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#### SUMMARY

Saskatoon serviceberry (Amelanchier alnifolia) twigs were administered to cattle in feed rations and by intraruminal dosing to determine the toxicity of the cyanogenic shrub. When chopped twigs were fed as 75% of the diet, cattle exhibited restlessness, shivering, loss of weight, scours and shortness of breath. Low level dosing experiments indicated a rapid turnover of hydrogen cyanide in the rumen. When potential doses were increased to 5 mg hydrogen cyanide/kg (body weight) progressive stages of poisoning developed rapidly, rumen hydrogen cyanide levels were elevated for at least three hours and the increase in heart rate correlated with the rise in blood hydrogen cyanide concentration.

#### RÉSUMÉ

# Toxicité de l'amélanchier Saskatoon pour les bovins

Cette expérience consistait à incorporer des brindilles de l'amélanchier Saskatoon, Amelanchier alnifolia, dans les aliments de bovins expérimentaux, ou à les introduire directement dans leur rumen, afin de déterminer la toxicité de cette plante cyanogénétique. L'addition de brindilles hachées aux aliments, dans une proportion de 75%, amena les bovins à manifester de l'anxiété, des tremblements, une perte de poids, de la diarrhée et une respiration courte. L'introduction dans le rumen de faibles quantités de cette plante révéla une élimination rapide de l'acide cvanhydrique de ce réservoir gastrique. L'augmentation de la dose jusqu'à 5 mg d'acide cyanhydrique/kg de poids vif provoqua le développement rapide d'une intoxication progressive; la teneur du contenu du rumen en acide cyanhydrique se révéla élevée pour au moins trois heures et le rythme cardiaque devint de plus en plus accéléré, à mesure qu'augmentait la teneur du sang en acide cyanhydrique.

#### INTRODUCTION

Saskatoon serviceberry (Amelanchier alnifolia Nutt.) is an important if not preferred browse for rangeland livestock and wildlife (4, 8). Recently however, it was recognized that the shrub was capable of producing toxic levels of hydrogen cyanide (HCN) or prussic acid (6). In North America, chokecherries (Prunus virginiana, P. caroliniana and P. serotina), arrowgrasses (Triglochin maritima and T. palustris), and Sorghum species are known to be poisonous to livestock through the action of HCN (9). The HCN is generated enzymatically from cyanogenic glycosides when plant tissue is fragmented during mastication and rumination. In contrast to ruminal metabolism of cyanogenic glycosides, abomasal release of HCN by acid hydrolysis appears to be unlikely (2). Hydrogen cyanide is extremely toxic because it blocks aerobic cellular respiration.

The cyanide potential of saskatoon was revealed during a digestion trial in winter with penned, mature mule deer (*Odocoileus hemionus hemionus*) and the shrub was found to be lethal when twigs were fed at the rate of 1 kg (fresh weight)/day for a week (6). The HCN was detected in the chopped feed and subsequently prunasin ([R]-mandelonitrile- $\beta$ -D-glucoside) was isolated and identified as the cyanogenic glycoside (6). The glycoside can occur as 1.67% of the dry weight in winter twigs (7) and this is equivalent to a cyanide potential of 152 mg HCN/100 g (dry weight). The deer were absorbing cyanide at a potential rate of 11 mg HCN/kg/day or close to the 24 hour tolerance level for ruminants (9).

The present study was undertaken to demonstrate the toxicity of saskatoon serviceberry to cattle. Our objectives were (a) to monitor cyanide concentrations in rumen fluid and blood when saskatoon twigs were given at different levels (b) to induce clinical signs of cyanide poisoning with saskatoon and (c) to determine if the shrub is a potential hazard to cattle.

#### MATERIALS AND METHODS

Saskatoon twigs were collected in the North Thompson River valley near Kamloops, British Columbia during January and February, 1979. Terminal ends of branches representing previous year's growth were snipped and stored at  $-20^{\circ}$  C. Twigs were chopped with a garden mulcher immediately before feeding or intraruminal administration.

In vivo experiments — A yearling Hereford (No. 78-3) and a three year old Hereford-Jersey crossbred (H4) were used in feeding trials to determine saskatoon consumption rates and to induce early signs of poisoning. Chopped saskatoon twigs were combined with regular orchard grass (*Dactylis glomerata*) hay rations and the proportion of saskatoon in the diet increased from 25 to 75% in four days. The animals had free access to water.

Intraruminal dosing experiments were conducted initially with a three year old fistulated Hereford-Jersey crossbred (H3) to determine ruminal cyanide turnover rates when saskatoon twigs were administered at low levels. Chopped twigs were introduced through the cannula in single doses equivalent to approximately one-tenth the estimated daily intake. Rumen fluid was collected before each dose and sequentially sampled after dosing.

Three fistulated Hereford-Jersey cows (H1, H2 and H3) three years old, were used in the final experiments where intraruminal doses were increased to about one-half the estimated daily intake. Ruminal cyanide levels and cyanide concentrations in

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venous blood were monitored immediately after dosing and concomitant clinical changes were recorded.

Fistulated animals were fasted for 20 h before intraruminal dosing.

In vitro procedures — Prunasin concentration (percent dry weight) in saskatoon twigs was determined after grinding subsamples in dry ice, incubating the ground powders with emulsin as described previously (6) and analyzing for cyanide by the method of Lambert *et al* (5).

Rumen fluid, 300 mL, was obtained from each fistulated cow by expressing the ingesta through one layer of cheesecloth and collecting the fluid in a preheated Thermos flask. The residual solids were returned to the rumen. An aliquot (1 to 5 mL) of the filtrate was incubated immediately in the main compartment of a 25 mL Erlenmeyer flask fitted with a centre well containing N NaOH. After 16 h at  $37^{\circ}$ C the centre well solution was analyzed for cyanide by the method of Lambert *et al* (5).

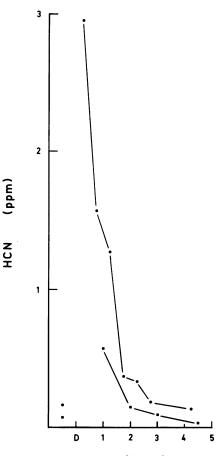
Heparinized venous blood (1 to 2 mL), obtained with the aid of a jugular catheter, was incubated and analyzed for cyanide as described above.

#### **RESULTS AND DISCUSSION**

Twigs collected in the winter of 1979 contained 1.30 to 1.49% prunasin, equivalent to an average cyanide potential of 130 mg HCN/100 g. The distinct odour of almonds was prominent when twigs were chopped, but the loss of HCN was minimal at the prevailing temperatures  $(-15 \text{ to } +5^{\circ} \text{ C})$ . If chopped twigs were maintained at 4° C for 24 h for example, the average prunasin level dropped from 1.43% to 1.22% and the HCN potential was reduced by 15%. In plant tissue, cyanogenic glycosides appear to be isolated in vacuoles (10). The chopping process destroys some cellular structure, glycosides are released and prunasin is hydrolyzed by endogenous plant enzymes to yield equimolar quantities of HCN, glucose and benzaldahyde. Benzaldahyde is the aromatic product which emits the smell of almonds. Fragmentation of cells, lysis of vacuoles, and glycoside hydrolysis continue in ruminants during mastication and through the action of rumen microbial enzymes (3). Whether ingested plant enzymes retain their activity in the rumen remains to be seen.

The animals' consumption rate of saskatoon varied from 0.67 to 1.05 kg (dry weight)/100 kg (body weight) /day when chopped twigs were fed as 75% of the diet. Early signs of poisoning were evident at the 75% level of saskatoon. The yearling heifer (78-3) exhibited restlessness, shivering, loss of weight, scours and shortness of breath. Heart rate increased from 56/min to 85/min after the morning feeding, but by afternoon it returned to normal. Physical signs were less pronounced in the other animal (H4), but restlessness, shivering and loss of weight were evident. In two days of the 75% saskatoon feeding regime the potential rate of cyanide absorption was 9 to 11 mg HCN/kg/day or close to the 24 h tolerance level for ruminants (9).

Figure 1 illustrates the change in rumen fluid cyanide levels after dosing an animal (H3) with 68 or 100 g saskatoon/100 kg body weight, approximately one-tenth the estimated daily intake. Cyanide levels in rumen fluid



Hours after dose

FIGURE 1. Turnover of HCN in the rumen after dosing with saskatoon. Dose (D) was administered as 68 g/100 kg (squares) and 100 g/100 kg (circles).

were elevated dramatically in both experiments and normal levels were restored within three hours of dosing. The cyanide turnover shown in Figure 1 is extremely rapid with the greatest change in concentration occurring within two hours of dosing. An extremely rapid rate of absorption is inferred and the volatility of HCN (boiling point  $25.6^{\circ}$ C) at rumen temperature ( $39^{\circ}$ C) must be considered a contributing factor.

The quantities of saskatoon dispensed in the preceding experiments were equivalent to potential doses of 0.86 and 1.32 mg HCN/kg. At the higher level an increase in heart rate (100/min) was recorded 1.5h after dosing, but the normal rate (80/min) was restored an hour later. Subsequent experiments were designed to increase potential doses to 5 mg HCN/kg.

Table I lists the elevated saskatoon doses and cyanide potentials administered to three fistulated cows. In addition, the cyanide turnover pattern in the rumen is indicated during the initial three-hour period. In contrast to the preceding experiments, normal rumen cyanide levels (< 0.2 mg/L) were not restored rapidly when saskatoon twigs were dosed at 127 to 401 g/100 kg. Instead, the turnover pattern in the rumen pointed to a steady release of HCN with a gradual decline in concentration. The rate of release and the development of toxic cyanide levels would be related to the digestibility of saskatoon in the rumen. Unlike dormant winter twigs, one would expect new succulent shoots to be more digestible. The release of cyanide would be accelerated and conditions for poisoning could be enhanced.

Progressive stages of poisoning developed rapidly. Shivering, frothy salivation, abdominal contractions and a dramatic increase in heart rate were manifest during the first hour. The distinct smell of almonds emanated from the rumen. It required only one to two hours from the time of placing saskatoon browse in the rumen for peak cyanide levels to occur in the blood (Figure 2). The spectacular increase in heart rate correlated with the dynamic rise in blood HCN concentration (Figure 2). The rapid change in heart rate reflected the increased demand for oxygen at the cellular level where oxygen utilization was being inhibited by cyanide.

Cows H3 and H1 recovered, but in

 TABLE I

 Cyanide Content of Rumen Fluid at Intervals After Dosing With Saskatoon Twigs

Cow	Saskatoon dose	Potential cyanide dose	Minutes after dosing					
No			15	30	60	120	180	
	g/100 kg	mg HCN/kg						
				Rumen fluid HCN (mg/liter)				
Н3	127	1.62	7	12	9	5	3	
H1	269	3.43	37	28	28	9	7	
H2	401	5.12	173	58	64	63	47	

the case of the latter, the escape of HCN through a leaky fistula may have reduced the effect of the dose. These animals showed no change in respiration rates and only a slight increase in the pinkness of mucus membranes. Cow H2 on the other hand, showed all the classical signs of cyanide poisoning. The progressive stages developed as follows during the first two hours (minutes after dose indicated in brackets): increased heart rate (see Figure 2); muscle fasiculation in pelvic area (30); inspiratory stertor (40); staggering, perspiration and increased respiration rate (50); lateral recumbency and vertical nystagmus (70); increase in pinkness of mucus membrances and agonal struggle (95); rectal temperature, 36.7°C (135). At the three hour interval, mucus membranes were bright pink, rectal temperature,  $35.5^{\circ}$  C and 45 minutes later tonic convulsive contractions were recorded every five minutes. The rumen was emptied four hours and fifteen minutes after dosing and the classical antidote (NaNO<sub>2</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) was administered intravenously (1). The rumen was lavaged, fresh inocculum was dispensed and cow H2 recovered by next day.

The experiment with cow H2 demonstrated that a single dose of saskatoon browse (1.43% prunasin) can be lethal to cattle (Table I). Recent surveys on the prunasin content of saskatoon twigs have shown that values in excess of two or three percent prunasin are not uncommon in spring (W. Majak, unpublished results, 1979). Accordingly, a single lethal dose under these conditions could be  $\leq 200 \text{ g}/100$ kg. Furthermore, the potential for poi-

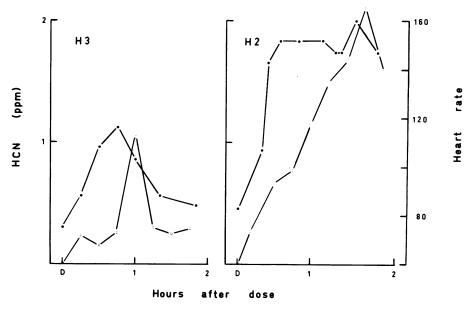


FIGURE 2. Increase in blood HCN concentration (open circles) and change in heart rate (solid circles) after dosing with saskatoon. Dose (D) was administered as 127 g/100 kg for cow H3 and 401 g/100 kg for cow H2.

soning increases significantly with new growth in spring. Values in excess of four percent prunasin have been recorded for leaves and shoots initiated in 1979 (W. Majak, unpublished results, 1979).

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