## *AMINO ACIDS & PROTEINS*

### **Definitions** [adapted from Lewis' Dictionary of Toxicology 1998]

**Amino acid**: Any of a class of organic acids with the general formula R-CH($NH_2$)-COOH ($\alpha$-amino acids) where R is a distinguishing group. They occur as optically active D- and L- isomers, the latter predominating in living organisms. Some 24 distinct amino acids occur in proteins. There are a number of non-protein amino acids.

**Protein**: Any of a large variety of complex nitrogenous macromolecules composed of polypeptide chains comprising amino acids connected by peptide linkages (-CO.NH -) formed by elimination of $H_2O$ between the $NH_2$ group and COOH group of successive amino acid residues.

## *Thiaminase*

### Core data

*Common sources:*

- *Marsilea drummondii* (nardoo)
- *Cheilanthes sieberi* (mulga or rock fern)
- *Pteridium esculentum* (bracken)

*Animals affected:* horses, sheep
*Mode of action:* induced thiamine deficiency
*Poisoning circumstances:*

- grazing nardoo- or mulga fern-dominant pasture
- hay contaminated by bracken (horses)

*Main effects:*

- horse: incoordination exacerbated by exercise
- sheep: polioencephalomalacia

*Diagnosis:* access, syndrome, blood thiamine (horse), pathology (sheep)
*Therapy:* parenteral thiamine
*Prevention:* deny access

See also sulphur-induced polioencephalomalacia
Syndrome names:

Horses: equine staggers, bracken staggers
[Domestic carnivores: Chastek's paralysis]
[Human thiamine deficiency: beriberi (dry & wet) & Wernicke-Korsakoff syndrome]

Chemistry:

Thiaminases are proteins of molecular weight around 100 000. Those from ferns have optimal activity at pH 8.0-9.0, are stable between pH 3 and pH12 at 4$^{o}$C for 24 hr, are denatured by 50% at temperatures of 60-65 $^{o}$C and are inhibited by certain metal ions [1 mM concentration: $Ag^{+}$ 100%; $Fe^{2+}$ and $Fe^{3+}$ 70%; $Cu^{2+}$ 50%] (McCleary & Chick 1977).

Sources:

**Plant** sources of thiaminase are certain species of **ferns and horsetails** (primitive relatives of ferns); Thiaminase was first recorded in *Pteridium aquilinum* and *Equisetum arvense* in UK by Evans *et al.* (1950)

In Australia, known plant sources are:

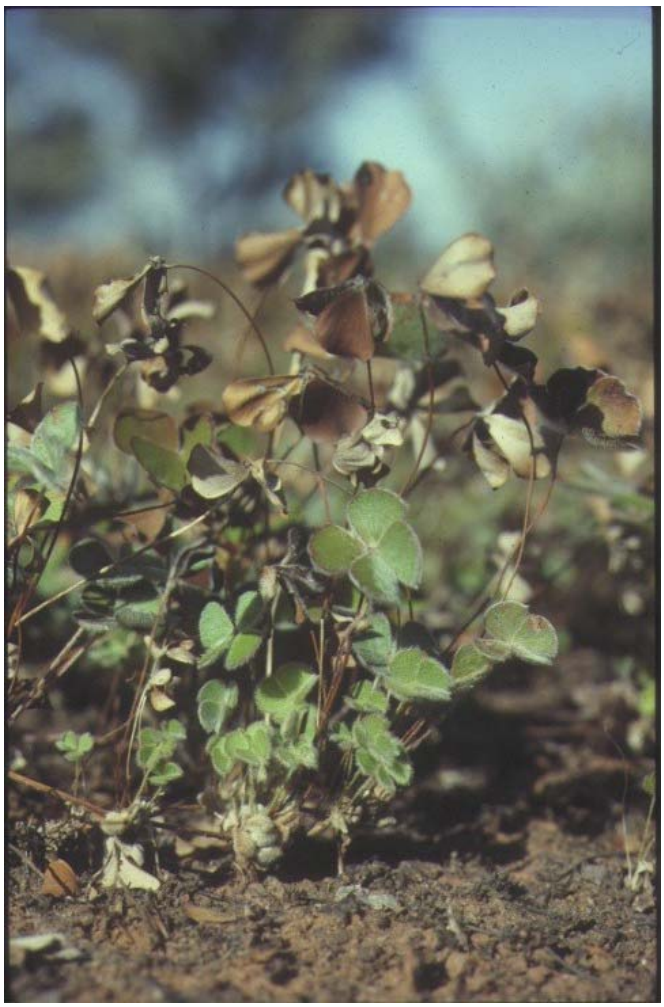
***Marsilea drummondii*** (common nardoo) – inland distribution on flood-plains and in and around semi-permanent and transient water bodies; thiaminase I detected/assayed by McCleary & Chick (1977)

*Marsilea angustifolia* (narrow-leaved nardoo); thiaminase I detected/assayed by McCleary & Chick (1977)

*Marsilea mutica* (smooth nardoo); thiaminase I detected/assayed by McCleary & Chick (1977)

- 8 *Marsilea* spp. are recognised in Australia, *ca.* 60 worldwide (Jones 1998)

*Marsilea drummondii* (common nardoo) [RAM Photo]



*Marsilea hirsuta* with sporocarps [RAM Photo]

***Cheilanthes sieberi*** (mulga fern or rock fern) – both coastal & inland distribution; thiaminase I detected/assayed by McCleary & Chick (1977)

***Pteridium esculentum*** (austral bracken) – coastal distribution; thiaminase I detected/assayed by McCleary & Chick (1977)

*Equisetum arvense* (common horsetail) – very limited distribution in central coastal NSW (Chinnock 1998)

- Europe: *Pteridium aquilinum, Equisetum arvense, Equisetum palustre* (Evans *et al.* 1951)

**Microbial** sources: Clinically-significant amounts of thiaminase are produced intermittently in the rumen by microbes (including *Clostridium sporogenes* [Shreeve & Edwin 1974], *Bacillus* sp. [Morgan & Lawson 1974]) and may account for some cases of sporadic polioencephalomalacia in ruminants

**Animal** sources: Fish and shellfish (molluscs) also contain thiaminase I and if they dominate the diet are known to cause thiamine deficiency with bilaterally-symmetrical lesions of the brainstem nuclei in cats, dogs and farmed foxes and mink (Chastek's paralysis) (Summers *et al.* 1995). Whole flesh of Australian freshwater mussels (*Velesunio ambiguus*) has thiaminase activity measured as 90 mg thiamine hydrolysed/hr/g dry weight by McCleary & Chick (1977).

Toxicity:

- lush young fronds have the highest concentration of thiaminase (McCleary & Chick 1977); thiaminase activity in nardoo = 100 x that in young bracken (McCleary & Chick 1977); bracken rhizomes contain 10-30 x more thiaminase than fronds (Evans *et al.* 1975)

| **Fern species** | **Plant part** | **Thiaminase activity †** | **Thiaminase activity ‡** | Reference |
|---|---|---|---|---|
| *Marsilea drummondii* | lush fronds | 460 | 7682 | McCleary & Chick 1977 |
| | rhizomes (lush growth) | 100 | 1670 | McCleary & Chick 1977 |
| | old fronds | 50 | 835 | McCleary & Chick 1977 |
| | sporocarps | 10 | 167 | McCleary & Chick 1977 |
| *Marsilea mutica* | lush fronds | 43 | 718 | McCleary & Chick 1977 |
| *Marsilea angustifolia* | lush fronds | 2 | 33 | McCleary & Chick 1977 |
| *Cheilanthes sieberi* | lush fronds | 110 | 1837 | McCleary & Chick 1977 |
| *Pteridium esculentum* | young fronds | 4 | 67 | McCleary & Chick 1977 |
| | old fronds | 3 | 50 | McCleary & Chick 1977 |
| | rhizomes | 3 | 50 | McCleary & Chick 1977 |
| *Pteridium aquilinum* | green fronds | 0-1.8 | 0-30 | Evans *et al.* 1975 |
| | stems | 0-0.2 | 0-4 | Evans *et al.* 1975 |
| | rhizomes | 0.6-2.4 | 10-40 | Evans *et al.* 1975 |

† = mg thiamine hydrolysed/hr/g dry weight (units used by McCleary & Chick 1977) (1 mg/hr= 16.7 µg/min)
‡ = µg thiamine destroyed/min/g dry weight (units used by Evans *et al* 1975)

- a thermostable anti-thiamine compound in bracken additional to and distinct from thiaminase has been shown experimentally to produce intoxication in horses (Konishi & Ichijo 1984)
- monogastrics have no endogenous synthesis of thiamine, although it possibly may occur in horse colon (reviews– Evans *et al.* 1982, Fenwick 1988)
- ruminants - thiamine is normally produced by rumen flora
- the syndromes described below have been experimentally reproduced in
  - horses by feeding bracken (Roberts *et al.* 1949, Fernandes *et al.* 1990)
  - pigs by feeding bracken rhizomes (Evans *et al.* 1963, 1972)
  - sheep by feeding bracken rhizomes (Evans *et al.* 1975)
  - sheep by feeding *Cheilanthes sieberi* (Hurst 1942) but without recognising polioencephalomalacia. Dosing *C. sieberi* collected in 1969 near Ipswich,

south-eastern Queensland, PO at 8.1 g/kg/day for 56 days to a wether produced no clinical illness (Clark & Dimmock 1971).

- feeding trials with *Marsilea drummondii* in sheep have been unsuccessful in reproducing the syndrome (QDPI Poisonous Plant Files unpublished data 1958, McCleary *et al.* 1980).

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*Nardoo and the Burke & Wills Expedition*

Ingestion of flour made from sporocarps (reproductive structures) of nardoo (*Marsilea* sp.) has been claimed as a major influence on the health of the leaders of the Burke & Wills expedition through central Australia in 1860-61 (Earl & McCleary 1994, Grant 1995). Human thiamine deficiency, currently usually associated either with scanty diets dominated by white (polished) rice or with chronic alcoholism, targets

- peripheral nerves causing a polyneuropathy (axonal neuropathy) with myelin degeneration and axon disruption and presenting as sensory loss and muscle weakness of legs (footdrop), then arms (wristdrop) (called neuropathic or dry beriberi),
- the cardiovascular system causing peripheral vasodilation, cardiac failure and peripheral oedema (called wet beriberi), and
- the brain causing haemorrhages and necrosis with neurone degeneration of the mamilliary bodies, periventricular thalamus (medial dorsal nucleus), floor of the 4th ventricle and anterior cerebellum (called Wernicke encephalopathy, Korsakoff psychosis or Wernicke-Korsakoff syndrome)

Typically, the three syndromes appear in this sequence, but occasionally a syndrome may manifest in isolation (Cotran *et al.* 1999).

Note the similarity of these effects to the various syndromes in domestic animals.

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Mode of action:

Thiamine (vitamin $B_1$, aneurin) is essential in carbohydrate metabolism. During absorption from the intestines, thiamine is phosphorylated to thiamine pyrophosphate which

- is needed as a cofactor for transketolase for enzymic conversion of pyruvate to acetyl-CoA. A deficiency of thiamine produces an increased concentration of pyruvate in blood.
- regulates oxidative carboxylation of α-keto acids, leading to the synthesis of adenosine triphosphate (ATP)
- maintains neural membranes and normal nerve conduction, mainly of peripheral nerves (Cotran *et al.* 1999)

Thiaminase I (the form of the enzyme in ferns) requires various co-substrates to catalyse the transformation of thiamine into a thiamine analogue. The availability of co-substrates is critical to the development of clinical disease. The thiamine analogue so produced is itself a thiamine antagonist, and its presence further adds to the direct loss of thiamine through thiaminase action. The lack of thiamine may have its CNS effects through interference with transketolase activity in oligodendrocytes, but the link to formation of lesions is unclear.

Conditions of poisoning:

*Horses*

- fed **hay contaminated by bracken fronds or horsetails**
- consume bracken when used as bedding
- grazing bracken or horsetails in pasture
- grazing **nardoo-dominated pastures**.
- access for several weeks required for bracken thiaminase poisoning, probably less for nardoo

*Sheep*

- grazing **pastures dominated by nardoo or mulga fern** (western NSW, Q) may produce numerous cases in a flock;

  **Nardoo**: Cases were first documented in 1911, 1934 and 1938 (Henry & Massy 1911, Hurst 1942). Further incidents occurred in the Moree district in 1974 after a series of floods promoted widespread growth of nardoo (Eggleston 1975) and the association with thiaminase was first made during 1974-5

investigations in the Gwydir basin area west of Moree, NSW, where sheep grazed dense nardoo populations (up to 100% of available pasture) leading to death of 2200 of 57000 sheep on 13 properties (Eggleston 1975, Pritchard *et al.* 1978). Cases occurred most commonly following periods of waterlogging or flooding of pasture, but were not restricted to these conditions and were recorded commonly from actively-growing nardoo, but also from hayed-off and apparently almost dead plants. Affected sheep were of all classes and ages greater than 6 weeks and had grazed nardoo for at least 2 weeks. Thiaminase peaks in nardoo during February-May, but grazing of nardoo at any time of year can result in regrowth of young fronds with high thiaminase activity. (McCleary *et al.* 1980)

**Mulga fern**: Since 1924, cases have been recognised in sheep in NSW and Qld (Hurst 1942, Clark & Dimmock 1971). Cases were documented during 1979 at Yass, Armidale, Kingston and Bingara when sheep were introduced to pastures containing mulga fern or grazed pastures containing mulga fern after the pasture grasses had dried off (McCleary *et al.* 1980).

- grazing adequate to lush pasture containing patches of nardoo (western NSW) may produce sporadic cases in a flock (Slattery 1999)
- rumen thiamine synthesis swamped by large influx of thiaminase (nardoo = 100 x young bracken)

*Pigs*

- eating bracken rhizomes @ > 25% of diet (thiaminase conc. rhizomes 10-30 times that in fronds)

Clinical signs:

*Horses*

- anorexia
- **marked incoordination exacerbated by exercise**
- **hindlimbs wide apart** (characteristic)
- hyperaesthesia, excitement
- low carriage of head
- head nodding, ear twitching
- yawning, vocalisation
- ± partial blindness
- ± nystagmus
- tachycardia, cardiac arrhythmia
- collapse with clonic convulsions, opisthotonus
- death within 2-10 days of onset

*Sheep* (Pritchard *et al.* 1978)

- separate from the flock, aimless walking/stand motionless
- **apparent blindness**
- "star-gazing"
- ± head pressing
- intermittent head shaking becoming more vigorous with onset of recumbency
- recumbency
- ± teeth grinding, saliva frothing
- nystagmus
- ± muscle tremor
- clonic-tonic convulsions or opisthotonus, particularly if touched
- recumbency with death in 2-4 days

forced exercise → dyspnoea, collapse, death in 6-12 hours

*Pigs*

- anorexia, listlessness
- **bradycardia, heart block**
- ± sudden death
- recumbency
- dyspnoea
- death within 2-14 days of onset

Pathology:

*Horses*

- ↓ blood thiamine (normal 8.5 μg/100 ml → 2.5)

- ↑ blood pyruvate (normal 2.2 mg/100 ml → 6.2)
- ± neutrophilia, thrombocytopaenia
- necropsy lesions consistent with heart failure described (Evans *et al.* 1951)
    - myocardial oedema and degeneration
    - hydropericardium, hydrothorax
    - brain congestion, but no other histological abnormality

*Sheep*

- large amount of fern in the rumen
- **polioencephalomalacia**: all cerebral lobes affected, sometimes sparing the temporal lobes
    - autofluorescence of lesions under UV illumination @ 365 nm wavelength; autofluorescent substance localised in mitochondria (Shibahara *et al.* 1999)

*Pigs*

- ↑ blood pyruvate, ↓ blood transketolase
- enlarged mottled heart
- congestion of lungs, liver (heart failure)

Diagnosis:

*Horses*: Access + syndrome; assay blood thiamine (normal 5-23 ng/ml whole blood [Puls 1994])

*Sheep*: access + pathology

*Pigs*: access + syndrome

Therapy:

*Horses*

- prognosis good for non-recumbent cases
- **thiamine** hydrochloride parenterally @ 100 mg twice on Day 1, then 100 mg daily for 7 days (double this dose rate in severe cases)

*Sheep*

- thiamine hydrochloride IV @ 10 mg/kg every 3 hours for 5 treatments (ideal)
- practically, treat twice and monitor response. If none in 6-8 hours, prognosis bad → euthanasia/slaughter
- Pritchard *et al.* (1978) reported recovery in 16 of 22 affected sheep treated with a single SC dose of 200 mg thiamine hydrochloride, noting that the 6 failing to respond had all been recumbent for over 24 hr before treatment.

*Pigs*

- thiamine as above

Prevention & control:

*Horses*, Pigs: deny access to bracken

*Sheep*: move flock to alternative pasture free of nardoo

Moist heat (steam for 30 mins or autoclaving) inactivates thiaminase (Evans *et al.* 1951)

References:

*Review literature*

Se18, DM38-40

Chick BF, McCleary BV, Beckett RJ (1989) Thiaminases. Chapter 3 in Cheeke PR (ed.) *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids.* CRC Press, Boca Raton, Florida. pp.73-91.

*General literature*

Beckett RJ (1984) Effects of rock fern on sheep and cattle. PhD Thesis, University of Sydney.

Chinnock RJ (1998) *Equisetum. Flora of Australia* **48**:97-98.

Clark IA, Dimmock CK (1971) The toxicity of *Cheilanthes sieberi* to cattle and sheep. *Aust. Vet. J.* **47**:149-152.

Cotran RS, Kumar V, Collins T (1999) *Robbins' Pathologic Basis of Disease.* 6th edition. W.B.Saunders Company, Philadelphia. pp.447-448, 1279, 1341.

Earl JW, McCleary BV (1994) Mystery of the poisoned expedition. *Nature* **368**:683-684.

Eggleston GW (1975) Nardoo poisoning in sheep. *NSW Vet. Proc.* **11**:29-30.

Evans ETR, Evans WC, Roberts HE (1951) Studies on bracken poisoning in the horse. *Br. Vet. J.* **107**:364-371, 399-411.

Evans IA, Humphreys DJ, Goulden L, Thomas AJ, Evans WC (1963) Effects of bracken rhizomes on the pig. *J. Comp. Path.* **73**:229-243.

Evans WC, Jones NR, Evans RA (1950) The mechanism of the anti-aneurin activity of bracken (*Pteris aquilina*). *Proc. Biochem. Soc. (Biochem J. )* **46**:xxxviii-xxxix.

Evans WC, Widdop B, Harding JDJ (1972) Experimental poisoning by bracken rhizomes in pigs. *Vet. Rec.* **90**:471-475.

Evans WC, Evans IA, Humphreys DJ, Lewin B, Davies WEJ, Axford RFE (1975) Induction of thiamine deficiency in sheep, with lesions similar to those of cerebrocortical necrosis. *J. Comp. Path.* **85**:253-267.

Evans WC, Patel MC, Koohy Y (1982) Acute bracken poisoning in homogastric and ruminant animals. *Proc. Roy. Soc. Edinburgh* **81B**:29-64.

Fenwick GR (1988) Bracken (*Pteridium aquilinum*) – toxic effects and toxic constituents. *J. Sci. Food Agric.* **46**:147-173.

Fernandes WR, Garcia RCM, Medeiros RMA, Birgel EH (1990) [Experimental *Pteridium aquilinum* intoxication of horses.] *Arquivos da Escola de Medicina Veterinaria da Universidade Federal da Bahia* **13**:112-124. [in Portuguese, English summary; Abstract in *Vet. Bull.*]
Gould DH (1998) Polioencephalomalacia. *J. Anim. Sci.* **76**:309-314.
Grant M (1995) *Marsilea* poisoning in 19th century Australia. *Pteridologist* **2**:6.
Henry M, Massy AE (1911) *Agric. Gaz., N.S.W.* **22**:109
Jones DL (1998) *Marsilea. Flora of Australia* **48**:166-172
Jubb KVF, Huxtable CR (1993) The Nervous System. Chapter 3 in *Pathology of Domestic Animals* 4th edition, edited by KVF Jubb, PC Kennedy and N Palmer, Academic Press, New York & London **1**:340-344.
Konishi T, Ichijo S (1984) [Experimentally induced equine bracken poisoning by thermostable antithiamine factor (SF factor) extracted from dried bracken.] *J. Jap. Vet. Med. Assoc.* **37**:730-734. [in Japanese, English summary; Abstract 3784 in *Vet. Bull.* **55**, 1985]
McCleary BV, Chick BF (1977) The purification and properties of a thiaminase I enzyme from nardoo (*Marsilea drummondii*). *Phytochemistry* **16**:207-213.
McCleary BV, Kennedy CA, Chick BF (1980) Nardoo, bracken and rock ferns cause vitamin $B_1$ deficiency in sheep. *Agric. Gaz. NSW* **91**(5):40-43.
Morgan KT, Lawson GHK (1974) Thiaminase type 1-producing bacilli and ovine polioencephalomalacia. *Vet. Rec.* **95**:361-363.
Pritchard D, Eggleston GW, Macadam JF (1978) Nardoo fern and polioencephalomalacia. *Aust. Vet. J.* **54**:204-205.
Puls R (1994) *Vitamin Levels in Animal Health. Diagnostic Data and Bibliographies.* Sherpa International, Clearbrook, BC Canada. p.39.
Roberts HE, Evans ETR, Evans WC (1949) The production of "bracken staggers" in the horse and its treatment with vitamin $B_1$ therapy. *Vet. Rec.* **61**:549-550.
Shibahara T, Horino R, Taniguchi T, Ando Y (1999) Autofluorescent substance and neurocyte necrosis in thiamine deficiency in cattle. *Aust. Vet. J.* **77**:329-330.
Shreeve JE, Edwin EE (1974) Thiaminase-producing strains of *Clostridium sporogenes* associated with outbreaks of cerebrocortical necrosis. *Vet Rec.* **94**:330.
Slattery S (1999) Nardoo poisoning as a sporadic condition. *Aust. Sheep Veterinary Soc. Newsletter,* April 1999, pp.4-5.
SummersBA, Cummings JF, de Lahunta A (1995) *Veterinary Neuropathology.* Mosby, St.Louis. pp.277-280, 341-342.

## ☑ *Mimosine*

## Core data

*Common sources:*
- *Leucaena leucocephala*
- *Mimosa pudica*

*Animals affected:* cattle, sheep, horse
*Mode of action:* unclear
*Poisoning circumstances:* > 10% leucaena in diet
*Main effects:*
- hair or fleece loss
- stomatitis
- goitre
- cataracts

*Diagnosis:* syndrome + access
*Therapy:* nil
*Prevention:* inoculate ruminants with mimosine-degrading anaerobic bacterium to degrade toxins

Chemical structure:

**non-protein amino acid** mimosine + enzyme in plant tissue
→ 3,4 dihydroxypyridone (3,4-DHP) on mastication
→ 2,3-DHP through rumen flora

Sources:

- in Australia:
  Family Mimosaceae
  - ***Leucaena leucocephala*** (leucaena, subabool [India], jumbey or jimbey [Bahamas], lamtoro [Indonesia], koa haole [Hawaii]) [DM148];The 22 species of *Leucaena* originate in Central & South America (Hughes 1998)
    - Mexican origin (Owen 1958)
  - *Mimosa pudica* (common sensitive plant)

Toxicity:

- monogastrics: >10% leucaena in diet → toxicity
- ruminants:
    - with appropriate ruminal bacteria fully destroy mimosine and metabolites
    - without appropriate ruminal bacteria → toxicity
- **mimosine and dihydroxypyridone metabolites are all toxic**
- mimosine → depilatory effects, mucosal erosion
- 3,4- & 2,3-DHP → prevent organic iodine binding → goitrogenic & other effects
- effects not reversible by iodine supplementation
- DHP → depression of feed intake → growth depression in unadapted animals

Mode of action: **unclear**

- mimosine ? amino acid antagonist
- mimosine binds Zn: ? → induced Zn deficiency → hair loss
- mimosine inhibits cystathionine synthetase & cystathionase which convert methionine to cystine: ? → alopecia
- 3,4- & 2,3-DHP → prevent organic iodine binding → goitre

Conditions of poisoning:

- prolonged ingestion of sources
- women in Indonesia eating young pods (Kraneveld & Djaenoedin 1950 cited by Owen 1958)

Clinical signs & Pathology:

*Cattle*

Rapid effects (days):

- **stomatitis**, mucosal erosions (mouth, pharynx, tongue), drool saliva
- **hair loss** (tail switch, other parts of coat)

Chronic effects (months):

- hyperplastic **goitre** (including congenital goitre – Hamilton *et al.* 1968)
- oesophageal erosion
- ↓ fertility, low birth weight, congenital goitre
- bilateral **cataracts**

*Sheep*

- **fleece shedding**

*Horses*

- ↓ appetite, ↓ weight gain
- ↓ fertility, abortion
- mane & tail alopecia, hoof shedding (Owen 1958)

Diagnosis: syndrome + access

Therapy: nil

Prevention & control:

- strict anaerobe ruminal bacteria (*Synergistes jonesii*) capable of breaking down mimosine and DHP isolated from Hawaiian goats and used for prevention of toxicity in Australian and American (Florida) cattle (Jones & Megarrity 1986, Allison *et al.* 1992)
    - inocula are available to cattle producers through Queensland DPI Brian Pastures Research Station, Gayndah; cultures produced at Animal Research Institute, Yeerongpilly, in continuous culture system using leucaena as sole nutrient source (artificial rumen), dispensed in culture medium + 25% glycerol in 500 ml bottles, frozen and stored at –20°C until required, supplied to producers with a drenching gun [Athol Klieve, personal communication 1998; Klieve & Roberton 1996; Klieve *et al.* 2002]
    - inoculating 1 in 5 head with 100 ml → protects whole herd
- animal-to-animal transmission of mimosine-degrading capacity occurs within a herd; *S. jonesii* excreted in cattle faeces → transmission through dust in cattle yards and other areas of cattle concentration
- mimosine-degrading capacity is maintained as long as leucaena is part of the diet; capacity will survive up to 9 months without access to leucaena
- maintain continuous protection in a herd by managing leucaena access and contact of protected with newly-introduced stock
- Zn supplementation → reduced effects in cattle (worth trying with horses?)
- ferrous sulphate supplementation → reduced effects in monogastrics; mimosine complexes with Fe → faecal excretion

References:

*Review literature*
Se71; Cheeke 289
Romeo JT (1989) Heterocyclic amino and imino acids (mimosine, azetidine-2-carboxylic acid and pipecolic acid). Chapter 6 in Cheeke PR (ed.) *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids*. CRC Press, Boca Raton, Florida. pp.125-139.

*General literature*
Allison MJ, Mayberry WR, McSweeney CS, Stahl DA (1992) *Synergistes jonesii*, gen. nov., sp. nov.: a rumen bacterium that degrades toxic pyridinediols. *System. Appl. Microbiol.* **15**:522-529
Falvey L (1976) The effects of *Leucaena leucocephala* on cattle in the Northern Territory. *Aust. Vet. J.* **52**:243.
Hamilton RI, Donaldson LE, Lambourne LJ (1968) Enlarged thyroid glands in calves born to heifers fed a sole diet of *Leucaena leucocephala*. *Aust. Vet. J.* **44**:484.
Hammond AC (1995) *Leucaena* toxicosis and its control in ruminants. *J. Anim. Sci.* **73**:1487-1492.
Hegarty MP, Court RD, Christie GS, Lee CP (1976) Mimosine in *Leucaena leucocephala* is metabolised to a goitrogen in ruminants. *Aust. Vet. J.* **52**:490.
Hughes CE (1998) Taxonomy of *Leucaena*. in Shelton HM, Gutteridge RC, Mullen BF, Bray RA (eds.) *Leucaena – Adaption, Quality and Farming Systems*. Proceedings of a Workshop held in Hanoi, Vietnam 9-14 Feb 1998. pp.27-38.
Jones RJ, Blunt CG, Nurnberg BI (1978) Toxicity of *Leucaena leucocephala*. The effect of iodine and mineral supplements on penned steers fed a sole diet of *Leucaena*. *Aust. Vet. J.* **54**:387-392.
Jones RJ, Megarrity RG (1986) Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of leucaena. *Aust. Vet. J.* **63**:259-262.
Klieve AV, Roberton R (1996) A fermentor-grown bacterial culture for cattle fed a diet of *Leucaena leucocephala*. *Microbiology Australia* **17**:A36 Symposium S26.3 (Abstract)
Klieve AV, Ouwerkerk D, Turner A, Roberton R (2002) The production and storage of a fermentor-grown bacterial culture containing *Stnergistes jonesii*, for protecting cattle against mimosine and 3-hydroxy-4(1H)-pyridone toxicity from feeding on *Leucaena leucocephala*. *Aust. J. Agric. Res.* **53**:1-5.
Owen LN (1958) Hair loss and other toxic effects of *Leucaena glauca* ("jumbey"). *Vet. Rec.* **70**:454-457.

## ☑ S-methylcysteine sulphoxide (SMCO) & N-propyl disulphide / thiosulphates

### Core data

*Common source:*
- SMCO: *Brassica* spp. (kale, rape, canola, cabbage, swede)
- N-propyl disulphide & thiosulphates: *Allium* spp. (onions, garlic); toxicity survives cooking

*Main Animals affected:*
- ruminants
- dogs, cats

*Mode of action:*
- SMCO in rumen → dimethyl disulphide → oxidation of haemoglobin (haemolysis + Heinz body formation)
- N-propyl disulphide & thiosulphates → oxidation of haemoglobin (as above)

*Poisoning circumstances:*
- grazing *Brassica* crops 1-3 weeks; mature or stressed crops
- fed onions, garlic (raw or cooked)

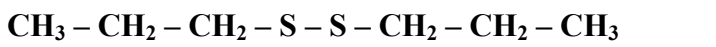
*Main effects:* Heinz body haemolytic anaemia
*Diagnosis:* access + Heinz bodies in erythrocytes
*Therapy:* remove from crop; antioxidant (ascorbic acid)?

Syndrome names:
**Kale anaemia**
**Onion poisoning**

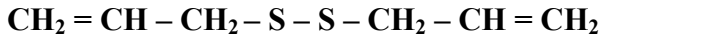
Chemical structure:
S-methylcysteine sulphoxide (SMCO) is a rare **non-protein amino acid** occurring in plants of Family Brassicaceae. S-propenylcysteine sulphoxide (SPCO) is also toxic.

Prop(en)ylcysteine sulphoxides in *Allium* spp. decompose to prop(en)yl thiosulphinates and then to
Prop(en)yl disulphides (N-propyl disulphide, di(2-propenyl) disulphide, di(1-propenyl) disulphide) & thiosulphates which are the sources of toxicity in *Allium* spp (Munday & Manns 1994, Yamato *et al.* 1998). N-propyl disulphide is volatile and in only small concentrations in

cooked onions. Cooked onions contain sodium *n*-propylthiosulphate, sodium *trans*-1-propenylthiosulphate & sodium *cis*-1-propenylthiosulphate, the first of which has been demonstrated to produce haemolysis in dogs (Yamato *et al.* 1998).



Dipropyl disulphide from *Allium* spp.



Di(2-propenyl) disulphide from *Allium* spp.

Di(1-propenyl) disulphide from *Allium* spp.

Sources:

- SMCO/SPCO
  - Family Brassicaceae (Cruciferae):
    - *Brassica napobrassica* (swede turnip, rutabaga) (Ev210)
    - ***Brassica napus*** (rape, canola) (Ev210)
    - ***Brassica oleracea*** (Ev212)
      - *Brassica oleraceae* var. *acephala* (common kale)
      - *Brassica oleraceae* var. *botrytis* (broccoli)
      - *Brassica oleraceae* var. *capitata* (cabbage)
      - *Brassica oleraceae* var. *gemmifera* (Brussels sprouts)
    - *Raphanus raphanistrum* (wild radish, jointed charlock) (Ev213, Parkinson & Sutherland 1954, Craig 1955, R.A.McKenzie unpublished data) – SMCO presence is unconfirmed, but the syndrome seen in association with the plant's consumption is consistent with its effects
- N-propyl disulphide & thiosulphates
  - Family Liliaceae:
    - ***Allium cepa*** (cultivated onions)
    - *Allium sativa* (garlic)
    - *Allium schoenoprasum* (chives) (Cheeke 1998)
    - *Allium* spp. (wild onions)
      - *Allium canadense* (Pierce *et al.* 1972 - horses)
      - *Allium ursinum* (Fenwick & Hanley 1985)
      - *Allium validum* (van Kampen *et al.* 1970 – sheep)
  - Family Fabaceae:
    - *Vicia narbonensis* (narbon bean) – seeds contain γ-glutamyl-*S*-ethenyl-cysteine and have caused haemolysis when fed to pigs in WA (Enneking 1995)

Toxicity:

- brassicas
  - cattle, sheep, goats
  - 1-3 weeks of access before signs occur
  - toxicity retained by dried plants
- onions (*Allium cepa*)
  - Susceptibility: cattle, cats > horses, dogs > sheep, goats (Knight *et al.* 2000).

  - **Cattle** (Goldsmith 1909, Koger 1956, Hutchison 1977, Gill & Sergeant 1981)
  - Horses (Thorp & Harshfield 1939)
  - Sheep: toxic dose 9 kg/head/day (Kirk & Bulgin 1979) (Fredrickson *et al.* 1995)
  - Fowls (experimental toxicity – Baldissera Nordio 1952b)

Rabbits (experimental toxicity – Baldissera Nordio 1952a)
Guinea pigs (experimental toxicity – Majori & Squeri 1954)
Rat (experimental toxicity – Majori & Squeri 1954)
**Dogs** (Gruhzit 1931a, Kalser *et al.* 1951, Spice 1976, Stallbaumer 1981, Harvey & Rackear 1985, Smith & Ellison 1986, Yamoto & Maede 1992, Edwards & Belford 1996
toxic doses reported for dogs
- 600-800 g raw onion (single dose)
- 15 + g raw onion/kg for 2-3 days → anaemia of sudden onset
- 11 g raw onion/kg for several days → anaemia of gradual onset

Cats (Kobayashi 1981, Edwards & Belford 1996)
toxic dose reported for cats: 28 g raw onion/kg once daily for 3 days

**Raw** or **cooked** onions are toxic to dogs and cats (Edwards & Belford 1996)
Effects are detectable within 1 day of feeding (Harvey & Rackear 1985)

garlic (*Allium sativum*)
dogs, cats (Lee *et al.* 1994; Edwards & Belford 1996)
diet containing 7% raw garlic toxic to dogs; fed for 50 days; time to onset of anaemia not stated (Lee *et al.* 1994)

wild onions (*Allium* spp.)
Horses (Pierce *et al.* 1972)
Sheep (van Kampen *et al.* 1970)

Mode of action:

ruminal metabolism of SMCO, SPCO (themselves practically non-toxic)
→ dimethyl disulphide
→ oxidation of haemoglobin (Heinz-Ehrlich body formation) + haemolysis

Heinz bodies are granules of oxidatively denatured haemoglobin retained in erythrocytes. Their formation indicates an oxidising toxin of some kind present in blood (Harley & Mauer 1961). Intraerythrocytic redox cycling of disulphides leads to formation of "active oxygen" species responsible for denaturation of haemoglobin and cell lysis (Munday 1989).

N-propyl disulphide & thiosulphates produce a similar oxidative effect on haemoglobin. N-propyl disulphide has been shown to be haemolytic in dogs (Gruhzit 1931b, Williams *et al.* 1941). Di (1-propenyl) and di(2-propenyl) disulphides are haemolytic in rats and more potent than N-propyl disulphide (Munday & Mamms 1994).

Species susceptibility is related to the relative sensitivity of erythrocytes to oxidative damage . In general, human erythrocytes resist oxidative damage (Munday & Mamms 1994).

Conditions of poisoning:

↑ SMCO concentrations from
↑ maturity of plants
nitrogenous fertilizers
stress (e.g. frost damage)

cattle forced to eat large quantities of *Raphanus raphanistrum* (Craig 1955)
cattle fed 20 kg onions/head/day for 6 weeks (Rae 1999)
cattle with access to dumped onions (Talcott PA, personal communication VETTOX Discussion Group 13 Mar 1997)
pets fed material containing onions/garlic
kitchen scraps, human meal residues (pizza, mince, Chinese recipes)
commercial baby food containing onion powder

Clinical signs:

haemoglobinuria
inappetence
weakness, lethargy
mucosal pallor

± jaundice
± tachycardia
± diarrhoea
± abortions (cattle) (Rae 1999, Talcott PA, personal communication VETTOX Discussion Group 13 Mar 1997)
± sudden death (cattle) (Rae 1999)

Pathology:

*Clinical pathology*

Heinz body anaemia, ± eccentrocytes (erythrocytes with haemoglobin contracted to one side of the cell) (Harvey & Rackear 1985)
± azoturia
± increased concentrations of liver-associated enzymes & bilirubin

*Necropsy*

haemoglobinuria (dark red-brown urine in bladder; red-brown-black kidneys)
± jaundiced carcase
± strong small of onions from the carcase (if fed onions)

*Histopathology*

haemoglobinuric nephrosis
± periacinar hepatocyte necrosis (hypoxia)
splenic haemosiderosis

Diagnosis:

access + Heinz bodies in erythrocytes
dogs: differential diagnosis of haemoglobinuria includes autoimmune haemolytic anaemia (Searle 1990), protozoa (*Babesia canis, Ehrlichia canis, Haemobartonella canis*), leptospirosis

Therapy:

removal from source, monitor haemogram → recovery in 3-4 weeks
no specific therapy
antioxidant effect of ascorbic acid (vitamin C) may be helpful

Prevention & control:

ruminant daily intakes of SMCO should be < 5 g/100 kg live weight , but note that it is possible to adapt sheep to an exclusive onion diet, presumed due to their ruminal sulphate-reducing bacteria's ability to rapidly adapt to metabolise disulphides [high S did not cause PEM in fed sheep] (Knight *et al.* 2000). Previously, sheep were fed 50% onions without illness (Fredrickson *et al.* 1995).
onions should not contribute > 25% of cattle diets (Lincoln *et al.* 1992)

Reference:

*Review literature*

Benevenga NJ, Case GL, Steele RD (1989) Occurrence and metabolism of *S*-methyl-L-cysteine and *S*-methyl-L-cysteine sulfoxide in plants and their toxicity and metabolism in animals. Chapter 9 in Cheeke PR (ed.) *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids.* CRC Press, Boca Raton, Florida. pp. 203-228.

Munday R (1989) Toxicity of thiols and disulphides: involvement of free-radical species. *Free Radical Biol. Med.* **7**:659-673.

Seawright AA (1989) *Animal Health in Australia. Volume 2 (Second Edition). Chemical and Plant Poisons.* Australian Government Publishing Service, Canberra. pp. 38-40.

Smith RH (1977) Kale and brassica poisoning. In Grundsell CSG, Hill FWG (eds.) *The Veterinary Annual.* 17th issue. Wright-Scientechnia, Bristol. pp. 28-33.

*General literature*

Baldissera Nordio C (1952a) Myelotoxic anaemia resulting from feeding onions (*Allium cepa*). *Boll. Soc. Ital. Biol. Sper.* **28**:53-56.

Baldissera Nordio C (1952b) Haematology of chickens treated with *Allium cepa. Boll. Soc. Ital. Biol. Sper.* **28**:1008-1009.

Button C (1987) Onion poisoning [cattle]. *Vet. Pathol. Report. (Newsletter ASVP)* No.15 p.13.

Craig J (1955) Unpublished report, Western Australian Department of Agriculture. [cited by Gardner CA, Bennetts HW (1956) *The Toxic Plants of Western Australia.* Western Australian Newspapers Ltd., Perth. pp.32-34.]

Earl CRA, Smith RH (1982) Dimethyl disulphide in the blood of cattle fed brassicas. *J. Sci. Food Agric.* **34**:23-28.

Edwards CM, Belford CJ (1996) Six cases of Heinz body haemolytic anaemia induced by onion and/or garlic ingestion. *Aust. Vet. Practit.* **26**:18-22 [cat + 5 dogs]

Enneking D (1995) The toxicity of *Vicia* species and their utilisation as grain legumes. *Centre for Legumes in Mediterranean Agriculture (CLIMA) Occasional Publication No. 6,* University of Western Australia, Nedlands. [cited by Cheeke 1998, p.197.]

Fenwick GR, Hanley AB (1985) *Allium* species poisoning. *Vet. Rec.* **116**:28.

Fredrickson EL, Estell RE, Havstad KM, Shupe WL, Murray LW (1995) Potential toxicity and feed value of onions for sheep. *Livestock Prod. Sci.* **42**:45-54.

Gill PA, Sergeant ESG (1981) Onion poisoning in a bull. *Aust. Vet. J.* **57**:484.
Goldsmith WW (1909) Onion poisoning in cattle. *J. Comp. Pathol. Ther.* **22**:151.
Gruhzit OM (1931a) Anemia of dogs produced by feeding of the whole onions and of onion fractions. *Am. J. Med. Sci.* **181**:813-815.
Gruhzit OM (1931b) Anemia in dogs produced by feeding disulphide compounds. *Am. J. Med. Sci.* **181**:815-820.
Harley JD, Mauer AM (1961) Studies on the formation of Heinz bodies. II. The nature and significance of Heinz bodies. *Blood* **17**:418-433.
Harvey JW, Rackear D (1985) Experimental onion-induced hemolytic anemia in dogs. *Vet. Pathol.* **22**:387-392. [haematological study; dehydrated onions fed @ 5.5 g/kg in single dose]
Hutchison TWS (1977) Onions as a cause of Heinz body anaemia and death in cattle. *Can. Vet. J.* **18**:358-360.
Kalser M, Ivy HK, Magee DF, Ivy AC (1951) Haemolytic anaemia produced by onions. *Fed. Proc.* **1**:71-72.
Kirk JH, Bulgin MS (1979) Effects of feeding cull domestic onions (*Allium cepa*) to sheep. *Am. J. Vet. Res.* **40**:397-399.
Knight AP, Lassen D, McBride T, Marsh D, Kimberling C, Delgardo MG, Gould D (2000) Adaption of pregnant ewes to an exclusive onion diet. *Vet. Human Toxicol.* **42**:1-4.
Kobayashi K (1981) Onion poisoning in the cat. *Feline Pract.* **11**:22-27.
Koger LM (1956) Onion poisoning in cattle. *J. Am. Vet. Med. Assoc.* **129**:75.
Lee BJ, Sung EJ, Lee MS, Jang IH, Lee HB (1994) [Effects of garlic on cadmium accumulation in the tissue of dogs.] *Korean J. Vet. Res.* **34**:635-645 [*Nutrition Abstracts & Reviews* Series B (1996) Abstract 664]
Lincoln SD, Howell ME, Combs JJ, Hinman DD (1992) Hematologic effects and feeding performance in cattle fed cull domestic onoins (*Allium cepa*). *J. Am. Vet. Med. Assoc.* **200**:1090-1094.
Majori L, Squeri L (1954) haematological modifications in the guinea pig and albino rat produced by ingestion of *Allium cepa. Boll. Soc. Ital. Biol. Sper.* **30**:791-792.
Munday R, Mamms E (1994) Comparative toxicity of prop(en)yldisulfides derived from Alliaceae: possible involvement of 1-propenyl disulfides in onion-induced haemolytic anaemia. *J. Agric. Food Chem.* **429**:59-62.
Parkinson B, Sutherland AK (1954) Post-parturient haemoglobinuria of dairy cows. *Aust. Vet. J.* **30**:232-236.
Parton K (2000) Onion toxicity in farmed animals. *N. Z. Vet. J.* **48**:89. [Short review; no original data presented]
Pierce KR, Joyce JR, England RB, Jones LP (1972) Acute hemolytic anemia caused by wild onion poisoning in horses. *J. Am. Vet. Med. Assoc.* **160**:323-327.
Prache S (1994) Haemolytic anaemia in ruminants fed forage brassicas: a review. *Vet Res.***25**:497-520.
Rae HA (1999) Onion toxicosis in a herd of beef cows. *Can. Vet. J.* **40**:55-57.
Robertson JE, Christopher MM, Rogers QR (1998) Heinz body formation in cats fed baby food containing onion powder. *J. Am. Vet. Med. Assoc.* **212**:1260-1266.
Searle A (1990) Diagnostic challenge (Autoimmune haemolytic anaemia). *Aust. Vet. Practit.* **20**:36, 40-41.
Smith CH, Ellison RS (1986) Concurrent onion poisoning and haematuria in a dog. *N. Z. Vet. J.* **34**:77-78.
Spice RN (1976) Hemolytic anemia associated with ingestion of onions in a dog. *Can. Vet. J.* **17**:181-183.
Stallbaumer M (1981) Onion poisoning in a dog. *Vet. Rec.* **108**:523-524.
Thorp F, Harshfield GS (1939) Onion poisoning in horses. *J. Am. Vet. Med. Assoc.* **94**:52-53.
van Kampen KR, James LF, Johnson AE (1970) Hemolytic anemia in sheep fed wild onion (*Allium validum*). *J. Am. Vet. Med. Assoc.* **156**:328-332.
Verhoeff J, Hajer R, van den Ingh TSGAM (1985) Onion poisoning of young cattle. *Vet. Rec.* **117**:497-498.
Williams HH, Erickson BN, Beach EF, Macy IG (1941) Biochemical studies of the blood of dogs with n-propyl disulfide anemia. *J. Lab. Clin. Med.* **26**:996-1008.
Yamato O, Maede Y (1992) Susceptibility to onion-induced hemolysis in dogs with hereditary high erythrocyte reduced glutathione and potassium concentrations. *Am. J. Vet. Res.* **53**:134-137.
Yamato O, Hayashi M, Yamasaki M, Maede Y (1998) Induction of onion-induced haemolytic anaemia in dogs with sodium *n*-propylthiosulphate. *Vet. Rec.* **142**:216-219

## *Indospicine*

Chemical structure:

Indospicine (L-6-amidino-2-aminohexanoic acid) is a **non-protein amino acid** first isolated and characterised by Hegarty & Pound (1968, 1970)
Teratogenic toxin presumed to be indospicine

Sources:

**plants** in Australia:
Family Fabaceae

*Indigofera spicata* Forsk. [= *Indigofera hendecaphylla* Jacq. (misspelled as *endecaphylla)*] (creeping indigo); leaves contain 0.1-0.5% indospicine (dry matter basis), seeds 0.1-2.0% (Morton 1989)
*Indigofera linnaei* (Birdsville indigo) [DM78]
*Indigofera suffruticosa* Mill. [= *Indigofera anil* L.]; indospicine detected (E.A.Bell personal communication to Morton 1989); aqueous extract of fruit hepatotoxic in mice (Ribiero *et al.* 1991)

**meat** from horses that have grazed *Indigofera linnaei* contains up to 30 mg indospicine/kg

Toxicity:

dogs: hepatotoxicity of contaminated horse meat and pure indospicine confirmed (Kelly *et al.* 1992)

cattle, sheep: hepatotoxicity, abortion. Feeding experiments in Hawaii and Fiji caused abortions in sheep and cattle; rations contained 25-50% *Indigofera spicata.* (Nordfeldt & Younge 1949, Nordfeldt *et al.* 1952, Yelf 1959)

horses: "grove disease" of horses in Florida has been attributed to grazing pastures dominated by *Indigofera spicata.* Horses preferentially graze the plant. Signs are of CNS disturbance: ataxia, staggering, difficulty in turning, inability to walk a straight line, recurring seizures, collapse, sometimes accompanied by corneal opacity or streaks in the eyes and ulceration and redness of the gums. Some mares have had abortions. No necropsy findings were reported. (Morton 1989) See Birdsville horse disease.

rats: An extract of *Indigofera spicata* seeds given to rats caused cleft palate in offspring (Pearn 1967). The teratogen was presumed to be indospicine.

Mode of action:

indospicine is an arginine analogue, interfering with hepatic protein synthesis → liver damage

Conditions of poisoning:

dogs fed on meat from horses that graze *Indigofera linnaei* (Birdsville horse disease) develop severe liver damage (Hegarty *et al.* 1988). One case has been reported where the putative source of indospicine was the flesh of galahs (*Eolophus roseicapillus*) that had been feeding on pods of *Indigofera linnaei* (Phillips 2001).

cattle grazing *Indigofera spicata* in Hawaii (Norfeldt *et al.* 1952)

Clinical signs (dogs):

anorexia
repeated vomiting over several days
progressive depression
jaundice
± nervous derangement (hepatic encephalopathy)

Pathology:

↑ serum liver-associated enzymes, ↑ bilirubin
liver small, pale, firm, bile-stained, nodular
periacinar hepatocyte necrosis, bile stasis, fibrosis, hypertrophy of islands of surviving hepatocytes
ascites
haemorrhage in alimentary tract

Diagnosis:

history of horse meat consumption from *Indigofera linnaei* area + syndrome
long-term ingestion of indospicine by dogs results in tissue concentrations in muscle, liver and pancreas of 10, 20 and 30 mg/kg respectively (Hegarty *et al.* 1988)
An HPLC assay has been developed (Pollitt *et al.* 1999) and can be applied to serum and muscle samples. Liver samples should be able to be assayed by this method.

Therapy:

all severely-affected dogs die
those with anorexia, liver enlargement and slightly increased serum liver-associated enzyme concentrations have recovered slowly on low protein diets

Prevention/Control:

source dog food from unexposed horses
Screening of meat using the HPLC assay (Pollitt *et al.* 1999) is theoretically possible, but the availability of the test is in doubt.
*Indigofera spicata* should not exceed 25% of ration for ruminants [account should also be taken of the hepatotoxicity of the plant]

References: Se62

Hegarty MP, Pound AW (1968) Indospicine, an hepatotoxic amino acid from *Indigofera spicata. Nature* **217**:354.

Hegarty MP, Pound AW (1970) Indospicine, a hepatotoxic amino acid from *Indigofera spicata*: isolation, structure, and biological studies. *Aust. J. Biol. Sci.* **23**:831-842.

Hegarty MP, Kelly WR, McEwan D, Williams OJ, Cameron R (1988) Hepatotoxicity to dogs of horse meat contaminated with indospicine. *Aust. Vet. J.* **65**:337-340.

Kelly WR, Young MP, Hegarty MP, Simpson GD (1992) The hepatotoxicity of indospicine in dogs. In James LF, Keeler RF, Bailey EM Jr, Cheeke PR, Hegarty MP (eds.) *Poisonous Plants. Proceedings of the Third International Symposium.* Iowa State University Press, Ames, Iowa. pp. 126-130.

Morton JF (1989) Creeping indigo (*Indigofera spicata* Forsk.) (Fabaceae_ - a hazard to herbivores in Florida. *Economic Botany* **43**:314-327.

Nordfeldt S, Younge OR (1949) *Hawaii Agricultural Experiment Station Progress Note* **55**:1-2. [cited by Everist 451]
Norfeldt S, Henke LA< Morita K, Matsumoto H, Takahashi M, Younge OR, Willers EH, Cross RF (1952) Feeding tests with *Indigofera endecaphylla* Jacq. (creeping indigo) and some observations on its poisonous effects on domestic animals. University of Hawaii Agricultural Experimental Station Technical Bulletin 15. pp. 5-23. [cited by Everist 451]
Pearn JH (1967) *Nature* **215**:980-981. [cited by Everist 451]
Phillips P (2001) Indospicine toxicity in a dog. *Vet. Path. Report (ASVP Newsletter)* No.61, p.26.
Pollitt S, Hegarty MP, Pass MA (1999) Analysis of the amino acid indospicine in biological samples by high performance liquid chromatography. *Natural Toxins* **7**:233-240.
Ribeiro LR, Bautista ARPL, Silva AR, Sales LA, Salvadore DMF, Maia PC (1991) Toxicological and toxicogenetic effects of plants used in popular medicine and in cattle food. *Memorias do Instituto Oswaldo Cruz* **86** (Suppl.2):89-91.
Yelf JD (1959) *Fiji Agric. J.* **29**:9. [cited by Everist 451]

## *Toxalbumins (lectins)*

### Core data

*Common sources:*
- seeds of *Ricinus communis, Abrus precatorius, Robinia pseudoacacia*
- bark of *R. pseudoacacia*

*Animals affected:* ruminants, horses
*Mode of action:* inhibit protein synthesis
*Poisoning circumstances:*
- feed grain contamination by *R. communis seeds*
- horses chewing bark of *R. pseudoacacia*

*Main effects:* severe gastroenteritis
*Diagnosis:* syndrome + access
*Therapy:*
- remove source
- rehydration, adsorbent, demulcent

*Prevention:* deny access to seeds, trees

Chemical structure:

highly toxic proteins in **seeds** [roots]
**ricin** in *Ricinus communis* (Olsnes & Kozlov 2001)
**abrin** in *Abrus precatorius*
robin in *Robinia pseudoacacia* (also present in toxic amounts in bark)
curcin in *Jatropha curcas* (Morgue 1961)
modeccin in *Adenia digitata* roots (Refsnes *et al.* 1977)
volkensin in *Adenia volkensii* (Stirpe *et al.* 1985)
ebulin in *Sambucus ebulus* (Citores *et al.* 1997)
viscumin in *Viscus album* (a European mistletoe) (Stirpe *et al.* 1982)
phasin in *Phaseolus vulgaris*
Ricin, abrin & modeccin consist of **2 peptide chains (A & B) linked by a disulphide bridge**

Sources:

**seeds** of
***Ricinus communis*** L. (castor oil plant, castor bean, mole bean, Palma Christi, wonder tree, African coffee tree, Mexico weed)
***Abrus precatorius*** L. (coral pea, crab's eye, gidee-gidee, precatory bean, rosary pea, jequirity bean, lucky bean, Paternoster bean, Indian liquorice)
***Robinia pseudoacacia*** L. (black locust, false acacia, black acacia) (Barnes 1921)
*Jatropha curcas* (physic nut)
*Adenia digitata* - African plant
*Adenia volkensii*
*Viscus album* (a European mistletoe)
*Phaseolus vulgaris* (common bean)
**bark** of *Robinia pseudoacacia*
rhizomes of *Sambucus ebulus* (dwarf elder)


*Ricinus communis* (castor oil plant): seeds [RAM Image 2002]


*Abrus precatorius*: seeds [RAM Image2002]

Toxicity:

horses, pigs, ruminants, dogs (Albretsen *et al.* 2000, Soto-Blanco *et al.* 2002), humans (Alpin & Eliseo 1997)

**poisoning is rare**

*Ricinus communis* seed single oral lethal dose (g/kg) = horse 0.1; pig 1.0-2.5; ruminants 1.0-5.5; fowl 14.0 (N.B. there is considerable individual variation)

ricin dog oral lethal dose = 1 µg/kg (one *R. communis* seed weighs about 0.25g and contains about 0.25 mg ricin) [Frohne & Pfänder 1984]

unbroken *Ricinus communis* or *Abrus precatorius* seeds are said to pass through the gut without releasing toxin

cooking denatures toxin in *Phaseolus vulgaris*, heat denatures toxins in *Ricinus communis* seeds

Mode of action (Olsnes & Kozlov 2001):

Toxalbumins (toxic lectins) **inhibit protein synthesis** and thus cause cell necrosis. The B peptide chain (the haptomer; receptor-binding moiety) attaches the molecule of toxin to the cell surface. The A peptide chain (the effectomer; enzymically-active moiety) is a glycosidase that removes an adenine residue from an exposed loop of 28 S ribosomal RNA, making the

depurinated RNA susceptible to hydrolysis, leading to inactivation of the elongation factor EF2 and halting protein synthesis. One molecule of the A peptide inactivates a few thousand ribosomes per minute, thus inactivating ribosomes faster than the cell can replace them and killing the cell.

Ricin is more toxic to animal cells than to plant cells

Conditions of poisoning:

contamination of feed grains with *R. communis* seeds

horses, cattle chewing bark of *R. pseudoacacia* (Landolt *et al.* 1997; Hopper 1999; Thursby-Pelham 1999)

dogs: eating castor bean cake (residual after oil extraction used in South America with bone flour as a fertiliser for ornamental plants); 27 dogs in 2 years in Sao Paulo, Brazil (Soto-Blanco *et al.* 2002)

Clinical signs:

after a **latent period** of from a few hours up to 2-3 days

**severe diarrhoea**

colic

± dysentery

alternatively (horses/*Robinia pseudoacacia*) → abdominal pain, dilated pupils, muscle trembling, tongue paresis/dysphagia, constipation (Hopper 1999; Thursby-Pelham 1999)

syndrome in 98 dogs ingesting *Ricinus communis* seed (descending order of frequency %): vomiting (80), depression (45), diarrhoea (37), diarrhoea with blood (24), abdominal pain (14), anorexia (16), vomiting with blood (10), death/euthanasia (9), weakness (8), hyperthermia (7), ataxia (5), hypersalivation (5), recumbency (5), tachycardia (5). Other less-frequent signs included coma, tremors, seizures, dehydration, pallor, dyspnoea, polydipsia, jaundice. (Albretson *et al.* 2000)

Pathology:

severe **gastroenteritis**

± liver & kidney degeneration/necrosis

Diagnosis: syndrome + access

Therapy:

remove source

rehydration + adsorbent + demulcent

Prevention & control:

deny access to seeds (or bark)

It is possible to immunise animals against ricin and abrin, but this has no practical application in preventing poisoning.

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*Immunotoxins as anti-neoplastic drugs*

The A-chain of ricin has been experimentally linked to different types of antibody molecules replacing the B-chain. These antibodies are directed against particular types of neoplastic cells. The synthetic molecule then introduces the A-chain into these target cells to kill them. Work is required on improving the fit of the synthetic molecules to the transportation mechanisms in the Golgi apparatus and endoplasmic reticulum that move ricin to its site of action of the A-chain in cells. (Olsnes & Kozlov 2001).

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References:

*Review literature*

Humphreys 266; Cheeke 310

Pusztai A (1989) Lectins. Chapter 2 in Cheeke PR (ed.) *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids.* CRC Press, Boca Raton, Florida. pp. 29-71.

*General literature*

Albretsen JC, Gwaltney-Brant SM, Khan SA (2000) Evaluation of castor bean toxicosis in dogs: 98 cases. *J. Am. Anim. Hosp. Assoc.* **36**:229-233.

Alpin PJ, Eliseo T (1997) Ingestion of castor oil plant seeds. *Med. J. Aust.* **168**:423-424.

Barnes MF (1921) Black locust poisoning of chickens. *J. Am. Vet. Med. Assoc.* **59** (New Series **12** No.3):370-372. [cited by Hurst 1942]

Citores L, De Benito FM, Iglesais R, Ferreras JM, Argueso P, Jimenez P, Testera A, Camafeita E, Mendez E, Girbes T (1997) Characterisation of a new non-toxic two-chain ribosome-inactivating protein and a structurally-

related lectin from rhizomes of dwarf elder (*Sambucus ebulus* L.). *Cell Mol. Biol. (Noisy-le-grand)* **43**:485-499.
Frohne D, Pfänder HJ (1984) *A Colour Atlas of Poisonous Plants.* Wolfe Publishing Ltd., London. pp.114-116, 121-129
Hopper DW (1999) False acacia poisoning in horses. *Vet. Rec.* **145**:115.
Landolt G, Feige K, Schöberl M (1997) [Poisoning with *Robinia pseudoacacia* in two horses.] *Schweizer Archiv für Tierheilkunde* **139**:363-366.
Morgue M (1961) *Bull. Soc. Chim. Biol.* **43**:517. [curcin]
Olsnes S, Kozlov JV (2001) Ricin. *Toxicon* **39**:1723-1728.
Pusztai A (1989) Lectins. Chapter 2 in Cheeke PR (ed.) *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids.* CRC Press Inc., Boca Raton, Florida. pp. 29-71.
Refsnes K, Haylett T, Sandvig K, Olsnes S (1977) Modeccin – a plant toxin inhibiting protein synthesis. *Biochem. Biophys. Res. Commun.* **79**:1176-1183.
Soto-Blanco B, Sinhorini IL, Gorniak SL, Schumaher-Henrique B (2002) *Ricinus communis* cake poisoning in a dog. *Vet. Human Toxicol.* **44**:155-156.
Stirpe F, Sandvig K, Olsnes S, Pihl A (1982) Action of viscumin, a toxic lectin from mistletoe, on cells in culture. *J. Biol. Chem.* **257**:13271-13277.
Stirpe F, Barbieri L, Abbondanza A, Falasca AI, Brown AN, Sandvig K, Olsnes S, Pihl A (1985) Properties of volkensin, a toxic lectin from *Adenia volkensii*. *J. Biol. Chem.* **260**:14589-14595.
Thursby-Pelham RHC (1999) False acacia poisoning in horses. *Vet. Rec.* **145**:148.
Wiley RG, Oeltmann TN (1991) Ricin and related plant toxins: mechanism of action and neurobiological applications. Chapter 12 in Keeler RF, Tu AT (eds) *Handbook of Natural Toxins. Vol.6. Toxicology of Plant and Fungal Compounds.* Marcel Dekker, Inc., New York. pp. 243-268.

## *Lathyrogens (neurolathyrism* - Lathyrus *spp. )*

Horses. North America, Europe
Similar syndrome to `Australian' stringhalt
Plants associated with equine disease: *Lathyrus odorata* (sweet pea), *Lathyrus latifolius* (everlasting pea)

Humans suffer from natural neurolathyrism (spastic paraparesis) through consumption of seeds of *Lathyrus sativus* (chickling vetch, chickling pea, grass pea, almorta [Spain], khesari or batura [India], gilban [Sudan, Egypt], guaya [Ethiopia], matri [Pakistan], gesette [France], pisello bretonne [Italy]), *Lathyrus cicera* (flat-podded vetch), *Lathyrus clymenum* (Spanish vetchling) or *Lathyrus latifolius* usually when other more nourishing pulses are unavailable during famines through drought or flood (Roy & Spencer 1989). Cases result when the diet contains more than 2/3 *Lathyrus* sp. seeds for 3-6 months. Historically, cases are known from many parts of the world including the Indian subcontinent, North Africa, the Middle East, Russia and Europe. Currently, cases occur in India, Bangladesh and Ethiopia. The neurotoxic non-protein amino β-N-oxalyl-L-α-β-diaminopropionic acid (ODAP) [=acid β-N-oxalylamino-L-alanine (BOAA)] is responsible for the syndrome.

The International Centre for Agricultural Research in the Dry Areas [ICARDA] is developing a low-toxin variety that retains drought tolerance (Aletor *et al.* 1994, Mayell 2000). Evaluation of *Lathyrus sativus, Lathyrus cicera* and *Lathyrus ochrus* in Western Australia may lead to development of lines with acceptable toxin concentrations (Siddique *et al.* 1996).

ODAP has also been detected in seeds of 13 species of *Crotalaria*, some of which occur in Australia (*C. incana, C. pallida*), and 17 species of *Acacia*, none of which occur in Australia (Bell 1968; Quereshi *et al.* 1977).

Osteolathyrism and angiolathyrism are diseases of experimental animals induced during studies attempting to elucidate the causes of human neurolathyrism (Roy & Spencer 1989). β-aminopropionitrile (BAPN) is largely responsible for these forms through → disorders of collagen and elastin metabolism.

References:

*Review literature*
Roy DN, Spencer PS (1989) Lathyrogens. Chapter 8 in *Toxicants of Plant Origin. Volume III. Proteins and Amino Acids.* Cheeke PR (editor), CRC Press, Boca Raton, Florida. pp. 169-201.
*General literature*
Aletor VA, Ali Abd El-Moneim, Goodchild AV (1994) Evaluation of the seeds of selected lines of three *Lathyrus* spp for β-*N*-oxalylamino-L-alanine (BOAA), tannins, trypsin inhibitor activity and certain *in vitro* characteristics. *J. Sci. Food Agric.* **65**:143-151.
Bell EA (1968) *Nature* **218**:197
Mayell H (2000) Cultivated crop turns lethal during times of famine. *National Geographic* [http://www.ngnews.com/news/2000/07/07052000/grasspea_2814.asp]

Quereshi MY, Pilbeam DJ, Evans CS, Bell EA (1977) The neurolathyrogen, α-amino-β-oxalylaminopropionic acid in legume seeds. *Phytochem.* **16**:477-479.
Siddique KHM, Loss SP, Herwig SP, Wilson JM (1996) Growth, yield and neurotoxin (ODAP) concentration of three *Lathyrus* species in mediterranean-type environments of Western Australia. *Aust. J. Exp. Agric.* **36**:209-218.

## *Sinapine*

*Egg taint*: **Trimethylamine** is formed in the caecae of birds by bacterial metabolism of sinapine (an ester of the B vitamin choline and sinapic acid) from *Brassica napus* (rape, canola) meal. Absorbed trimethylamine is converted in the liver to trimethylamine oxide by trimethylamine oxidase (Cheeke 1998). Brown-shelled-egg-laying strains of hen have a congenital absence of the trimethylamine oxidase and thus allow accumulation of the compound in egg yolks (Bolton *et al.* 1976). The fish odour taint in the yolks is due to the amine. The odour does not develop if the diet contains less than 0.1% sinapine; canola meal contains 2.5-3.0% sinapine (Bell 1993).

References:

Bell JM (1993) Factors affecting the nutritional value of canole meal: a review. *Can. J. Anim. Sci.* **73**:679-697.
Bolton W, Carter TC, Morley-Jones R (1976) The hen's egg. Genetics of taints in eggs from hens fed on rape seed meal. *Brit. Poultry Sci.* **17**:313-320.
Cheeke PR (1998) *Natural Toxicants in Feeds, Forages and Poisonous Plants.* 2nd edition. Interstate Publishers Inc., Danville, Illinois. pp. 175-180.

## *Polyamines of gousiekte-inducing plants*

A plant poisoning of ruminants unique to southern Africa, first described by Theiler (1906-1907) and first linked with ingestion of *Pachystigma pygmaeum* by Theiler *et al.* (1923) and one of the six major plant poisonings of livestock in southern Africa (Fourie *et al.* 1995).

Syndrome names: gousiekte [Afrikaans = "quick" disease]

Chemical structure:

A polyamine toxin, pavetamine, has been isolated, first from *Pavetta harborii* and then from *Pavetta schumanniana, Fadogia homblei* and *Pachystigma pygmaeum*, that produced gousiekte when given IV to goats (Fourie *et al.* 1995).

Sources:

All source plants are in the Family Rubiaceae and are from southern Africa.

*Pachystigma pygmaeum* (Schultr.) Robyns [= *Vangueria pygmaea* Schultr.] (hairy gousiektebossie)
*Pachystigma latifolium* Sond.
*Pachystigma thamnus* Robyns (Natal gousiektebossie, smooth gousiektebossie)
*Fadogia homblei* de Wild. [= *F. monitcola* Robyns] (wild date, wildedadel)
*Pavetta harborii* S.Moore (pavetta, tonnabossie)
*Pavetta schumanniana* F.Hoffm. (poisonous bride's bush, gousiekte tree, gousiekte-boom)

All but *Pavetta schumanniana* are perennial shrublets with a taproot and subterranean branches from which aerial stems grow. *Pavetta schumanniana* is a perennial shrub. All a deciduous during the dry winter season.

Organ systems affected: heart

Toxicity:

Sheep, goats, cattle
The plants induce acute heart failure without premonitory signs, 4-8 weeks after the initial ingestion.

Mode of action:

Detail undescribed. The disease pathogenesis results in damage to myocardial fibres with replacement fibrosis.

Conditions of poisoning:

Sprouting stems of the gousiekte plants often emerge before grass growth in spring, providing the first green material available to grazing livestock for some months.
Intake of toxic amounts may occur during dry spells when grass is wilted.
Exercise usually precipitates deaths from gousiekte, but some are spontaneous.

Clinical signs:

sudden death, spontaneous or precipitated by exercise; some animals struggle briefly; some still have a bolus of food in the mouth after death

± signs of congestive heart failure: weakness, lagging behind the group, gasping, dyspnoea, oedema of the head

Close examination of experimentally-induced gousiekte cases has revealed signs of cardiac abnormality in most animals: arrhythmias (tachycardia, dropped beats, irregular rhythm), ECG abnormalities (wandering P waves, inversion of T waves, changed polarity of QRS waves). These and other changes have been interpreted as indicating

- functional cardiac dilation causing signs of AV valve insufficiency, gallop rhythm, bundle branch block and increased P wave duration
- cardiac ischaemia causing wandering pacemaker, bundle branch block and ectopic ventricular beats
- decreased myocardial contractility causing signs of congestive heart failure

Pathology:

± irregular areas of pallor, particularly in the endocardium which may be greyish
± thin & tough ventricular walls > dilation of the heart
± extracardiac lesions of heart failure: cyanosis, congestion, pulmonary oedema, hydrothorax, hydropericardium, ascites

Cardiac lesions are most consistently seen in the apex of the heart and in the left ventricle, then the septum, then the right ventricle. Special histopathological stains for connective tissue are useful in determining the extent of fibrosis. Major lesions are **pronounced endocardial fibrosis with atrophy of myocardial fibres** and **multifocal mononuclear inflammatory cell infiltration of the myocardium**. Experimental cases have early focal to diffuse myocardial fibre degeneration/necrosis with lymphocytic infiltration and fibrosis.

Diagnosis:

syndrome + pathology + history of access to sources
differential diagnoses include gifblaar (*Dichapetalum cymosum*) poisoning (fluoroacetate)

Therapy: nil

Prevention & control:

References:

Fourie N, Erasmus GL, Schultz RA, Prozesky L (1995) Isolation of the toxin responsible for gousiekte, a plant-induced cardiomyopathy of ruminants in southern Africa. *Onderstepoort J. Vet. Res.* **62**:77-87.

Kellerman TS, Coetzer JAW, Naudé TW (1988) *Plant Poisonings and Mycotoxicoses of Livestock in Southern Africa.* Oxford University Press, Cape Town. pp.114-121.

Theiler A (1906-1907) Gouw-Ziekte. *Report of the Government Veterinary Bacteriologist of the Transvaal, 1906-1907*. pp. 21-22 [cited by Kellerman *et al.* 1988]

Theiler A, Du Toit PJ, Mitchell DT (1923) Gousiekte in sheep. *Report on Veterinary Research, Union of South Africa. Nos 9 & 10.* pp.9-105. [cited by Kellerman *et al.* 1988]