kidneys revealed vacuolated epithelial cells at distal convoluted tubules. These findings indicate that guinea fowl are very sensitive to poisoning by *Crotalaria retusa*.

Keywords: Poisonous plants, pyrrolizidine alkaloids, monocrotaline, avian

Introduction

Plant species from the genus *Crotalaria*, including *C. retusa* (wedge-leaf rattlebox), are used as soil builders and green manure (Williams and Molyneux 1987) and for management of plant-parasitic nematodes (Thoden and Boppré 2010). However, these plants contain pyrrolizidine alkaloids (PAs), mainly monocrotaline (Williams and Molyneux 1987). *C. retusa* poisoning has been described in several species, including horses (Nobre et al. 2004), sheep (Nobre et al. 2005), pigs (Hooper and Scanlan 1977), chickens (Hooper and Scanlan 1977, Alfonso et al. 1993, Hatayde et al. 2008), and geese (Alfonso et al. 1993). Poultry are frequent victims of poisoning by such contamination under field conditions (Williams and Molyneux 1987); however, other avian species may often be affected. In addition to intentional ingestion of the plant, avians are poisoned by *Crotalaria* seeds through contamination of corn, sorghum, and soybean during harvest (Nakage et al. 2000).

There is a great variation in susceptibility to PA toxicity between animals of different species. This variation is a result of many different factors including differing rates of metabolic activation, hydroxylation, N-oxidation, hydrolysis, and glucuronidation of PAs (Huan et al. 1998, He et al. 2010). This objective of this study was to determine the pathological changes produced by the ingestion of *Crotalaria retusa* seeds by guinea fowl (*Numida meleagris*) for 7 days.

Material and Methods

Seeds from *Crotalaria retusa* L. were collected at Mossoró city, RN, northeastern Brazil (5°11'15"S and 37°20'39"W), at an altitude of 16 m above sea level. The climate in this region is characterized as semi-arid. The mean annual temperature in this region is 27.4°C, and the mean annual rainfall and mean relative humidity are 674 mm and 68.9%, respectively. The botanical identification was performed by Prof. Odaci Fernandes de Oliveira, Mossoró, RN, Brazil. Only mature seeds without any sign of lesion were used, and they were dried at room temperature and then powdered before use.

Eighteen 3- to 5-day-old guinea fowl (*N. meleagris*) were housed one animal per cage with free access to food and water. The fowl were randomly divided into 3 groups of 6 and treated with 0 (control), 1.0 mg/kg BW, or 5.0 mg/kg BW crushed *C. retusa* seeds that were mixed into their daily ration for 7 consecutive days. After treatment, blood samples were collected from all the guinea fowl from the jugular vein and serum was collected for a complete biochemical panel. The serum concentrations of albumin, cholesterol, urea, and uric acid, and the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercially available kits (Katal, Belo Horizonte, MG, Brazil) and an automatic analyzer SBA-2000 (Celm, Barueri, SP, Brazil) using recommended reagents and following the manufacturer's directions. Immediately after blood collection, the guinea fowl were euthanized and necropsied. At necropsy all the gross lesions were noted and small fragments of liver, kidney, and heart were collected, fixed in buffered formalin, imbedded in paraffin, and processed for routine histologic studies.

The study used a completely randomized design with three treatments. Statistical analyses were performed by analysis of variance (ANOVA) plus a posthoc Dunnett's test using the software BioEstat 5.0. Statistical significance was set at $P < 0.05$. Results are presented as means with standard errors.

Results

During the experimental period mortality occurred in all groups, two in the control group and those treated with 1.0 mg/kg of *C. retusa* and three in the group treated with 5.0 mg/kg of *C. retusa*. Clinical signs were observed in guinea fowl treated with 5.0 mg/kg of *C. retusa*, and consisted of anorexia, emaciation, muscular weakness, trembling, and depression in the 8 hours before death. Fowls from other groups presented anorexia before death, and the necropsy revealed congestion of liver, lungs, and intestines. There were no significant differences between the various biochemical parameters (table 1).

At necropsy all animals treated with 5.0 mg/kg of *C. retusa* presented mild ascites (clear fluid); the livers were uniformly pale, icteric, and soft; and the gall bladders were distended and filled with bile but the bile aspect and the gall bladder wall were normal. The necropsy of the other animals (controls and those treated with 1.0 mg/kg) killed at the end of the experiment revealed no gross lesion.

Table 1. Serum biochemical panel from guinea fowl (*Numida meleagris*) treated with 0 (control), 1.0 (G1.0), or 5.0 (G5.0) mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days

Parameter	Control	G1.0	G5.0
ALT (U/l)	11.3±5.46	10.0±1.67	13.0±8.13
AST (U/l)	360.1±68.6	407.5±87.7	313.2±105.9
Urea (mg/dl)	4.65±1.47	0.80±1.60	6.13±5.38
Uric acid (mg/dl)	7.94±1.15	6.15±1.98	9.24±1.80
Cholesterol (mg/dl)	136.1±43.5	162.3±30.3	138.3±43.7
Albumin (g/dl)	0.93±0.20	1.12±0.10	0.94±0.20

Data are presented as mean±SEM.
ALT: alanine aminotransferase. AST: aspartate aminotransferase.

Table 2. Pathological findings in guinea fowl (*Numida meleagris*) treated with 0 (control), 1.0 (G1.0), or 5.0 (G5.0) mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days

Lesion	Control	G1.0	G5.0
Centrilobular swollen and vacuolated hepatocytes	-	+	++
Centrilobular necrosis	-	-	++ [1]
Hepatomegalocytosis	-	-	+ [1]
Vacuolated renal tubular epithelial cells	-	+	+ / ++

- No lesion + mild ++ moderate +++ severe
[1] present in just one animal.

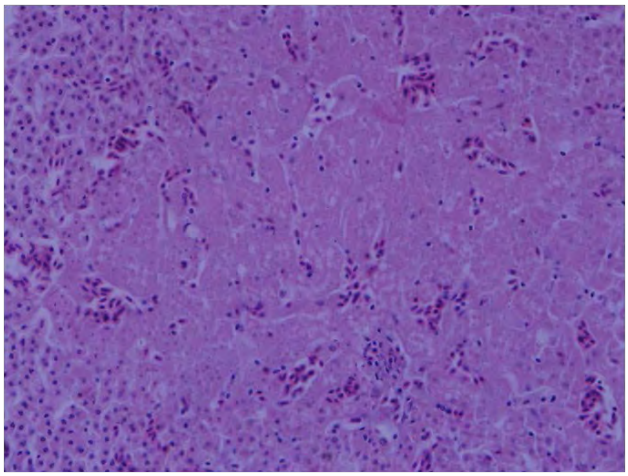

Figure 1. Liver of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing vacuolated hepatocytes (HE, 40x).

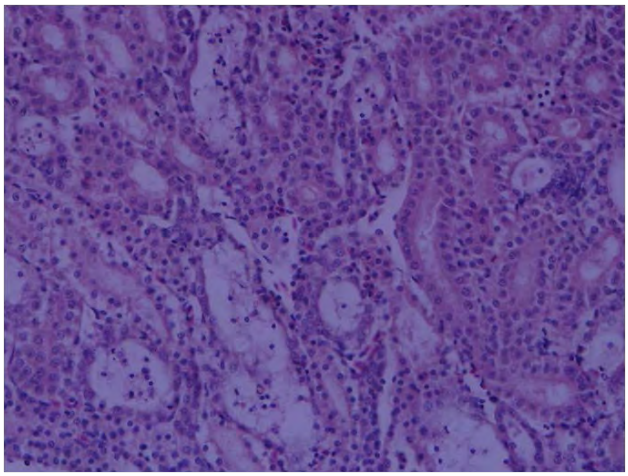

Figure 2. Liver of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing centrilobular necrosis (HE, 40x).

The histopathological examination (table 2) of the livers from *C. retusa*-treated animals revealed centrilobular swollen and vacuolated hepatocytes (figure 1), moderate in guinea fowl treated with 5.0 mg/kg and mild in those treated with 1.0 mg/kg. One animal treated with 5.0 mg/kg BW presented centrilobular necrosis (figure 2) and hepatomegalocytosis with prominent nucleolus. The histological examination of kidneys revealed vacuolated epithelial cells at distal convoluted tubules (figure 3).

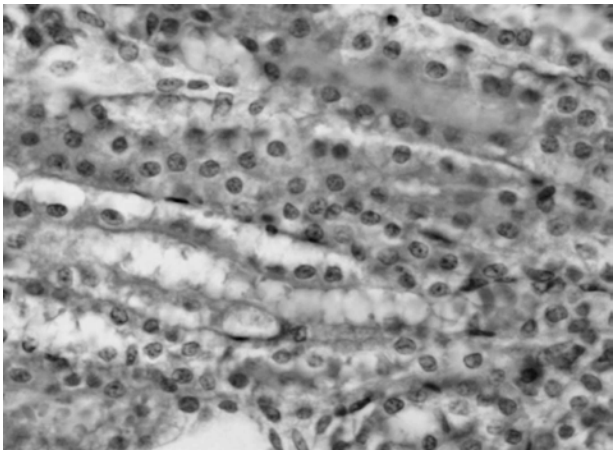

Figure 3. Kidney of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing vacuolated renal tubular epithelial cells (HE, 100x).

Discussion

Natural cases of poisoning of birds by PA-containing plants have been reported in chickens (Hooper and Scanlan 1977, Pass et al. 1979, Alfonso et al. 1993, Gaul et al. 1994), ducks (Pass et al. 1979), and geese (Alfonso et al. 1993). The gross examination of chickens experimentally fed *C. spectabilis* seeds for 28 days revealed ascites and cachexia with liver volume increased or reduced with fibrin or subcapsule hematomas. The reported microscopic lesions of PA poisoning in birds include fatty degeneration, congestion, hemorrhage, and necrosis and megalocytosis of hepatocytes (Hatayde et al. 2008). The variations of observed lesions in the present study with those observed in chicken could be attributed to differences on administration period. In fact, the observed lesions in guinea fowl were

very similar to acute poisoning of sheep by *C. retusa* (Nobre et al. 2005).

Ascites is a common finding in PA poisoning, and was attributed to impaired serum protein synthesis in the liver and portal hypertension caused by damaged liver (Petterson and Culvenor 1983). However, both mechanisms should be excluded in affected guinea fowl in the present work because there was no hypoalbuminemia and no cirrhosis. From the several other known causes of ascites (McHutchison 1997, Hou and Sanyal 2009), the most plausible explanation for the cases in the present study is heart failure.

The evaluation of modes of cell death induced by monocrotaline in rats revealed that the hepatic parenchymal cells underwent coagulative oncosis in centrilobular regions and apoptosis in other regions (Copple et al. 2004). The observed centrilobular necrosis of hepatocytes in a guinea fowl from the present study was a possible result of that coagulative oncosis.

We found no significant difference between these treatment groups in any of the biochemical parameters that we analyzed. Since no biochemical evaluations were made in the guinea fowl that died earlier in the experiment, which could produce artifacts that were impossible to be identified or excluded, this assessment was made only in fowl that survived. The surviving guinea fowl could have become resistant to monocrotaline and other PAs present in *C. retusa*, and this resistance could have occurred through increased rate of hepatic conjugation of the toxin. In fact, it was observed that sheep treated with sub-lethal quantities of seeds of *C. retusa*, the same kind used in this study, became resistant (Anjos et al. 2010). However, this possible resistance should be further studied.

Among the various animal species, it is well known that there is a great variation in sensitivity to the toxic effects of PAs. Sheep, Japanese quail, rabbits, gerbils, hamsters, and guinea pigs are resistant to PA chronic toxicity, whereas chickens and turkeys are susceptible (Pierson et al. 1977, Cheeke and Pierson-Goeger 1983, Cheeke 1988, Cheeke and Huan 1988, Alfonso et al. 1993). The species variation in sensitivity is attributed to the need for bioactivation of these alkaloids to promote toxicity. The bioactivation occurs primarily through the cytochrome P450 enzyme complex, forming a highly reactive compound, the pyrrole, which combines with macromolecules such as proteins and DNA (Cheeke and Huan 1998, Huan et al. 1998, Kosogof et al. 2001). One form of variation in the sensitivity of animal species is the rate of bioactivation of PAs. In fact, Japanese quail (Buckmaster et al. 1977) and sheep (Huan et al. 1998) are highly resistant species because they have a low rate of pyrrole formation. Another feature of change is the detoxification of pyrrole compound that may occur by conjugation with glutathione or enzymatic degradation by hepatic esterases (Cheeke and Huan 1998, Huan et al. 1998, He et al. 2010). In sheep, the high efficiency in conjugation of pyrrole derivatives with GSH contributes to the resistance to PA poisoning (Swick et al. 1983, Huan et al. 1998).

In conclusion, guinea fowl (*N. meleagris*) are sensitive to the toxicity of PAs from *Crotalaria retusa*. Further, guinea fowl that survive the initial toxic insult may develop some resistance to PA intoxication.

References

Alfonso, H.A., L.M. Sanchez, M.A. Figueredo, and B.C. Gómez. 1993. Intoxication due to *Crotalaria retusa* and *C. spectabilis* in chickens and geese. *Veterinary and Human Toxicology* 35:539.

Anjos, B.L., V.M.T. Nobre, A.F.M. Dantas, et al. 2010. Poisoning of sheep by seeds of *Crotalaria retusa*: aquired resistance by continuous administration of low doses. *Toxicon* 55:28-32.

Buckmaster, G.W., P.R. Cheeke, G.H. Arscott, et al. 1977. Response of Japanese quail to dietary and injected pyrrolizidine (*Senecio*) alkaloid. *Journal of Animal Science* 45:1322-1325.

Cheeke, P.R. 1988. Toxicity and metabolism of pyrropizidine alkaloids. *Journal of Animal Science* 66:2343-2350.

Cheeke, P.R., and J. Huan. 1998. Species differences in bioactivation and detoxification of pyrrolizidine alkaloids. *In* T. Garland and A.C. Barr, eds., Toxic Plants and Other Natural Toxicants, pp. 559-563, CAB International, Wallingford, U.K.

Cheeke, P.R., and M.L. Pierson-Goeger. 1983. Toxicity of *Senecio jacobea* and pyrrolizidine alkaloids in various laboratory animals and avian species. *Toxicology Letters* 18:343-349.

Copple, B.L., C.M. Rondelli, J.F. Maddox, et al. 2004. Modes of cell death in rat liver after monocrotaline exposure. *Toxicological Sciences* 77:172-182.

Gaul, K.L., P.F. Gallagherm, D. Reyes, et al. 1994. Poisoning of pigs and poultry by stock feed contaminated with heliotrope seeds. *In* S.M. Colegate and P.R. Dorling, eds., Plant-Associated Toxins: Agricultural, Phytochemical and Economic Aspects, pp. 137-142, CAB International, Wallingford, U.K.

Hatayde, M.R., W.A.B. Pereira, G.S. Godoy, and A.C. Alessi. 2008. Efeitos da ingestão de sementes de *Crotalaria spectabilis* sobre o desempenho produtivo de galinhas poedeiras. *Veterinária Notícias* 14:19-28.

He, Y.Q., L. Yang, H.X. Liu, et al. 2010. Glucuronidation, a new metabolic pathway for pyrrolizidine alkaloids. *Chemical Research in Toxicology* 23:591-599.

Hooper, P.T., and W.A. Scanlan. 1977. *Crotalaria retusa* poisoning of pigs and poultry. *Australian Veterinary Journal* 53:109-114.

Hou, W., and A.J. Sanyal. 2009. Ascites: diagnosis and management. *The Medical Clinics of North America* 93:801-817.

Huan, J.Y., C.L. Miranda, D.R. Buhler, and P.R. Cheeke. 1998. Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicology Letters* 99:127-137.

Kosogof, C., J.J. Tepe, and R.M. Williams. 2001. DNA cross-linking by a photo-triggered pyrrolic progenitor developed from monocrotaline. *Tetrahedron Letters* 42:6641-6643.

McHutchison, J.G. 1997. Differential diagnosis of ascites. *Seminars in Liver Disease* 17: 191-202.

Nakage, A.P.M., M. Macari, L.S.O. Nakaghi, et al. 2000. Estudos hematológico e hormonal de frangos de corte tratados com contaminantes do milho: *Crotalaria spectabilis* e *Senna occidentalis*. *Brazilian Journal of Veterinary Research and Animal Science* 37:125-132.

Nobre, V.M.T., F. Riet-Correa, J.M. Barbosa Filho, et al. 2004. Intoxicação por *Crotalaria retusa* (Fabaceae) em eqüídeos no semiárido da Paraíba. *Pesquisa Veterinária Brasileira* 24:132-143.

Nobre, V.M.T., A.F.M. Dantas, F. Riet-Correa, et al. 2005. Acute intoxication by *Crotalaria retusa* in sheep. *Toxicon* 45:347-352.

Pass, D.A., G.G. Hogg, R.G. Russell, et al. 1979. Poisoning of chickens and ducks by pyrrolizidine alkaloids of *Heliotropium*. *Australian Veterinary Journal* 55:283-288.

Petterson, J.E., and C.C.J. Culvenor. 1983. Hepatotoxic pyrrolizidine alkaloids. *In* R.F. Keeler and A.T. Tu, eds., Handbook of Natural Toxins, pp. 637-671, Marcel Dekker, New York, NY.

Pierson, M.L., P.R. Cheeke, and E.O. Dickinson. 1977. Resistance of the rabbit to dietary pyrrolizidine (*Senecio*) alkaloid. *Research Communications in Chemical Pathology and Pharmacology* 16:561-564.

Swick, R.A., C.L. Miranda, P.R. Cheeke, and D.R. Buhler. 1983. Effect of phenobarbital on toxicity of pyrrolizidine (*Senecio*) alkaloids in sheep. *Journal of Animal Science* 56:887-894.

Thoden, T.C., and M. Boppré. 2010. Plants producing pyrrolizidine alkaloids: Sustainable tools for nematode management? *Nematology* 12:1-24.

Williams, M.C., and R.J. Molyneux. 1987. Occurrence, concentration, and toxicity of pyrrolizidine alkaloids in *Crotalaria* seeds. *Weed Science* 35:479-481.

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Short Communication

Lithium Carbonate as a Potential Aversive Agent

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Lithium chloride, an emetic widely used in animal behavioral studies and in clinical applications in humans (Ralphs and Provenza 1999), has been rated as the most effective agent to induce aversion in cattle, sheep, and horses (Ralphs et al. 2001). Lithium chloride causes nausea without dangerous side effects (Provenza et al. 1994). Owing to its caustic nature and the relatively large amount required to induce aversion in livestock, it must be administered into the rumen either in solution by stomach tube or in gelatin capsules or boluses, allowing dilution in the digestive fluid (Ralphs et al. 2001). The boluses, however, may break during insertion into the esophagus, resulting in acute tracheitis when coughed up and aspirated, leaving dosing by stomach tube as the safer option (Ralphs et al. 2001). Administering lithium chloride orally via a stomach tube, however, is a time-consuming, labor-intensive process that must be performed by a skilled person. This renders it impractical to apply when large numbers of cattle have to be treated.

Lithium carbonate (Merck, Pty Ltd), a lithium salt with low solubility in water and rather tasteless, was easily drenched orally without harmful effects or unwillingness by the animal to swallow. The efficacy of lithium carbonate as aversive agent was demonstrated by the following observations:

(1) Four naïve steers were placed individually in pens to induce aversion to high-quality feed pellets by oral drenching with 200 mg lithium carbonate/kg body weight (BW). All the steers refused to eat the feed pellets that were presented for an hour a day for 3 consecutive days following aversion treatment. Aversion to the feed pellets seemed to be induced quite rapidly, as all the steers refused to eat the feed pellets when offered 30 minutes after aversion treatment. When retested in the same pens after spending 3 months on pasture, they continued to refuse to eat the feed pellets (L.D. Snyman and R.A. Schultz, 2006, unpublished data).

(2) Six naïve steers, individually penned and subjected to aversion treatment to high-quality feed pellets by oral drenching with 75, 100, 125, 150, 175, and 200 mg lithium carbonate/kg BW, refused 0%, 87%, 66%, 98%, 97%, and 100%, respectively, of 500 g feed pellets offered for a period of 3 days, commencing the day after aversion treatment (Snyman et al. 2011).

(3) Inducing aversion in cattle to yellow tulp (*Moraea pallida*) on yellow tulp-infested grass pasture by administering lithium carbonate (80 mg/kg BW, drenched per os) in combination with epoxyscillirosidin (0.005 mg/kg BW, administered intramuscularly) resulted in effective aversion of all six animals, compared to three of six untreated control animals that were poisoned. The trial was performed over 2 days (L.D. Snyman and R.A. Schultz, 2007, unpublished data).

(4) Lithium carbonate was also successfully used to induce aversion in 15 sheep to *Geigeria ornativa* (Snyman et al. 2011). This was achieved by oral drenching with

lithium carbonate (160 mg /kg BW), followed by continuous access to an aversive mixture that resulted in consumption of 20 mg lithium carbonate/kg BW/day. The treated sheep, unlike 7 of the untreated controls, remained unaffected while grazing on a *G. ornativa*-infested pasture for the duration of the 62-day trial.

The results suggest that lithium carbonate might be an effective agent for inducing aversion to poisonous plants in livestock. The more practical means of administration renders lithium carbonate an attractive option for inducing aversion in large numbers of stock in the field. Since lithium carbonate can be used as the unprocessed raw material as mined, it might also be a cheaper alternative to lithium chloride.

References

Provenza, F.D., L. Ortega-Reyes, C.B. Scott, et al. 1994. Antiemetic drugs attenuate food aversions in sheep. *Journal of Animal Science* 72:1989-1994.

Ralphs, M.H., and F.D. Provenza. 1999. Conditioned food aversions: principles and practices, with special reference to social facilitation. *Proceedings of the Nutrition Society* 58:813-820.

Ralphs, M.H., F.D. Provenza, J.A. Pfister, D. Graham, G.C. Duff, and G. Greathouse. 2001. Conditioned food aversion: from theory to practice. *Rangelands* 23:14-18.

Snyman, L.D., R.A. Schultz, A. Theunissen, and K. Mosia. 2011. Maintaining aversion to *Geigeria ornativa* (vermeerbos) in sheep by means of continuous exposure to an aversive mixture presented in a self-feeder. *In* F. Riet-Correa, J. Pfister, A.L. Schild, and T.L. Wierenga, eds., *Poisoning by Plants, Mycotoxins, and Related Toxins*, pp. 631-636. CAB International, Wallingford, Oxfordshire, U.K.

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Western Juniper-Induced Abortions in Beef Cattle

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Abstract

It has been known for many years that ponderosa pine needles can induce late-term abortions in cattle. Labdane acids including isocupressic acid (ICA) and agathic acid are responsible for initiating abortions. Recent research demonstrated that a number of trees including many species of pine, juniper, and cedar contain either isocupressic acid or agathic acid at concentrations sufficient to be a risk for causing abortions in late-term cattle. The objective of this study was to determine if the bark from western juniper (*Juniperus occidentalis*) will induce late-term abortions in cattle. Pregnant cows were dosed starting on day 250 of gestation with 2.3 kg of ground plant material twice daily for 10 days or until abortions occurred. Western juniper bark used in this study contained approximately 0.7% labdane acids, on a dry weight basis, including isocupressic acid (0.025%), agathic acid (0.43%), imbricatoloic acid (0.15%), and dihydroagathic acid (0.05%). Two cows aborted 4-5 days after the start of the treatment. Both cows had retained placental membranes. The remaining 4 cows calved at full term (26-31 days after the start of treatment) and had no retained placental membranes. Results from this study indicated that western juniper trees contain compounds known to be abortifacient in cattle and that consumption of large amounts of bark in the third trimester of gestation may induce abortions. Although the risk of abortion from eating western juniper bark appears to be less than that from eating ponderosa pine needles, livestock producers should be aware of this potential.

Keywords: agathic acid, imbricatoloic acid, cattle, abortion, western juniper

Abbreviations

AA	Agathic Acid
DHAA	Dihydroagathic Acid
IMB	Imbricatoloic Acid
ICA	Isocupressic Acid
Rt	Retention Time
THAA	Tetrahydroagathic Acid

Introduction

Since the early 1900s, ponderosa pine (*Pinus ponderosa*) needles have a long history of causing abortions in cattle (MacDonald 1952, Stevenson et al. 1972, James et al. 1977, Panter et al. 1990). Pine needle related-abortions occur most frequently in the last trimester of gestation. Affected cattle often have incomplete cervical dilation and weak uterine contractions resulting in difficult calving (dystocia), followed by retained fetal membranes. Retained fetal membranes can result in endometritis and pyometria. The combination of early parturition, dystocia, and retained placental membranes forms the defining characteristics of pine needle-caused abortion (James et al. 1994). Calves are viable if the abortion is late in gestation; however, they are weak, often require assistance to suckle, and are prone to increased respiratory problems and disease.

Several years ago, isocupressic acid (ICA; figure 1), a labdane resin acid in the pine needles and bark of ponderosa pine (*Pinus ponderosa*), was identified as the major abortifacient agent in ponderosa pine needles (Gardner et al. 1994). Isocupressic acid was found to be metabolized in both the rumen and the liver through oxidative and reductive processes (Gardner et al. 1999). The major metabolites identified thus far include imbricatoloic acid (IMB), agathic acid (AA), dihydroagathic acid (DHAA), and tetrahydroagathic acid (THAA) (Lin et al. 1998, Gardner et al. 1999). Additional research has demonstrated that several other species of trees are also abortifacient (Panter et al. 2007). Current management recommendations indicate that any tree with a concentration greater than 0.5% ICA (on a dry weight basis) poses a risk for inducing abortions in late-term pregnant cattle. It recently was reported that the bark of Utah juniper (*Juniperus osteosperma*), which contains a high concentration of agathic acid (1.5% by dry weight) but no ICA, will induce abortions in cattle, suggesting that agathic acid is also abortifacient in cattle (Gardner et al. 2010). However, it remains uncertain as to whether the other metabolites, IMB, DHAA, or THAA, are biologically active as abortifacient compounds in late-term pregnant cattle.

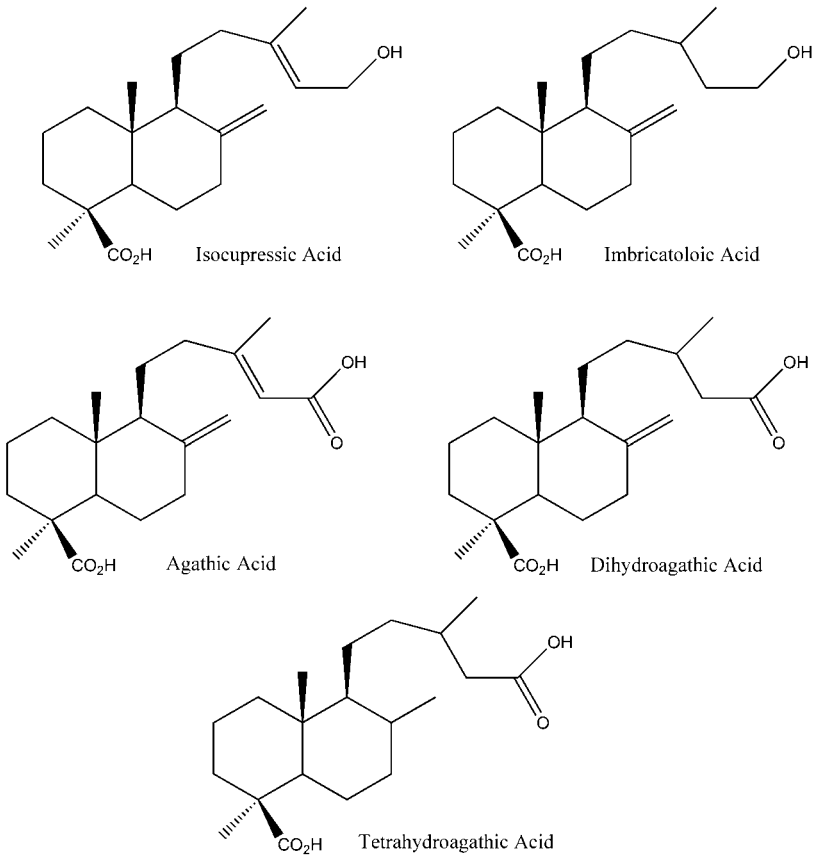

Figure 1. Chemical structures of several labdane acids.

During the late 1990s and early 2000s, many ranchers in Baker County, OR, were using western juniper trees in riparian restoration projects. These large, bushy trees were utilized as a source of large woody debris and placed in stream banks and beds to dissipate stream flow energy and to capture and store sediment within the stream channel. Shortly after the beginning of the riparian restoration project, several local ranchers reported atypical late-term abortions. There have been reports by several ranchers of 10 to 15 percent of their herds aborting after being pastured in these areas (personal communications). Ponderosa pine and other trees known to contain ICA were not found in the areas in which these abortions occurred. However, there was clear visual evidence that the cattle had been eating the bark of the downed western juniper trees. Therefore, samples of western juniper needles, berries, and bark were analyzed for labdane acid content. Both the needles and berries were found to contain low concentrations of labdane acids. However, preliminary analyses indicated that the bark of western juniper trees had a fairly high concentration of labdane acids (~ 1.0 %). Consequently, the objective of this study was to determine if the bark from western juniper trees could induce abortions in cattle.

Materials and Methods

Plant material—Bark from western juniper (*Juniperus occidentalis*) trees was collected in August 2009 from trees that had been cut down 1 and 2 years previously near Baker City, OR (N lat 44° 43. 281', W long 117° 53. 009'). The bark was allowed to dry at ambient temperature and then stored on a canvas tarp in an enclosed, non-heated, un-insulated building at ambient temperature until treatment. Prior to treatment, enough plant material for 2 days of treatments was ground to pass a 2mm screen and stored in a plastic bag in the dark at 4°C until used.

Animals—Six healthy, experimentally naïve, Angus heifer cows (mean ± SD weight, 599 ± 48 kg) were purchased from a herd with no history of abortion or reproductive disease. The cows were time bred and confirmed pregnant via palpation. The cows were fed alfalfa-grass hay and a dietary mineral supplement (standard diet provided to all cattle at the USDA-ARS Poisonous Plant Research Laboratory) and housed in outdoor paddocks. Pregnancies were monitored and verified via palpation prior to treatment. Ground plant material (2.3 kg) was administered twice each day (morning and afternoon) via stomach tube directly into the rumen starting on day 250 of gestation. This dose has been previously shown to be well tolerated and will consistently induce abortion with ponderosa pine needles containing 1.0% ICA or greater (James et al. 1994). Dosing continued daily until abortion or day 260 of gestation (maximum of 20 doses/animal). During treatment, the cattle were closely monitored for signs of parturition. In this study, if the placental membranes were not expelled within 24 hours after parturition, the animals were treated with uterine lavage and intrauterine antibiotic infusions.

Blood samples were collected via the jugular vein prior to treatment each morning. Blood samples were allowed to clot at room temperature for 30 min and then centrifuged at 1700 x g for 20 min to separate the serum from the cellular fractions. The serum was removed and stored in plastic vials at -20°C until analysis. All protocols for animals used in this research were performed under veterinary supervision and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, UT.

Analytical methods—The concentration of labdane acids in the plant material was measured by gas chromatography. The dry ground bark (0.100 g) was extracted in duplicate using the published procedure for isocupressic acid (Gardner and James 1999) to give a crude organic acid extract. The dry extract was dissolved in 2.0 mL of methylene chloride. A 0.200 mL aliquot was transferred to a GC autosample vial and the solvent evaporated under flow of nitrogen. Pyridine (0.200 mL) containing 0.200 g/mL of heptadecanoic acid and 0.050 mL of BSTFA silyation reagent (Pierce Biotechnology, Rockford, IL) was added and the sample capped and heated for 30 min at 60°C. Before analysis, 0.75 mL of methylene chloride was added. Calibration standards (isocupressic acid) were prepared at 25, 50, 75, and 100 µg and were treated as above for silyation and dissolution. Gas chromatography was performed as previously described (Gardner and James 1999).

For analysis of sera samples for labdane acids, 1.0 mL aliquot of sera was placed into an 8 mL screw cap vial and 1.0 mL of saturated KH_2PO_4 was added. The samples were then extracted twice with chloroform (2.0 mL). After each extraction, the samples were centrifuged to aid layer separation and the chloroform solution was withdrawn with a

Pasteur pipette and passed through a filter of anhydrous sodium sulfate into a second 8 mL vial. The combined chloroform extracts were evaporated to dryness under a flow of nitrogen in a heat block at 60°C. The samples were dissolved in 0.25 mL of pyridine (containing 40 ppm heptadecanoic acid as a reference standard) and 0.050 mL of BSTFA silyation reagent and heated at 60°C for 30 min. Samples were then analyzed for metabolites of isocupressic acid (agathic acid, dihydroagathic acid, and tetrahydroagathic acid) by gas chromatography/ mass spectrometry. Peak areas of the detected metabolites were measured from selected ion chromatograms, referenced against the C17 standard and then plotted versus day collected to measure relative concentration of metabolites in the sera samples.

Data analysis—Data are expressed as the mean ± SD. Statistical comparisons of serum labdane acid concentrations between cattle that aborted and those that calved at full term were made by use of a two-way ANOVA with a Bonferroni post-hoc test. Correlations between serum labdane acid concentrations and the number of days to parturition were determined using Pearson's correlation.

Results

Analysis of the western juniper bark by gas chromatography found the major labdane acid to be agathic acid (AA; figure 2). The total AA concentration was measured at 0.43% (dry weight basis). The bark also contained low concentrations of other labdane acids including ICA (0.025%), IMB (0.15%), and DHAA (0.05%), as well as abietane and pimarane acids as the major components in the extract. The western juniper bark used for this study contained approximately 0.7% total labdane acids, by dry weight. This concentration of labdane acids was greater than the threshold concentration (0.5%) thought to be required to produce abortions in cattle (Gardner and James 1999). However, to date the only labdane acids that have been shown experimentally to induce abortions are ICA and AA. The concentration of those two labdane acids in this plant material was approximately 0.46%, which is slightly below the estimated threshold concentration that poses a risk for abortion.

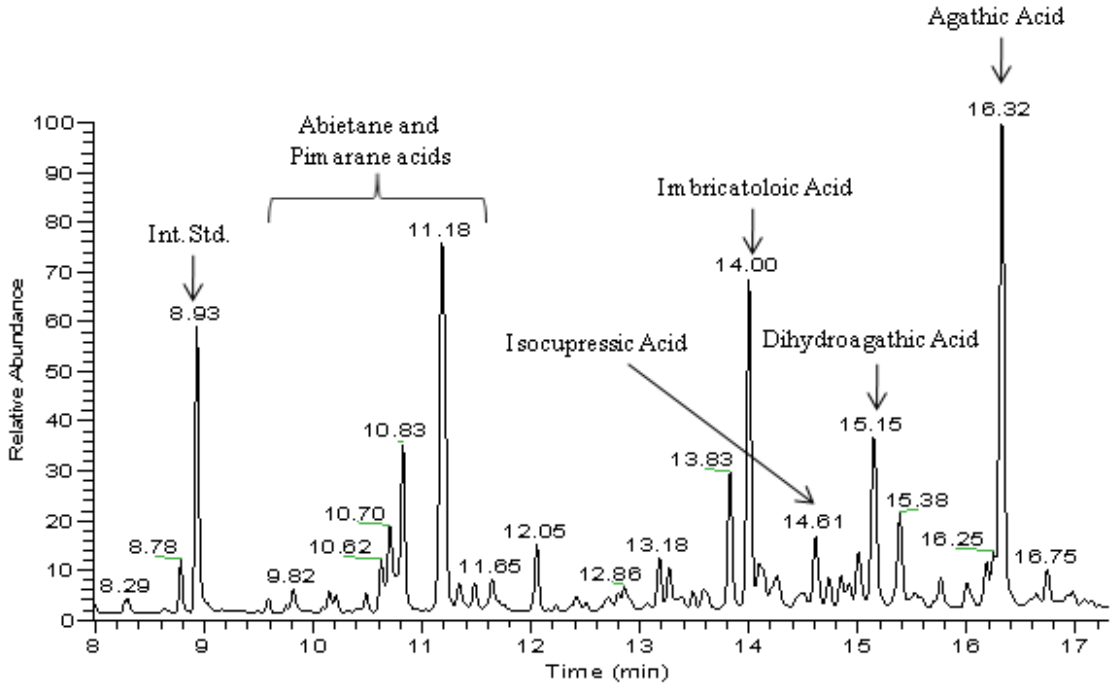

Figure 2. Chemical analysis of western juniper bark. Gas chromatography/mass spectrometry chromatograms and the major diterpene acids identified from the plant material. Specific peaks include Imbricatoloic Acid (Rt, 14.00 min), Isocupressic Acid (Rt, 14.61 min), Dihydroagathic Acid (Rt, 15.16 min), and Agathic Acid (Rt, 16.32 min).

Table 1. The amount of plant material dosed daily, the corresponding daily dose of labdane acids, and the number of days to parturition after start of treatment

Animal	Weight (kg)	Daily Plant Dose (g/kg)	Daily Labdane Acid Dose (mg/kg)	Daily Agathic Acid Dose (mg/kg)	Daily Imbricatoloic Acid Dose (mg/kg)	Days to Parturition
1	623	7.4	48.4	31.7	11.1	26
2	532	8.6	56.6	37.2	13.0	5
3	632	7.3	47.7	31.3	10.9	4
4	545	8.4	55.3	36.3	12.7	26
5	640	7.2	47.1	30.9	10.8	31
6	623	7.4	48.4	31.7	11.1	26

Two of the 6 cows aborted after 4 to 5 days of treatment (table 1). Both abortions were typical of pine needle-induced abortions, including parturition 4 to 5 days after treatment started as well as typical clinical signs including dystocia and retained placental membranes (James et al. 1994). The remaining 4 cows calved normally 26 to 31 days after the start of treatments on gestation days 276 to 281. There were no complications observed during parturition and no retained placental membranes in these four cows.

In this study, there was not a dose-response relationship observed between the amount of labdane acids dosed and the number of days to parturition (table 1). One of the cows that aborted received the highest daily dose of labdane acids (56.6 mg/kg). However, the other cow that aborted received one of the lowest daily doses of labdane acids (47.7 mg/kg). The cows that did not abort received a daily dose of labdane acids that was in between the range to those that did abort (47.1 – 55.3 mg/kg). Additionally, there was no correlation between the dose of labdane acids received and the number of days to parturition (P=0.54).

Sera samples were analyzed to determine if the variation in results observed in this study was due to the amount of bioavailable labdane acids in the cattle. Serum concentrations of ICA and IMB were essentially zero at all time points (figure 3A). Agathic acid, DHAA, and THAA were detected in blood serum samples of cows dosed with western juniper bark (figure 3). However, the animals that aborted did not have a higher concentration of any of the labdane acids in their sera; in fact, there was a trend for the two cows that aborted to have a lower sera concentration of agathic acid (figure 3A).

Discussion

It has been known for many years that pine needle-induced abortion is a problem, estimated at $4.5 million annually (Lacey et al. 1988), for ranchers that are forced to pasture their cattle in the last trimester of pregnancy in rangelands that contain ponderosa pine trees (Bruce 1927). Additionally, it has been demonstrated that labdane acids including ICA and AA are the compounds responsible for initiating the abortions (Gardner et al. 1994, Gardner et al. 2010). Recent research efforts have demonstrated that a large number of trees including many species of pine, juniper and cedar trees, contain either ICA or AA at concentrations sufficient to be a risk for causing abortions in late-term cattle (Panter et al. 2007). The results from the study presented here demonstrate that the bark from western juniper trees can also induce abortions in cattle.

Although western juniper bark is not likely a normal part of cattle's diet, large winter snow storms often force cattle to graze on non-typical forages, which could include slash piles of downed western junipers. The State of Oregon is currently clearing large areas of western juniper trees in an attempt to recover critical grasses in mountain ranges used by wildlife and livestock for grazing. As a result of these efforts, large piles of downed western juniper trees are available to cattle in many Oregon rangelands.

The results observed in this study were varied, in that only two of the six cattle aborted. Additionally, the variation cannot be explained by differences in the daily dose of labdane acids administered to the cattle. The variation may stem from the fact that the concentrations of ICA and AA in this plant material were too low to consistently cause abortions. Previous research in our laboratory has demonstrated that an ICA concentration of 0.5% or greater is required to induce abortions in cattle (Gardner and James 1999), with the incidence increasing with higher concentrations of ICA, with concentrations greater than 1.0% being significant risk (personal observations). The bark used in this study contained 0.7% labdane acids; however, IMB and DHAA have

not yet been shown to be abortifacient. Consequently, the concentration of known abortifacient labdane acids in this plant material was 0.46 percent, which is below the putative threshold.

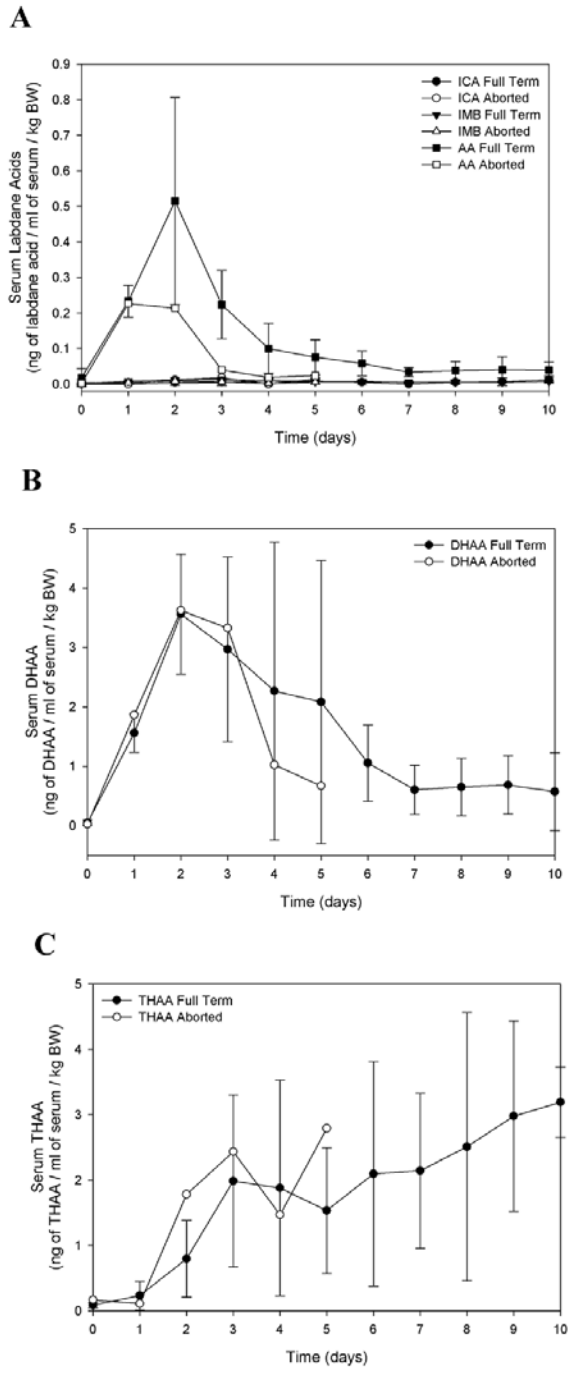

Figure 3. Comparison of labdane acid concentrations in sera of cows that aborted versus cows that calved at full term. A) Isocupressic (ICA), Imbricatoloic (IMB), and Agathic (AA) acids, B) Dihydroagathic Acid (DHAA), and C) Tetrahydroagathic Acid (THAA). Results represent the mean ± SD (n=4) for the full term group and the mean (n=2) for the aborted group.

Therefore, it is possible that the variation in response was due to the lack of abortifacient compounds in the bark and consequently only the two most susceptible animals aborted. These results are in accordance with most field observations wherein 10 to 20 percent of a herd will abort when they are exposed to pine needles during a large winter storm (personal communications), suggesting that normally only the most susceptible animals have a problem.

Future research efforts will include a study to sample bark from western juniper trees across Oregon and California to determine if there are areas that contain higher concentrations of ICA and/or AA. Areas where western juniper bark contains ICA and/or AA at 0.5 percent and greater would be considered a risk for causing abortions in cattle. Further, if samples of bark are found that contain ICA and/or AA at much higher concentrations, additional experiments could be performed to determine if abortions would occur at a higher incidence with western juniper bark that contains a higher ICA and/or AA concentration. There also remains a potential to find a sample of western juniper bark that would contain a high concentration of IMB and very little of the other labdane acids. This bark would be of high interest for determining if IMB is also abortifacient.

An additional interesting observation from this study was that after 3 to 4 days of continuous dosing, the serum AA concentrations dropped below the level of detection. Similar results have also been observed in previous studies (Gardner et al. 2010). Typically, when a compound is administered multiple times, it will lead to accumulation of the compound in the body (Shargel and Yu 1993, Shen 2008). For example, if a compound follows first-order elimination kinetics, the elimination rate increases as the body burden increases. Thus, with a fixed, continuous exposure, accumulation of a compound in the body reaches a point when the rate of intake equals the rate of elimination for that compound. From that point forward, the body burden remains constant, which is commonly referred to as a steady state (Shen 2008). However, with regard to the labdane acids ICA, AA, and IMB in cattle, a steady state does not appear to occur. One possible explanation for this observation is that multiple doses of these compounds could result in an induction of the enzymes/pathways that are involved in the elimination of these compounds from the body (Meyer and Gut 2002, Parkinson and Ogilvie 2008). Additionally, multiple dosing of these compounds orally may induce a change in the rumen microflora

such that these compounds are subsequently metabolized in the rumen to an extent that they are not absorbed by the cattle (James et al. 1967, Van Kampen and James 1969, James and Cronin 1974).

Additional experiments are needed to study this phenomenon in more detail. If it is determined that a multiple-dosing regimen of these compounds does enhance their elimination from the body, or inhibit their absorption of the GI tract, changes to management recommendations could be made. These changes would reflect the possibility that naïve cattle may be more susceptible to pine needle-induced abortions and that the grazing of naïve cattle in pastures or rangelands abundant in ponderosa pine trees (or other ICA- and related labdane acid-containing trees) should be even more limited during the latter part of pregnancy.

In conclusion, the bark from western juniper trees contains labdane acids that have been associated with abortion in cattle. Consequently, livestock producers should be aware of the potential for western juniper trees to induce abortions in late-term pregnant cattle, especially if grazing conditions deteriorate so that cattle are compelled to eat juniper.

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References

Bruce, E.A. 1927. *Astragalus serotinus* and other stock-poisoning plants of British Columbia. *In* Dominion of Canada. U.S. Department of Agriculture Bulletin No. 88, page 44.

Gardner, D.R., and James L.F. 1999. Pine needle abortion in cattle: analysis of isocupressic acid in North American gymnosperms. *Phytochemical Analysis* 10:132-136.

Gardner, D.R., R.J. Molyneux, L.F. James, et al. 1994. Ponderosa pine needle-induced abortion in beef cattle: identification of isocupressic acid as the principal active compound. *Journal of Agricultural and Food Chemistry* 42:756-761.

Gardner, D.R., K.E. Panter, and L.F. James. 1999. Pine needle abortion in cattle: metabolism of isocupressic acid. *Journal of Agricultural and Food Chemistry* 47:2891-2897.

Gardner, D.R., K.E. Panter, and B.L. Stegelmeier. 2010. Implication of agathic acid from Utah juniper bark as an abortifacient compound in cattle. *Journal of Applied Toxicology* 30:115-119.

James, L.F., J.W. Call, and A.H. Stevenson. 1977. Experimentally induced pine needle abortion in range cattle. *Cornell Veterinarian* 67:294-299.

James, L.F., and E.H. Cronin. 1974. Management practices to minimize death losses of sheep grazing halogeton-infested range. *Journal of Range Management* 27:424-426.

James, L.F., R.J. Molyneux, K.E. Panter, et al. 1994. Effect of feeding ponderosa pine needle extracts and their residues to pregnant cattle. *Cornell Veterinarian* 84:33-39.

James, L.F., J.C. Street, and J.E. Butcher. 1967. *In vitro* degradation of oxalate and of cellulose by rumen ingesta from sheep fed *Halogeton glomeratus*. *Journal of Animal Science* 26:1438-1444.

Lacey, J.R., L.F. James, and R.E. Short. 1988. Ponderosa pine: economic impact. *In* L.F. James, M.H. Ralphs, and D.B. Nielsen, eds, The Ecology and Economic Impact of Poisonous Plants on Livestock Production, pp. 95-106, Westview Press, Boulder, CO.

Lin, S.J., R.E. Short, S.P. Ford, et al. 1998. *In vitro* biotransformations of isocupressic acid by cow rumen preparations: formation of agathic and dihydroagathic acids. *Journal of Natural Products* 61:51-56.

MacDonald, M.A. 1952. Pine needle abortion in range beef cattle. *Journal of Range Management* 5:150-155.

Meyer, U.A., and J. Gut. 2002. Genomics and the prediction of xenobiotic toxicity. *Toxicology* 181-182:463-466.

Panter, K.E., D.R. Gardner, S.T. Lee, et al. 2007. Important poisonous plants of the United States. *In* R.C. Gupta, ed, Veterinary Toxicology: Basic and Clinical Principles, pp. 825-872, Academic Press, New York, NY.

Panter, K.E., L.F. James, R.J. Molyneux, et al. 1990. Premature bovine parturition induced by ponderosa pine: effects of pine needles, bark and branch tips. *Cornell Veterinarian* 80:329-338.

Parkinson, A., and B.W. Ogilvie. 2008. Biotransformation of Xenobiotics. *In* C.D. Klaassen, ed, Toxicology: The Basic Science of Poisons, pp. 161-304, McGraw-Hill, New York, NY.

Shargel, L., and A.B.C. Yu. 1993. Applied Biopharmaceutics and Pharmacokinetics. 3rd ed. Appleton & Lange, Norwalk, CT.

Shen, D.D. 2008. Toxicokinetics. *In* C.D. Klaassen, ed, Casarett & Doull's Toxicology: The Basic Science of Poisons, pp. 305-325, McGraw-Hill Medical, New York, NY.

Stevenson, A.H., L.F. James, and J.W. Call. 1972. Pine-needle (*Pinus ponderosa*)-induced abortion in range cattle. *Cornell Veterinarian* 62:519-524.

Van Kampen, K.R., and L.F. James. 1969. Acute halogeton poisoning of sheep: pathogenesis of lesions. *American Journal of Veterinary Research* 30:1779-1783.

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