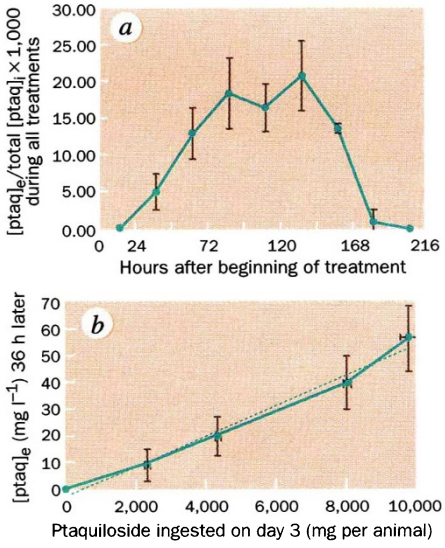
Bracken ptaquiloside in milk

SIR — Ptaquiloside, a norsesquiterpene[1] found in bracken (*Pteridium aquilinum*), is a potent carcinogen[2] that binds covalently to nucleotides, causing the splitting of uncoiled DNA[3]. Its potential for causing tumours and other ailments in farm animals is well established[4]. The observation that milk from cows feeding on a diet containing bracken fronds is carcinogenic and mutagenic to mice and rats[5,6] led to the suspicion that a bracken compound was present in this milk. When ptaquiloside was first discovered, it was the chief candidate for this carcinogen, but has never been demonstrated to be present in milk. Here we report our attempts to ascertain its presence in milk, to estimate the relationship between the amount of ptaquiloside ingested by cows via bracken and the amount excreted through milk, and to assess the risk of drinking raw milk from cows exposed to bracken. We find that ptaquiloside is excreted in milk at a concentration of about 8.6 ± 1.2% of the amount ingested by the cow, and is linearly dose-dependent.

We used milk from six two-year-old cows from an agricultural research station in Mérida, Venezuela, with an average milk production of 20±2 litres per day. For five consecutive days we gave the cows artificial diets containing 6.0 kg per day (2,400 to 10,000 mg per animal per day of ptaquiloside) of previously analysed[7] freshly collected young bracken fronds (neotropical var. *caudatum*). Milk samples were drawn each morning, frozen and analysed immediately[8] until all ptaquiloside disappeared. None of the cows showed signs of intoxication during treatment.



a, Ratio between ptaquiloside excreted through milk $[ptaq]_e$ and amount ingested $[ptaq]_i$ by cows (n=6) 38 h before, during 6 d of treatment with 6.0 kg fresh young bracken fronds containing an average of 4,422 ± 79 mg ptaquiloside. The approximate time required for ptaquiloside to appear in milk after the first ingestion of bracken fronds is 38 h. Error bars, s.d.. *b*, Variation of [ptaq]e (mg per l milk) by hour 86 in relation to the dose of $[ptaq]_i$ in the range 2,400–10,000 mg per animal per d, at a constant bracken frond intake of 6 kg per animal per d. Data for 5 animals showing the linear regression line (dotted). $[ptaq]_e$ (mg)= −2.62(±2.62) + 0.00562 (±4.3 × 10^{-4}) × $[ptaq]_i$ (mg) (r^2=0.9829, F= 171.08, P= 0.001).

We detected and quantified unstable, water-soluble ptaquiloside by selectively extracting it from deproteinated, defatted milk as stable pterosin B, the only alkali-induced decomposition product of ptaquiloside. Pterosin B does not occur naturally in milk from bracken-fed cows, and appears only after changing the pH of the defatted milk to 11 for 2 h at 40 °C. The per cent conversion curves of ptaquiloside in milk from bracken-fed cows and artificially ptaquiloside-contaminated milk in alkali at 38 °C overlap strikingly well over the 70 min of this experiment. We found no other secondary metabolite of bracken that could produce pterosin B under these mild conditions. Thus, pterosin B represents only its precursor, ptaquiloside. We performed quantitative assessment by dichloromethane extraction and reverse-phase HPLC analysis against standards. We also identified pterosin B in milk extracts by comparison of retention times in three mobile phases with pure compound and by the exact coincidence of the ultraviolet spectrum of the suspected peak (λ_{max}= 218, 260 nm) by stopped-flow scanning of the eluate.

We detected ptaquiloside in milk for the first time 38 h after feeding cows with bracken fronds. At a constant intake, the amount of ptaquiloside excreted daily increased progressively until the fourth day when it levelled off (*a* in the figure). After day five, ptaquiloside continued to be excreted at approximately the same rate for 62 h after the last treatment. Then, it decreased rapidly and disappeared from the milk by 86 h after the last intake. The average value of total ptaquiloside excreted $[ptaq]_e$ in milk is 8.6 ± 1.2 % of the total ptaquiloside ingested $[ptaq]_i$ over the entire feeding period. The daily $[ptaq]_e$ / $[ptaq]_i$ ratio during the first 38 h of feeding was independent of the dose ingested. However, there was a linear dependency of $[ptaq]_e$ on the dose given to the animal (*b* in the figure).

When we forced cows to eat bracken in two consecutive periods with a 48-h pause inbetween, we obtained a bimodal excretion curve. Thus, cattle exposed sporadically to bracken feeding will yield pulses of ptaquiloside in milk a few hours after starting to feed and for a period of more than 80 h after the animal has stopped eating bracken. This is a wide window for ptaquiloside to be present in milk. Despite the fact that cattle do not normally choose to eat bracken, they can be forced to eat it during periods of drought when bracken remains green, or when natural grasses are overgrazed, or when animals wander into dense bracken thickets. Therefore, there is a rather high risk to humans of ingesting milk containing ptaquiloside in areas where bracken is dominant. If a person drinks about 0.5 litres of milk daily from a cow producing 20 litres of milk per day, and this cow has eaten a subtoxic dose of ptaquiloside of 5,000 mg per day (as would be contained in 6 or 7 kg of fern, which typically might be eaten for a few days), this person will ingest 10.75 ± 1.45 mg ptaquiloside per day.

Although we cannot reach any definite conclusions at present about toxicity to humans at the intake levels reported here, as there are as yet no data on the toxicology of ptaquiloside in humans, the acute toxic effects of this chemical to rabbits at 50–100 mg per kg (ref. 9), as well as the expected consequences of ingesting a carcinogen and DNA-damaging compound at this concentration, strongly indicate cause for concern. It is certainly likely, in our view, that ptaquiloside in milk is responsible for the connection between bracken infestation and the incidence of gastric cancer in populations of farmers inhabiting cattle-range areas in Costa Rica[10] and in other countries where bracken growth is dense (Andean South America, Central America and southern Mexico).

Miguel E. Alonso-Amelot
Uvidelio Castillo
Grupo de Química Ecológica,
Departamento de Química,
Facultad de Ciencias,
Universidad de Los Andes,
Mérida 5101, Venezuela
Barry L. Smith
Denis R. Lauren
New Zealand Pastoral Agricultural Research Institute Ltd,
Ruakura Research Centre,
Hamilton, New Zealand

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