Case report: *Salvia reflexa*-contaminated hay poisoning in cattle

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Abstract

Salvia reflexa is an annual forb that has recently (2021) been reported to contaminate hay and cause fatal hepatotoxicity in cattle. It is possible that prior poisoning cases may have gone unrecognized due to the lack of information on fatal hepatotoxicity caused by *S. reflexa*. Two cases are reported. Case 1 occurred in December 2001 in Montana, in which 30 cows died out of 105. Case 2 occurred in 2023 in Wyoming, in which 111 cows out of 155 died. Poisoned cows in both cases had elevated aspartate aminotransferase and alkaline phosphatase activities with microscopic severe acute centrilobular necrosis with hemorrhage. These lesions were similar to those described previously in clinical and experimental *S. reflexa* trials. Hepatotoxic diterpenes of *S. reflexa*, salviarin and rhyacophiline, were detected in hay samples in both cases and in rumen contents from animals in Case 2. Plant specimens of *S. reflexa* were collected from 2 herbaria, representing collections from 12 states, and screened for salviarin and rhyacophiline. The *S. reflexa* toxins were found in herbarium samples suggesting if *S. reflexa* is present, it is potentially toxic to cattle.

Key words: *Salvia reflexa,* lanceleaf sage, sage mint, cattle, hepatotoxicity

Introduction

Salvia reflexa (Hornem.) is an annual forb in the *Lamiaceae* (mint) family. It is known by several common names such as lanceleaf sage, blue sage, lambsleaf sage, Rocky Mountain sage, mintweed, narrow-leaf sage, and sage mint. The plant grows up to 2 feet (0.6 meters) tall with square stems. The leaves are narrow and lance-shaped and are about 2 inches (51 millimeters) long. Blue flowers grow on spikes in whorls of 2 or 3. It often grows in dry fields, gravel-clay flats, and rocky soils where there is little competition. It has been reported to be a weed in cultivated crops[1] and has a large distribution across the United States.[2]

Salvia reflexa has been reported to cause heavy losses to livestock in Australia due to acute nitrate intoxication.[3,4] However, past reports of livestock losses in the U.S. list the toxic agent as unknown.[5] One confirmed case describes animals consuming alfalfa hay contaminated with about 10% *S. reflexa,* and the poisoned animals presented post-mortem evidence of poorly described gastrointestinal inflammation.[5] In an additional poisoning in Wyoming, alfalfa hay containing a high percentage of *Salvia* had been fed to 100 head of cattle in which 7 died. Poisoned cattle were affected by symptoms of muscular weakness with similarly uncharacterized inflammation of the gastrointestinal tract.[6] The cause of toxicity and the toxic principle was not identified.

In a recently reported livestock poisoning case, approximately 500 mixed-breed beef cattle were fed a weedy alfalfa hay.[7] About half of the cattle developed symptoms of lethargy, depression, recumbency with nearly 100 animals dying within 48 hrs. Several days later, a number of the poisoned animals developed hepatic encephalopathy with aberrant behavior, aggression, icterus, blindness and exhaustion resulting in death. In the end, a total of 165 cattle were fatally poisoned. The poisoned cattle developed liver disease characterized by altered serum biochemical analysis and microscopic hepatocellular degeneration and necrosis. Poisoned cattle that recovered presented lesions of hepatocellular degeneration and necrosis that resolved over several months with minimal remaining hepatic fibrosis and biochemical indications of altered hepatic functions. Gross and microscopic lesions of fatally poisoned animals were characterized as swollen, dark, mottled livers with massive centrilobular-to-panlobular hepatic necrosis, respectively.[7]

Panter et al.[7] were the first to definitively identify *S. reflexa* as causing fatal hepatotoxicity in livestock. However, there have been numerous epidemic-like outbreaks of hepatic necrosis that the cause has not been identified.[8-10] It is possible that *S. reflexa* might be responsible for other poisoning cases that have gone unrecognized. The objectives of this report are to document 2 additional cases of *S. reflexa* poisoning and better document the potential toxicity of *S. reflexa* in plant populations in the Western U.S. Also, an analysis of *S. reflexa* herbarium plant specimens was conducted to determine if hepatotoxic diterpenes occur in plants throughout its known distribution.

Case descriptions

Case 1

A case in which cattle death losses were suspected to be caused by *S. reflexa* poisoning occurred near Grass Range, Mont., in December 2001. Initially, 15 cattle died within 2 days with a total of 30 animals out of 105 dead after feed had been changed. Contaminated hay was suspected. Hay samples were examined by Montana State University Herbarium and reported to consist primarily of alfalfa (*Medicago sativa*) with small amounts of mint (*Salvia* spp.), wild oats (*Avena* spp.), blue mustard (*Chorispora tenella*) and a substantial amount of climbing nightshade (*Solanum dulcamara*). One dead cow, 1 calf, and 2 bulls were submitted to the State of Montana Diagnostic Laboratory for necropsy. Hay samples were sent to the Poisonous Plant Research Lab (Logan, UT) for chemical analysis.

Case 2

A separate case in which 111 cattle died out of a herd of 155 animals near Crowheart, Wyo., was reported in January 2023. Hay fed to animals at the time was reported to be the first cutting from a new seeding of alfalfa that was later found to be contaminated with *S. reflexa*. The first dead animal was found approximately 24 hrs. after the contaminated feed was introduced. Reports stated that animals started going down and were unable to get up. Animals were aggressive and then became convulsive and started to die. One cow calved, even though it was not due for another 30 days. Two animals were submitted to the Wyoming State Veterinary Laboratory for necropsy. Rumen contents and hay samples were sent to the Poisonous Plant Research Lab for chemical analysis.

Herbarium survey

Plant specimens of *S. reflexa* (n = 29; Table 1) were collected from the Intermountain Herbarium at Utah State University, Logan, UT, and the Stanley L. Welsh Herbarium at Brigham Young University, Provo, UT. States covered in the survey include Arizona, Colorado, Minnesota, Missouri, Nebraska, New Mexico, Nevada, Oregon, South Dakota, Texas, Utah and Wyoming. *Salvia reflexa* specimens were screened for the hepatotoxins salviarin and rhyacophiline using previously described techniques.[11]

Diagnostic findings

Case 1

Multiple sections of brain, lymph node, esophagus, heart, kidney, liver, spleen, lung and trachea were examined microscopically. Morphologic diagnosis included mild lymphoplasmacytic cellular tracheitis with congestion. Suppurative periportal hepatitis with biliary fibrosis and vacuolar degeneration with moderate to severe diffuse hyperplasia. Lymph node presented lymphoid hyperplasia with lympholysis. The most significant lesions occurred within the liver. The liver had moderate to severe acute centrilobular necrosis with hemorrhage. The report states that the lesion was suggestive of a toxic hepatopathy or circulatory disturbance.

A serum biochemistry, from a bull that was fatally poisoned in Case 1, had several increased enzymatic indicators of hepatic damage (Table 2). The aspartate aminotransferase (AST) activities (26X normal) were elevated with a mild elevation in alkaline phosphatase (ALKP) activities (4X normal). The gamma-glutamyl transpeptidase (GGT) and alanine aminotransferase (ALT) activities along with bilirubin concentrations were within normal ranges. The creatine phosphokinase (CPK) (133X normal) activities were increased indicating muscle damage. A second biochemistry from an affected, but non-fatally poisoned bull, had mild elevation of CPK (1.4X normal), AST (8X normal), GGT (2.2X normal), ALT (2.5X normal) and ALKP (11X normal) activities (Table 2). The bull developed bile stasis as the total bilirubin was 24X and direct bilirubin 10X above the normal concentrations.

Ocular fluid from a fatally poisoned cow was analyzed for nitrate and nitrite. Both concentrations were within normal ranges. Additionally, the liver from 2 poisoned bulls were evaluated for trace mineral concentrations (Se, Ba, Fe, P, Cr, Tl, Ca, Mg, Zn, Cd, Na, Cu, Mn, Sb, Hg, K, Co, Mo, As, Pb, B) and found to be within normal ranges.

Case 2

The first animal submitted was a three-year-old crossbred Angus cow of average body condition. Diagnostic findings of the liver were acute centrilobular and midzonal hepatocellular necrosis with marked congestion and hemorrhage (Figure 1A). Portal areas contain increased amounts of fibrous connective tissue and mild bile duct hyperplasia along with some loose aggregates of lymphocytes and plasma cells The pathological changes were consistent with an acute toxic hepatopathy with subsequent acute liver failure. The second animal submitted was a four-year-old Angus cow with above average body condition. The prominent diagnostic findings were acute centrilobular and midzonal hepatocellular necrosis with periportal vacuolar hepatocellular degeneration (Figure 1B). Remaining periportal hepatocytes had minimal changes of swelling and cytoplasmic vacuolation suggestive of degeneration. Some animals had small regenerative nodules with hepatocellular hyperplasia and proliferation with frequent mitoses. Infrequent abnormal mitotic figures were also observed. Portal areas contained increased amounts of fibrous connective tissue and mild bile duct hyperplasia along with some loose aggregates of lymphocytes and plasma cells. The hepatocellular changes were consistent with an acute toxic hepatopathy similar to the first animal. Serum biochemistries were not available on either animal.

Results and discussion

In Case 1, a total of 30 cattle died from of a herd of 105 animals. Fifteen animals died within a 2-day period after changing feed. Toxins of *S. reflexa*, salviarin and rhyacophiline, were detected in hay samples (Figure 2).

In Case 2, animals were found dead within 24 hours of changing feed. Hay was the first cutting from a new seeding of alfalfa. Though only fragments of contaminating plants were available for evaluation, they were closely examined by the plant taxonomist at the Utah State University Intermountain Herbarium and determined to be *S. reflexa*. Insufficient intact plant material was available to make an herbarium voucher. The contaminated hay sample was chemically analyzed and *S. reflexa* diterpenes, salviarin and rhyacophiline, were detected in the hay fed to the cattle. Salviarin and ryacophiline were also detected in the rumen contents available from 1 cow. Rhyacophiline was detected at low concentrations but salviarin was not detected in the rumen contents of a separate cow.

Cattle that consume a large initial dose of *S. reflexa* are fatally poisoned within 12-72 hr. of exposure.[7] Cattle that consume smaller doses may not be fatally poisoned, but they do develop disease with enough malaise that it has been proposed they develop an aversion to eating the plant.[7] This clinical disease includes depression, inappetence, colic, aggression and biochemical and microscopic changes indicative of liver disease. Aggression in cattle has been previously reported in cases with liver hepatotoxicity.[7,12-14] Severely affected animals require several months before biochemical and microscopic lesions resolve.[7] The inconsistent nature of hay contamination and difficulty in identifying non-fatal poisoning suggests that past poisonings may have been unrecognized.

Diagnostic laboratory reports from Case 1 list *S. dulcamara* as suspect in the cause of death of the cattle. *Solanum* species contain glycoalkaloids which cause gastrointestinal irritation, salivation, nasal discharge, drowsiness, progressive

weakness, paralysis, trembling and liver necrosis. However, the findings from this case are more similar to *S. reflexa* toxicosis than poisoning from *S. dulcamara*.

The hay fed in the case reported by Panter et al.[7] was contaminated with numerous weeds including annual kochia (*Bassia scoparia* synonym = *Kochia scoparia*) Russian thistle (*Salsola tragus*), sunflower (*Helianthus* spp.), red-root pigweed (*Amaranthus retroflexus*), lamb's quarter (*Chenopodium* spp.), halogeton (*Halogeton glomeratus*), barnyard grass (*Echinochloa* spp.), cocklebur (*Xanthium strumarium*) field pennycress (*Thlaspi arvense*), foxtail (*Setaria* spp.), corn stalks (*Zea mays*), and *S. reflexa*.

Carboxyatractyloside, the toxin in cocklebur, has been reported to cause similar massive hepatic necrosis,[15,16] and it was initially suspected as the cause in the Panter et al.[7] case. However, analysis of hay and rumen samples from dead cows failed to detect carboxyatractyloside. Annual kochia has also been anecdotally linked with hepatic disease and as it was a heavy contaminate of the toxic hay, it was also suspected, but kochia from the hay was not toxic in feeding trials. With the lack of a definite suspect, a bioassay-guided chemical fractionation process, using a mouse model, was performed to identify the hepatotoxic compounds in the hay.[11] The hepatotoxic diterpenes were identified as salviarin, salvianduline D,

Table 1: Salviarin and rhyacophiline detected in Salvia *reflexa* plant specimens collected from 2 herbaria.

Herbarium[1,2]	I.D. Number	County	State
UTC	75762	Apache	Arizona
UTC	137949	Navajo	Arizona
UTC	276344	Yavapai	Arizona
BRY	645677	Yavapai	Arizona
UTC	11531	Conejos	Colorado
UTC	11533	Jefferson	Colorado
UTC	137026	Jefferson	Colorado
BRY	634611	Jefferson	Colorado
UTC	78648	Las Animas	Colorado
UTC	78951	Yellow Medicine	Minnesota
BRY	86101	Shannon	Missouri
BRY	12672	–	Nebraska
BRY	499487	Cibola	New Mexico
UTC	139789 (287823)	San Miguel	New Mexico
UTC	54906	San Miguel	New Mexico
UTC	272332	San Miguel	New Mexico
BRY	12671	Lincoln	Nevada
BRY	434545	Lincoln	Oregon
BRY	394883	Stanley	South Dakota
UTC	153268	Randall	Texas
BRY	315869	Randall	Texas
UTC	87611	Travis	Texas
UTC	43153	Beaver	Utah
UTC	276867	Cache	Utah
BRY	227465	Utah	Utah
UTC	163793	Carbon	Wyoming
UTC	276946	Fremont	Wyoming
UTC	159765	Laramie	Wyoming
BRY	496000	Platte	Wyoming

[1] UTC (Index Herbariorum acronym) = Intermountain Herbarium, Utah State University, Logan, UT.

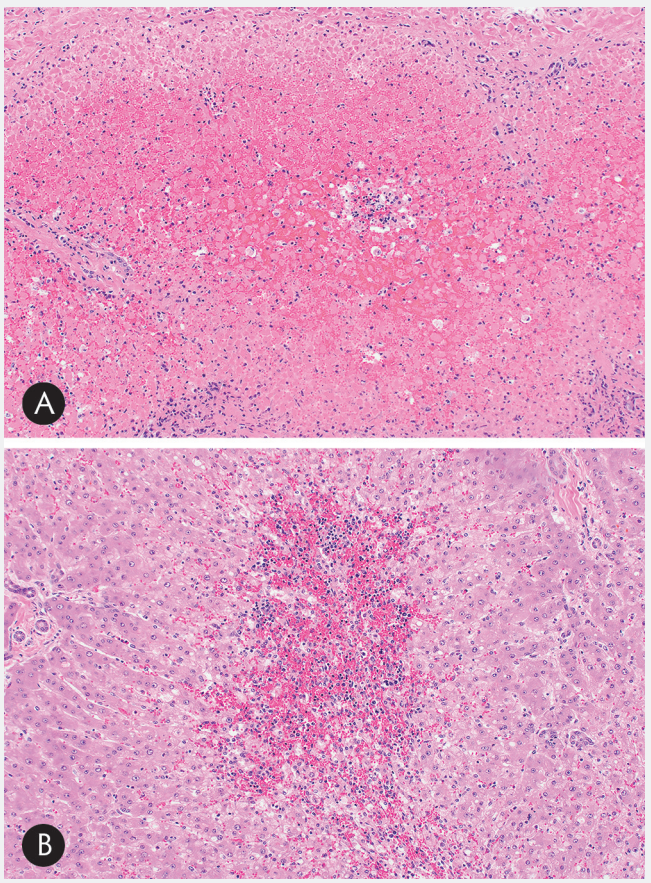
[2] BRY = Stanley L. Welsh Herbarium, Brigham Young University, Provo, UT.

Table 2: Select serum biochemistry results from a bull that was fatally poisoned and a second bull that was non-fatally poisoned in Case 1.

Test* (units)	Fatally poisoned bull	Non-fatally poisoned bull	Reference range
CPK (IU/L)	21,290	222	15-160
AST (IU/L)	3,986	1,189	60-150
GGT (IU/L)	39	108	15-48
ALT (IU/L)	74	173	10-70
ALKP (IU/L)	392	1,063	15-95
Total bilirubin (mg/dL)	0.3	9.7	0.1-0.4
Direct bilirubin (mg/dL)	0.3	4.0	0-0.4

* Creatine phosphokinase (CPK); aspartate aminotransferase (AST); gamma-glutamyl transpeptidase (GGT); alanine aminotransferase (ALT); alkaline phosphatase (ALKP).

Figure 1: Liver from 2 cows fatally poisoned with *Salvia reflexa*-contamintated hay (field Case 2). **A)** Liver, cow 1. Massive hepatocellular necrosis with hemorrhage. Only occasional hepatocytes surrounding portal tracts are spared. **B)** Liver, cow 2. There is centrilobular to midzonal hepatocellular necrosis. Moderate numbers of neutrophils and macrophages have infiltrated the affected area. Midzonal hepatocytes and periportal hepatocytes have vacuolar degeneration which is less severe closer to portal tracts. 100X magnification, Hematoxylin and Eosin.



rhyacophiline, and 7-hydroxyrhyacophiline, and with further examination, *S. reflexa* was identified in the hay responsible for the livestock poisoning.[7]

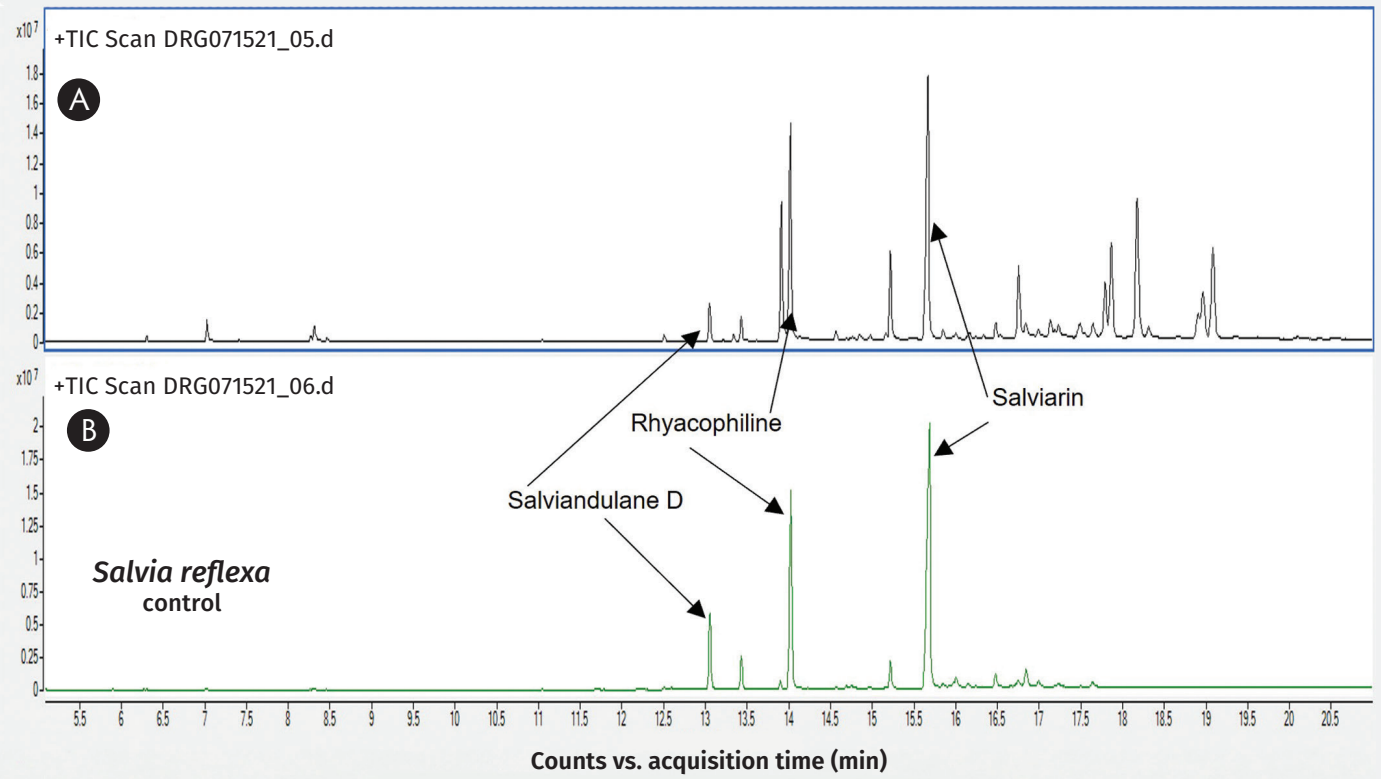
Annual kochia has been repeatedly suspected in similar poisoning cases involving a variety of livestock. Kochia has been reported to cause nitrate poisoning, hepatotoxicity, nephrotoxicity, polioencephalomalacia, and photosensitization.[17-19] Though kochia nitrate poisoning is well documented, the other disease syndromes are incidental, largely anecdotal, or combined with other toxins.[19,20] Hepatotoxicity from annual kochia has not been experimentally demonstrated and as it tends to grow in similar locations and conditions as *S. reflexa*, its association with hepatopathy might be responsible for kochia's association with liver disease. Kochia contaminated forages should be closely examined for other contaminates including *S. reflexa*.

Salvia reflexa has a large distribution across the U.S.,[2] but it is unknown if all *S. reflexa* plants contain the hepatotoxic diterpenes, thus an analysis of herbarium specimens was conducted. Salviarin and/or rhyacophiline were detected in all *S. reflexa* plant specimens surveyed from the Intermountain Herbarium at Utah State University and the Stanley L. Welsh Herbarium at Brigham Young University (Table 1). Plant specimens represented locations from 12 different states. Salviarin and rhyacophiline were detected in plant specimens throughout western and even midwestern states indicating that if *S. reflexa* is present, it has the potential to be toxic to cattle.

Conclusion

Salvia reflexa was reported to be present within the meadow hay harvested in Case 1. Salviarin and rhyacophiline were detected in the hay ,and thus *S. reflexa* is the most likely cause of poisoning. In Case 2, the hepatotoxic *S. reflexa* diterpenes were detected in rumen contents of both dead cows and in the hay fed to cattle. This also suggests *S. reflexa* is the most likely cause of poisoning. *Salvia reflexa* toxicity is largely dependent on an initial dose. Animals that are initially exposed to non-lethal doses of *S. reflexa* can become tolerant or averted to the plant.[7] The herbarium survey indicates that all *S. reflexa* populations are toxic. This suggests caution should be taken when *S. reflexa* is detected within or near hay crops. As weeds often grow in newly planted alfalfa, the first cutting should be closely examined for *S. reflexa* contamination.

Figure 2: Gas chromatography – mass spectrometry chromatogram of the hepatotoxic compounds, salviarin, rhyacophiline and salviandulane D, detected in hay sample **(A)** collected from livestock poisoning Case 1 and a *Salvia reflexa* control sample **(B)**.



Acknowledgment

The authors declare no conflicts of interest. The study was funded by the United States Department of Agriculture-Agricultural Research Service.

ARS is an equal-opportunity provider and employer.

Author contributions

Clinton A. Stonecipher contributed to conceptualization, investigation, interpretation of data, writing (original draft, review and editing), approval of final version of manuscript; Dale R. Gardner contributed to methodology, resources, writing (review and editing); Brett T. Webb contributed to interpretation of data, writing (review and editing); Will Laegreid contributed to interpretation of data, writing (review and editing); Kevin D. Welch contributed to conceptualization, interpretation of data, writing (review and editing); Bryan L. Stegelmeier contributed to conceptualization, interpretation of data, writing (original draft, review and editing); Daniel Cook contributed to conceptualization, investigation, interpretation of data, writing (original draft, review and editing) approval of final version of manuscript.

References

1. Odero DC, Mesbah AO, Miller SD, Kniss AR. Lanceleaf sage (*Salvia reflexa*) interference in sugarbeet. *Weed Technol* 2010;24(4):557-561. **https://doi:10.1614/WT-D-10-00015.1**

2. USDA, NRCS. 2023. The PLANTS Database (http://plants.usda.gov, 04/24/2023). National Plant Data Team, Greensboro, NC USA.

3. Williams CH, Hines HJG. The toxic properties of *Salvia reflexa*. *Aust Vet J* 1940;16(1):14-20. **https://doi: 10.1111/j.1751-0813.1940.tb01259.x**

4. Everist SL, *Poisonous Plants of Australia, 2nd ed*. Angus and Robertson, London, 1981.

5. Hart CR, Garland T, Barr AC, Carpenter BB, Reagor JC. Toxic plants of Texas: Integrated management strategies to prevent livestock losses. Texas Cooperative Extension B-6105. Texas A&M University Press, 2004:256 pp.

6. Beath OA, Gilbert CS, Eppson HF, Rosenfeld I. Poisonous Plants and Livestock Poisoning. *Wyo Agric Exp Stn Bull* 324, 1953.

7. Panter KE, Stegelmeier BL, Gardner DR, et al. Clinical, pathologic, and toxicologic characterization of *Salvia reflexa* (lance-leaf sage) poisoning in cattle fed contaminated hay. *J Vet Diagn Invest* 2021;33(3):538-547. **https://doi: 10.1177/1040638721995784**

8. Casteel SW, Rottinghaus GE, Johnson GC, Wicklow DT. Liver disease in cattle induced by consumption of moldy hay. *Vet Hum Toxicol* 1995;37:248-251.

9. Golder HM, Moss N, Rogers G, et al. Acute photosensitization and mortality in a herd of dairy cattle in Tasmania. *N Z Vet J* 2017;65(1):39-45. **https://doi: 10.1080/00480169.2016.1232181**

10. Read E, Edwards J, Deseo M, Rawlin G, Rochfort S. Current understanding of acute bovine liver disease in Australia. *Toxins* 2017 9(1):8. **https://doi:10.3390/toxins9010008**

11. Gardner DR, Panter KE, Stegelmeier BL, Stonecipher CA. Hepatotoxicity in cattle associated with *Salvia reflexa* diterpenes, including 7-Hydroxyrhyacophiline, a new seco-clerodane diterpene. *J Agric Food Chem* 2021;69(4):1251-1258. **https://doi: 10.1021/acs.jafc.0c06390**

12. Manthorpe EM, Jerrett IV, Rawlin GT, Woolford L. Clinical and pathologic features of acute bovine liver disease in *Aust J Vet Diagn Invest* 2021;33(5):875-883. **https://doi:10.1177/10406387211025829**

13. Manthorpe EM, Jerrett IV, Rawlin GT, Woolford L. Plant and fungal hepatotoxicities of cattle in Australia, with a focus on minimally understood toxins. *Toxins* 2020;12(11):707. **https://doi: 10.3390/toxins12110707**

14. Santos Alberti T dos, Zamboni R, Scheid HV, et al. Spontaneous poisoning by sprouts of *Xanthium* spp. in cattle in southern Rio Grande do Sul. *Acta Sci Vet* 2020;49 (Supple 1):507. **https://doi:10.22456/1679-9216.99161**

15. Cole RJ, Stuart BP, Lansden JA, Cox RH. Isolation and redefinition of the toxic agent from cocklebur (*Xanthium strumarium*). *J Agric Food Chem* 1980;28(6):1330-1332. **https://doi:10.1021/jf60232a043**

16. Wang Y, Han T, Xue LM, et al. 2001. Hepatotoxicity of kaurene glycosides from *Xanthium strumarium* L. fruits in mice. *Pharmazie* 2001;66 (6):445-449. **https://doi:10.1691/ph.2011.0844**

17. Thilsted J, Hibbs C, Kiesling H, et al. Kochia (*Kochia scoparia*) toxicosis in cattle: results of four experimental grazing trials. *Vet Hum Toxicol* 1989;31(1):34-41.

18. Dickie CW, Berryman JR. Polioencephalomalacia and photosensitization associated with *Kochia scoparia* consumption in range cattle. *J Am Vet Med Assoc* 1979;175(5);463-465.

19. Dickie CW, James LF. 1983. *Kochia scoparia* poisoning in cattle. *J Am Vet Med Assoc* 1983;183(7):765-768.

20. Rankins DL Jr. Evaluation of *Kochia scoparia* (L.) Schrad toxicosis in sheep and cattle. New Mexico State University Dissertation Publishing; 1989.

